RESEARCH ARTICLE

Effect of different photoperiods on oviposition and eclosion rhythm of Drosophila agumbensis and Drosophila nagarholensis

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Abstract

Light is the most important zeitgeber that synchronize the circadian rhythm of several insects. The daily cycles of light and darkness is the main environmental cue organisms use to synchronize circadian rhythms to the 24h day. A circadian rhythm of activity is a major characteristic of insects affecting their ecology. In the present study circadian rhythm of oviposition and eclosion of *D.agumbensis* and *D.nagarholensis* were determined at three different photoperiods (LD 8:16, 14:10 and 16:8h) and constant 22 ± 1^0C temperature with 75% relative humidity in the laboratory. The flies exhibited the circadian rhythmicity of oviposition and eclosion in all the three photoperiods. The more number of eggs were laid during evening period and eclosion was observed only during morning hours in all the photoperiods. Circadian rhythm of oviposition was affected by different photoperiods but numbers of eggs laid at different photoperiods were significantly decreased when compared to LD 12L/12D condition. But eclosion rhythm was not affected by different photoperiods. Statistical analysis revealed that there is a significant difference in the oviposition at different photoperiods in both the species.

INTRODUCTION

Circadian rhythms have been documented in a wide range of behavioral, physiological and molecular processes in a variety of organisms including bacteria, fungi, plants, birds and mammals (Zordan et al., 2000). Recent studies in different organisms have shown that circadian timing systems are likely to be far more complex than thought earlier (Preitner et al., 2002), for example there is evidence that light input pathways for photo entrainment of circadian clocks may be a part of central clock or under clock control (Emery et al., 1998) and overt rhythms may feed back on circadian clocks (Mrosovsky et al., 1992). Moreover, the circadian clock itself might be composed of several oscillators, such as putative, morning and evening oscillators (Daan et al., 2001; Helfrisch-Forster, 2000; de la Iglesia et al., 2000; Jagota et al., 2000; Pittendrigh, 1981) or separate master or slave oscillators (Pittendrigh and Daan, 1976). There is also evidence suggesting that circadian timing systems may consist of multiple oscillators, each controlling different rhythms (Aschoff et al., 1967; Ebihara and Gwinner, 1992; Engelmann and Mack, 1978; Helfrich and Engelmann, 1987; Roenneberg, 1996; Sheeba et al., 2001a). In alga *Gonyaulax polyedra*, two oscillatory systems with red/blue sensitive photoreceptors control cell aggregation and bioluminescence and together fulfill the criteria for true self-sustained circadian oscillators (Roenneberg, 1996). In the moth *Pectinophora gossypiella*, egg-hatching, eclosion, oviposition rhythms are controlled by separate oscillators (Pittendrigh and Minis, 1971), and three separate are believed to regulate the initiation of larval wandering, diapause induction and eclosion rhythms in the fleshy *Sarcophaga argyrostoma* (Saunders, 1986).

Indicators of multiple oscillators controlling different circadian rhythms have also come from the studies on fruit flies of the genus *Drosophila.* The light pulse phase response curve (PRC) and the free running period (τ) of

eclosion and locomotor rhythms in *Drosophila pseudoobscura* were observed to differ (Engelmann and Mack, 1978). Similarly in *D. melanogaster*, eclosion, locomotor and oviposition rhythms had significantly different and peak of oviposition and eclosion, as well as the onset of locomotor activity, occurred at different times under 24h 12:12h light/dark (LD) cycles (Sheeba et al., 2001a). A parallel line of evidence supporting multi oscillator organization for circadian clocks comes from the studies on mutant *D.melanogaster* lines. In ebony mutants, adult locomotor activity was arrhythmic, where as eclosion was rhythmic (Newby and Jackson, 1991). In lark mutants eclosion was arrhythmic but locomotor activity rhythm was normal (Newby and Jackson, 1993). Though the observation of (τ) differences among rhythms (Engelmann and Mack, 1978; Sheeba et al., 2001a), strongly suggests the involvement of different oscillators in governing various overt rhythms, evidence from studies on the phase relationship among rhythms (ψ) (Sheeba et al., 2001a) and ebony and lark mutants (Newby and Jackson, 1991, 1993) suggests differences in output pathways among rhythms controlled by the same oscillators.

