672..673aa and addition of aspartic acid (Table 2). The mechanism by which the sequence variations of *In(1)B^{M2}(reinverted)* may affect the phenotype is being currently studied.

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Circadian rhythm of locomotor behavior of *D. agumbensis* and *D. rajasekari* collected from Sakleshpur.

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Abstract

Circadian clock regulates physiological and behavioral processes in a wide variety of organisms ranging from unicellular organism to human beings. The present study is aimed to investigate circadian rhythm of locomotor behavior of *D. agumbensis* and *D. rajasekari* collected from Sakleshpur, Karnataka, India at 910m altitude. Locomotor activity behavior was assayed by using *Drosophila* activity monitor (Trikinetics IV) under laboratory conditions. Both the species exhibited unimodal activity. The range of activity was 126-851 in *D. agumbensis* and 114-964 in *D. rajasekari*. The period of activity of both the species was closer to 24h. Statistical analysis revealed that there is a significant difference in the locomotor behavior of the two species. Keywords: locomotor activity; *D. agumbensis*; *D. rajasekari.*

Introduction

Circadian rhythms characterize the physiological processes of organisms ranging from the unicellular dinoflagellate *Gonyaulax polyedra* (Hastings and Sweeney, 1958) to human beings (Wever, 1979; Aschoff, 1981; Brady, 1981). It is now reported that besides eukaryotes, even cynobacteria among eubacteria possess circadian clocks (Ouyang *et al.,* 1998). The ubiquitous occurrence of circadian clocks at various levels of organization and complexity suggests that they may be of adaptive value (Aschoff, 1994; Aschoff *et al.,* 1982; Hastings *et al.,* 1991; Pittendrigh, 1993; Sharma, 2003a). It is believed that circadian clocks benefit organisms by efficiently timing various behavioral and metabolic processes to appropriate times of the day in accordance with cyclic external and internal environments (Aschoff, 1964; Aschoff *et al.,* 1982; Hastings *et al.,* 1995;

Pittendrigh, 1993; Sharma, 2003a). Previous studies on fruit flies (*D. melanogaster*) (Pittendrigh and Minis, 1972), bollworm (*Pectinophora gossypiella*) (Von Saint Paul and Aschoff, 1978), and Cynobacteria (Ouyang *et al.,* 1998) have demonstrated that survival of organisms regularly maintained under light/dark (LD) cycles (12:12h) is enhanced considerably if their periods of activity closely match those of the (LD) cycles.

Several studies on the altitudinal variation in circadian physiology of *Drosophila* are rather few as compared to that of latitudinal variation. Altitude dramatically altered the basic parameters of eclosion rhythm of the Japanese strains (Pittendrigh and Takamura, 1989), *D. suboobscura* (Lankinen, 1993) in the locomotor and eclosion rhythms of *D. ananassae* from Srilanka. Molecular polymorphism in *period* gene of different latitudinal strains of *D. melanogaster*, *D. littoralis,* and *D. simulans* collected from Europe, Africa, and Australia was ascribed to natural selection (Costa *et al*., 1992; Sawyer *et al*., 1997; Weeks *et al*., 2006; Vanlhriatpuia *et al*., 2007). Consistent with the idea that there may be a link between circadian genes and latitudes, there is a significant latitude cline in the distribution of the Th/Gly polymorphisms in Europe and North Africa (Costa *et al*., 1992; Sawyer *et al*., 1997). Genetic components for the altitudinal differences in oviposition rhythm were analyzed by carrying out crosses within and between populations of *D. buzzatii* that originated from different altitudes in Argentina (Dahlgaard *et al*., 2002). Recently several aspects of circadian physiology of *Drosophila* have been investigated, such as the latitudinal variation in the circadian rhythm of eclosion (Joshi and Gore, 1999), adult locomotor activity (Joshi, 1999; Palaksha *et al.,* 2011), and altitudinal variation in Himalayan strains of *D. helvetica* (Vanlalhriatpuia *et al*., 2007). In view of this, the present study has been undertaken to investigate the circadian rhythm of locomotor behavior of *D. agumbensis* and *D. rajasekari.*

Materials and Methods

The species used in this experiment were *D. agumbensis* and *D. rajasekari,* collected from Sakleshpur, Karnataka, India at 910m altitude during the month of April 2011. Isofemale lines were maintained, and from the first generation flies adult locomotor activity was assayed. Activity was recorded under 12L/12D conditions.