Light and temperature are the most important zeitgebers that influence circadian physiology of several insects (Johnson et al., 2003; Rensing and Ruoff, 2002; Saunders, 1982). Effects of temperature on circadian rhythms of eclosion, locomotor activity and oviposition have been reported in few insects' species. For example temperature cycles were used to entrain the eclosion rhythm of *Drosophila pseudoobscura* (Zimmermann *et al.*, 1968) *D.melanogaster* (Jackson, 1983; Konopka, 1972; Yoshi et al., 2002) *D.ananassae* (Khare et al., 2002) *Chymomyza costa* (Lankinen and Riihimaa, 1997) and *D.subobscura* (Lankinen, 1993). The activity rhythms of many insects are governed by abiotic factors, but light is the basic factor that sets the timing of the activity phase in nearly every case (Harker, 1961; Edwards, 1964; Tshernyshev, 1996). The daily cycles of light and darkness is the main environmental cue organisms use to synchronize circadian rhythms to the 24h day. A circadian rhythm of activity is a major characteristic of insects affecting their ecology (Neville, 1967; Pittendrigh, 1972; Roberts, 1965, 1974; Beck, 1980, Tshernyshev, 1984) and it is important to select the optimal photoperiod for their maintenance. The time of peak activity is determined by physiological processes that trigger behavioral reactions in insects. The objectives of this study were to determine the effect of photoperiods on circadian rhythm of oviposition and eclosion of *D.agumbensis* and *D.nagarholensis*.

Materials and methods

To study the effect of different photoperiods on oviposition and eclosion rhythm, *Drosophila agumbensis* and *Drosophila nagarholensis* were used. Experimental flies were obtained from Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore and then flies were reared on the normal wheat cream agar media seeded with yeast granules under laboratory conditions at constant temperature of 22±1°C with 75% relative humidity with light/dark (12L: 12D) cycles.

Oviposition rhythm:

Oviposition rhythm was assayed by collecting virgin flies from the experimental stocks and aged for seven days, and then transferring male-female pairs into vials (9.5cm ×2.5 mm) containing 6ml of food medium. Twenty five such pairs were subjected to light/dark (LD) 8:16h, 14:10h and 16:8h cycles. The intensity of the light used during the light phase of LD cycles was about 200 lux. Red light of wavelength greater than 650nm was used to handling and observing the vials in dark condition. The flies were transferred to fresh media vials at every 4h and the number of eggs laid in each vial over the proceeding 4h period was recorded. This procedure was continued for 10 consecutive days in each LD cycles.

Eclosion rhythm:

To assess the eclosion rhythm, eggs were collected from experimental stocks and dispensing them into 75 vials containing about 3ml of food media, at a density of 50 eggs per vial. Twenty five vials each were subjected to light/dark (LD 8:16h, 14:10h and 16:8h) cycles. The intensity of the light used during the light phase of LD cycles was about 200 lux. Red light of wavelength greater than 650nm was used to handling and observing the vials in dark condition. When adults began to eclose, vials were inspected every 4h. Eclosing adults were collected and the number of flies in each vial eclosed during the proceeding 4h period was recorded. This procedure was continued for 10 consecutive days or until most pupae had eclosed in each LD cycles.

Statistical analysis: Data obtained from oviposition and eclosion rhythm at different photoperiods were subjected to ANOVA and period was analyzed by using circadian physiology software version 3.5. Student 't' test was performed to compare the two species.

Results

Oviposition:

Effect of long and short photoperiods on oviposition rhythm of *D.agumbensis* is depicted in the figure 1 and table 1. Flies exhibits rhythmic pattern of oviposition in all the photoperiods. The peak of oviposition was 14.00h (afternoon 2'o' clock) in LD 12:12h, 18.00h in LD 8:16h and 14:10h cycles and 22.00h in LD 16:08 cycles. The period of oviposition was 23.8h in LD 12:12h, 23.6h in LD 8:16h, 23.7h in LD 14:10h and 23.8h in 16:8h cycles. In LD 12:12h cycle's flies laid more eggs but total eggs significantly reduced in LD 8:16h, 14:10h and 16:8h cycles. Circadian rhythm of oviposition was not affected by long and short photoperiods. The total numbers of eggs laid were significantly reduced in long and short photoperiods when compared to LD12:12h cycles. Analysis of Variance revealed that there was a significant difference in oviposition among long and short photoperiods (P<0.05) (table 1).

Effect of long and short photoperiods on oviposition rhythm of *D.nagarholensis* is depicted in the figure 2 and table 2. Flies exhibits rhythmic pattern of oviposition in all the photoperiods. The peak of oviposition was observed at 18.00h (evening) in LD 12:12h and 8:16h cycles, 18.00h in LD 14:10h and 22.00h in LD 16:8h cycles. The period of oviposition was 23.6h in LD 12:12h, 23.9h in LD 8:16h, 23.8h in 14:10h and 23.7h in LD 16:8h cycles. In LD 12:12h cycles flies laid more eggs than LD 8:16h, 14:10h and 16:8h cycles. Circadian rhythm of oviposition was not affected by long and short photoperiods but total numbers of eggs laid were significantly reduced when compared to LD12:12h cycles. Analysis of Variance revealed that there was a significant difference in oviposition among long and short photoperiods (P<0.05) (table 2). Interspecies comparison revealed that there was a significant difference in oviposition between *D.agumbensis* and *D.nagarholensis* t=6.87 in LD 8:16h, t=5.0 in 14:10h and t=9.34 in 16:8h cycles.

Eclosion:

Effect of long and short photoperiods on eclosion rhythm of *D.agumbensis* is represented in the figure 3 and table 3. Emergence of flies was not observed throughout the day. Eclosion was restricted to particular time of the day. It occurred only during morning in all the photoperiods. The peak of eclosion was observed at10.0h (morning) in LD 12:12h, 14:10h and 16:8h and 6.00h (morning) in LD 8:16h cycles. The period of eclosion was 23.9h in LD 12:12h, 23.8h in LD 8:16h, 23.9h in 14:10h and 23.7h in 16:8h cycles. No emergence was observed in the scotophase (dark) in all the photoperiods. Circadian rhythm of eclosion was not affected by long and short photoperiods. Analysis of Variance revealed that there is no significant difference in eclosion among long and short photoperiods (P>0.05) (table 3).

Effect of long and short photoperiods on eclosion rhythm of *D.nagarholensis* is represented in the figure 4 and table 4. Emergence of flies was not observed throughout the day. It was restricted to particular time of the day. Eclosion occurred only during morning in all the photoperiods. The peak of eclosion was observed at 10.0h (morning) in LD 12:12h, 14:10h and 16:8h and 6.00h (morning) in LD 8:16h. The period of eclosion was 24h in LD 12:12h, 23.8h in LD 8:16h, 23.6h in 14:10h and 23.8h in 16:8h cycles. No emergence was observed in the scotophase (dark) in all the photoperiods. Circadian rhythm of eclosion was not affected by long and short photoperiods. Analysis of Variance revealed that there is no significant difference in eclosion among long and short photoperiods (P>0.05) (table 4). Interspecies comparison revealed that there was no significant difference in eclosion between *D.agumbensis* and *D.nagarholensis* t=1.73 in LD 8:16h, t=1.69 in 14:10h and t=1.90 in 16:8 cycles.

Table 1: Mean±SE of oviposition rhythm of *D.agumbensis* under LD 12:12h, 8:16h,   14:10h and 16:08h cycles.
* P<0.05

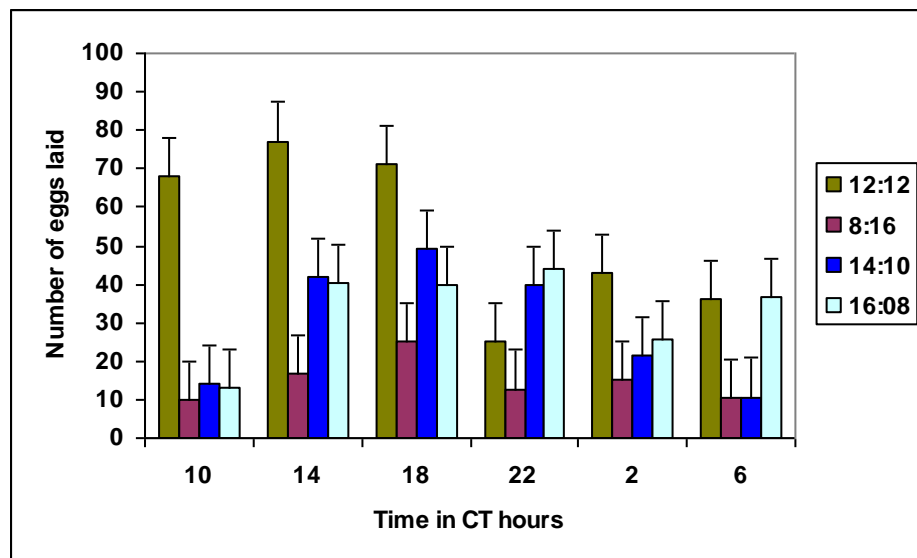| Time	LD 12:12	LD 8:16	LD 14:10	LD 16:8	F	Sig
10	66.9±9.7	10.1±3.9	14.3±5.6	12.9±1.5	20.4	0.00*
14	75.8±5.7	16.7±5.8	42±9.8	40.2±7.2	10.9	0.00*
18	69.2±6.1	25.3±5.2	49.2±6.9	40±5.0	9.7	0.00*
22	22.9±2.9	12.8±3.3	39.8±5.9	44.1±7.5	7.5	0.00*
2	42.5±6.7	15.3±5.0	21.3±3.8	25.7±5.0	4.9	0.00*
6	35.5±3.2	10.4±2.8	10.7±3.1	36.7±5.4	15	0.00*
F	4.3	1.56	66.0	4.3		
Sig	0.022*	0.18	0.00*	0.00*		

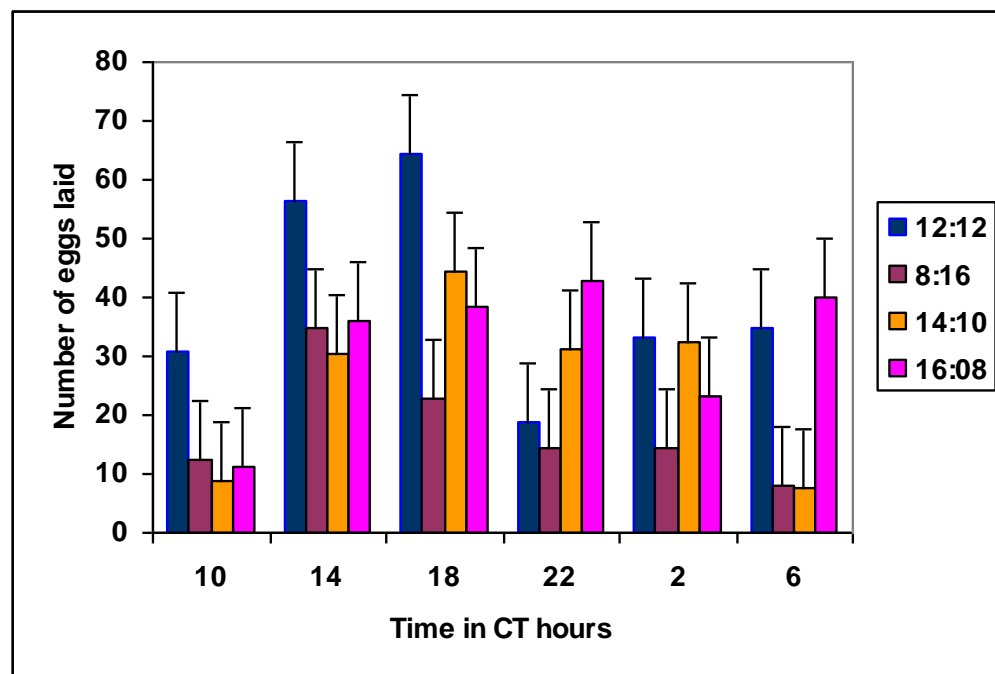Figure 1. Oviposition rhythm of *D.agumbensis under* 12:12, 8:16, 14:10 and 16:8 LD cycles

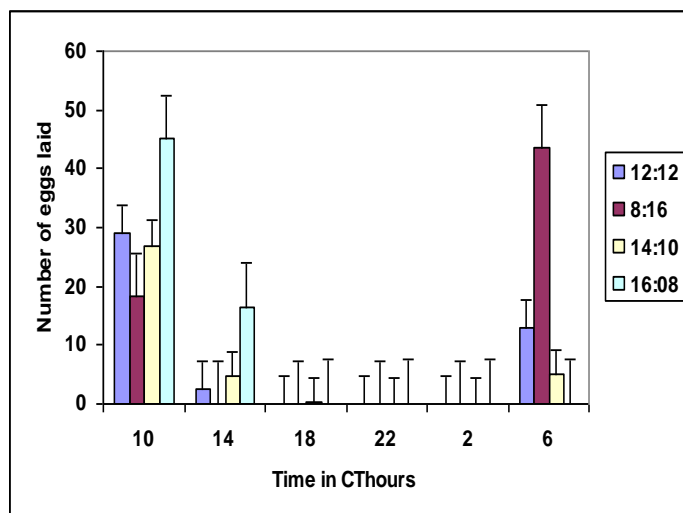Figure 2. Oviposition rhythm of *D.nagarholensis under* 12:12, 8:16, 14:10 and 16:8 LD cycles

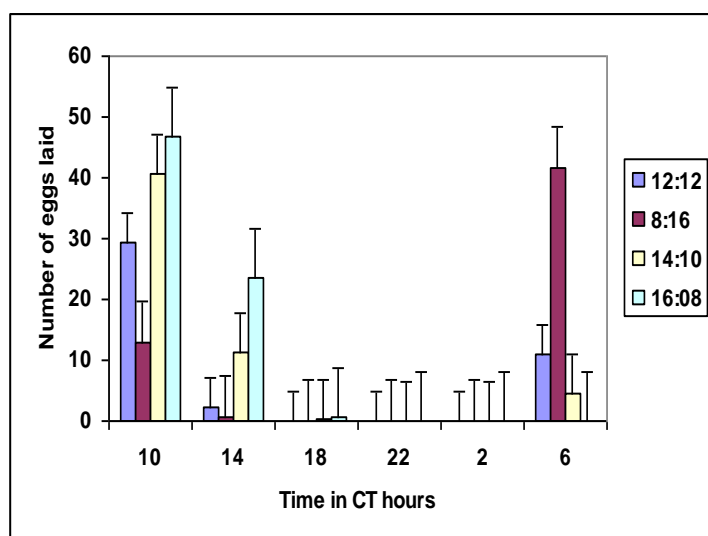Figure 3. Eclosion rhythm of *D.agumbensis* under 8:16, 14:10 and 16:8 LD cycles

Figure 4. Eclosion rhythm of *D.nagarholensis under* 8:16, 14:10 and 16:8 LD cycles

Table 2: Mean±SE of oviposition rhythm of *D.nagarholensis* under LD 12:12h, 8:16h,   14:10h and 16:08h cycles.

Time	LD 12:12	LD 8:16	LD 14:10	LD 16:8	F	Sig
10	31±6.9	12.6±4.9	8.9±3.4	11.2±3.7	4.1	0.01*
14	56.6±12.3	34.9±8.6	3.4±9.2	36±9.0	1.3	0.26
18	64.4±13.9	22.9±5.6	44.3±10.4	38.6±10.9	2.5	0.06
22	18.8±3.8	14.5±3.4	31.3±6.2	42.9±9.3	4.2	0.01*
2	33.4±8.2	14.4±4.9	32.6±8.9	23.1±4.4	0.6	0.57
6	34.8±7.4	8.2±2.1	7.8±2.4	39.9±9.3	7.4	0.01*
F	3.3	3.2	2.05	2.17		
Sig	0.01*	0.01*	0.08	0.07		

*P<0.05

Table 3: Mean±SE of eclosion rhythm of *D.agumbensis* under LD 12:12h, 8:16h,   14:10h and 16:08h cycles.

Time	LD 12:12	LD 8:16	LD 14:10	LD 16:8	F	Sig
10	29.1±1.6	18.4±2.1	27±4.7	45.1±2.5	11.1	0.00*
14	2.4±0.6	0.1±0.1	4.7±1.1	16.5±2.6	28	0.00*
18	0.1±0.1	0.0±0.0	0.2±0.2	0.1±0.1	0.73	0.53
22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	0.00
2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	0.00
6	12.8±1.5	43.6±3.7	4.9±2.3	0.0±0.0	89.6	0.00*
F	149.2	103	22.1	146.9		
Sig	0.00*	0.00*	0.00*	0.00*		

*P<0.05

Table 4: Mean±SE of eclosion rhythm of *D.nagarholensis* under LD 12:12h, 8:16h,   14:10h and 16:08h cycles.

Time	LD 12:12	LD 8:16	LD 14:10	LD 16:8	F	Sig
10	29.1±1.6	13±0.9	40.6±5.6	46.9±2.5	16.1	0.00*
14	2.4±0.6	2.0±0.7	11.3±1.4	23.6±2.4	51.8	0.00*
18	0.1±0.1	0.0±0.0	0.4±0.2	0.7±0.49	0.86	0.4
22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	0.00
2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.00	0.00
6	12.8±1.5	41.7±2.7	4.6±1.5	0.0±0.0	143.2	0.008*
F	149.2	204.9	41	175.6		
Sig	0.00*	0.00*	0.00*	0.00*		

* P<0.05

Discussion

Oviposition:

     In insects, the circadian system is responsible for imposing daily rhythmicity on a variety of processes, including locomotor activity, oviposition, hatching, pupation and ability to return at the appropriate time to a food source that is available only at a particular time of the day. The circadian system functions as a biological clock, capable of providing the individual with information on the time of day and the ability to measure lapses of time (Page, 2003). Daily light and dark cycles are the main environmental cues organisms use to synchronize circadian rhythms to a 24 hour of the day. The light-dark not only has a synchronizing function but also a direct excitatory or inhibitory influence. Together these two effects determine, to a great extent, the time of the day at which an animal active (Aschoff, 1960). *Drosophila* sps is the classic example of the influence of photophase on synchronization of emergence (Pittendrigh, 1967). Circadian rhythm of oviposition activity of several *Drosophila* sps on highland populations in Argentina were early in the afternoon in the field (1400-1600h) as opposed to populations from the much warmer lowland site, where flies were mainly active in the early morning prior to sunset (Dahlgaard et al., 2001). In each cross between genetically different strains of *Drosophila melanogaster*, the oviposition curves of F1and F2 were usually close to the midparent curve (Allemand and David, 1984). In the present study highest oviposition was occurred during evening period and more number of eggs was noticed in LD 12:12h than other photoperiods in both the species and interspecies comparison revealed that there is significant difference in oviposition between *D.agumbensis* and *D.nagarholensis.* Dhanashree et al., (2005) reported that *Drosophila melanogaster* laid more number of eggs during light to dark transition under LD cycles. Greenberg et al., (2006) have been demonstrated that oviposition was highest during morning period in boll weevil. The circadian rhythm of oviposition, emergence, photo and gravity-tropism of egg parasitoid *Trichogramma* sps have been reported (Afonina et al., 1985, 1986; Dakhiya et al., 1985; Greenberg, 1991). Tshernyshev (1984) reported that the cabbage fly, *Delia brassicae* Bouche by Howkes and flower flies, *Syrphidae* sps have maximum oviposition at the end of the day. Sharp drops in duration of the day light also stimulated oviposition by *Pyralidae* at dusk (Tshernyshev, 1984).

     Circadian rhythm of oviposition was endogenous because they were maintained during constant conditions of the light and temperature and could not be induced by direct influence of any control factors. Presence of endogenic rhythm and its expression depended completely on insect's genetics and did not disappear in relation to

the environment of the parents. Circadian rhythm has been expressed as a result of influence on the maternal organism. Similarly the transmission of rhythm from maternal organism to the daughter was shown for the fly *Dacus (Strumela) tryoni* Frogg (Bateman, 1955). The heritability of the circadian rhythm trait is independent of the previous environment of the maternal organisms. *Drosophila* reacted to the light cycle of the current environment but this was not reflected on the rhythms of their progeny (Tshernyshev, 1984). Moreover endogenic rhythms are more typical for insects of tropical and subtropical origin species, which has expanded only recently into temperate regions (Tshernyshev, 1984). The present study clearly showed that circadian rhythm of oviposition is not affected by different photoperiods but numbers of eggs laid by flies were significantly reduced when compared to 12L/12D condition.

Eclosion:

In several insect species adult eclosion is gated in a manner that it occurs only during a narrow window of time, generally around dawn, when environmental humidity is highest (Pittendrigh, 1974). In *D.melanogaster* the clocks that gate adult eclosion are located in the prothoracic gland and ventral lateral neuron (Myers et al., 2003) and it is believed that these clocks also play a key role in the regulation of pre-adult development (Qui and Hardin, 1996). Eclosion occurs once in a single fly's life time, the multiple events that occur over several days within a population are considered as rhythm (Skopik and Pittendrigh, 1967). In the present study eclosion events occurred only during morning hours in all the photoperiods in both the species. Tanaka and Watari (2009) also showed that peak of insects eclosion occurs during early morning. One of the possible explanations for the early morning adult eclosion in insects is that the relatively humid and cool air favours expansion of the wing in newly emerged adult (Tanaka and Watari, 2009; Pittendrigh, 1954). The dry and hot air around noon exerts a harmful effect on the wing by loosing water at a faster rate. Further, it has been found that desiccation does not prevent expansion of the wing in newly emerged onion flies (Diptera), but high temperature disturbed the process of wing expansion significantly. In the present study peak of emergence was observed only during morning hours in all the LD cycles, probably to prevent temperature. Interestingly the peak of emergence is restricted to photophase. Tshernyshev (1984) reported that butterflies emerge during morning hours, but moths emerge during evening. Greenberg et al., (2006) have demonstrated that highest emergence of boll weevil was observed during morning hours under different LD cycles. Dhanashree et al., (2005) reported that peak of eclosion was observed during morning hours in *D.melanogaster* under different photoperiods. In *D.pseudoobscura* (Pittendrigh, 1954), *D.littoralis* (Lankinen, 1986) and *D.subobscura* (Lankinen, 1993). It has been found that eclosion peaks occur close to lights on in light/dark cycles, but the lengths of light-dark phases may influence this timing. Even though eclosion occurs once in the life cycle of an individual insect, the timing of eclosion is controlled by circadian oscillation (Saunders, 1982). There is a persistence of circadian rhythmicity in an aperiodic environment for several generations (Paranjpe et al., 2003), so as in the present situation. Organisms living in periodic and constant environments confer adaptive value by coordinating various cyclic metabolic processes to external environmental cycles (Pittendrigh, 1993). The present study clearly showed that, the eclosion events are not affected by different photoperiods in both the species.

Acknowledgements

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