Locomotor activity was analyzed by using *Drosophila* activity monitor (Trikinetics IV). The activity of an individual fly was monitored by placing the fly into a glass tube (6.5 cm length × 1.5 cm diameter). The tube was inserted into a ring detector. One end of the tube was provided with culture media (2 g agar and 2 g sucrose and yeast), and the other end was plugged with cotton. The tube was placed in the path of an infrared beam. The culture medium was replenished once each two days. Every interruption of the infrafred beam by fly movement triggers all or none electric signal that was counted and registered. Every time the fly moves is referred as bout. It was summed at 10 minute intervals known as bins (10 min = 1 bin). This procedure was continued without interruption for ten days or 240 hrs.

Statistical analysis: The data were subjected to Student ‘t’ test, and period was calculated using Refinetti’s software, version 3.5.

Result

Circadian rhythms of locomotor behavior of *D. agumbensis* and *D. rajasekari* were depicted in Figures 1 and 2. Locomotor activity behavior of both species showed unimodal activity. Activity continued throughout the day (photophase) and exhibit rhythmic activity pattern. The peak of activity was observed at 14 h in *D. agumbensis* and 10 h in *D. rajasekari* (Figures 1 and 2). The period of activity was 23.8 h and 23.7 h, respectively, in *D. agumbensis* and *D. rajasekari*. The range of activity was 126-851 in *D. agumbensis* and 114-964 in *D. rajasekari*. The Student 't' test revealed that activity between two species was significant (t = 13.3, P < 0.05).

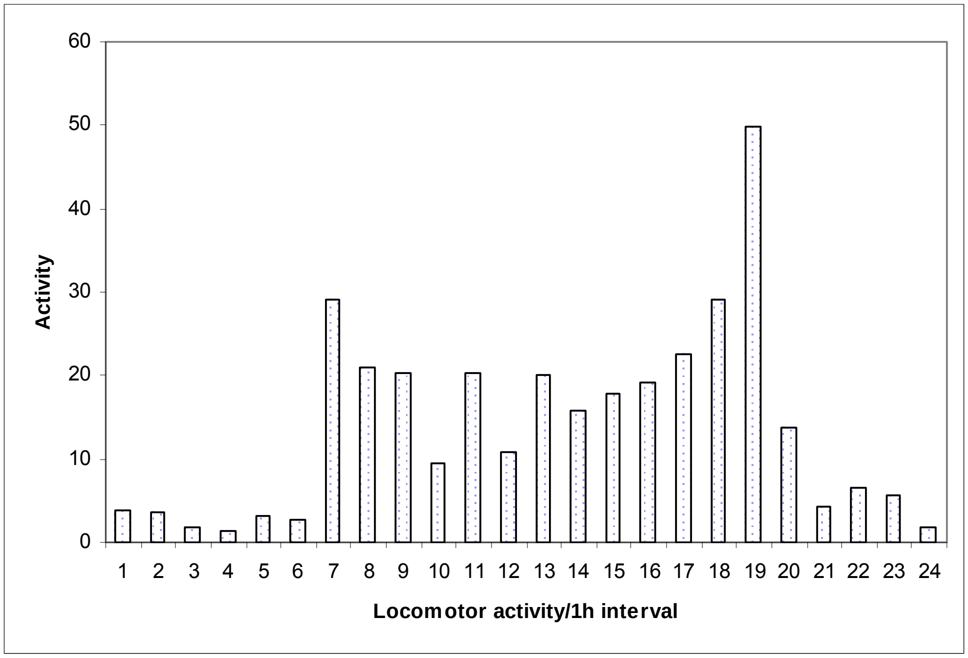

Figure 1. Average locomotor activity of *D. agumbensis* from Sakleshpur at 910m altitude.

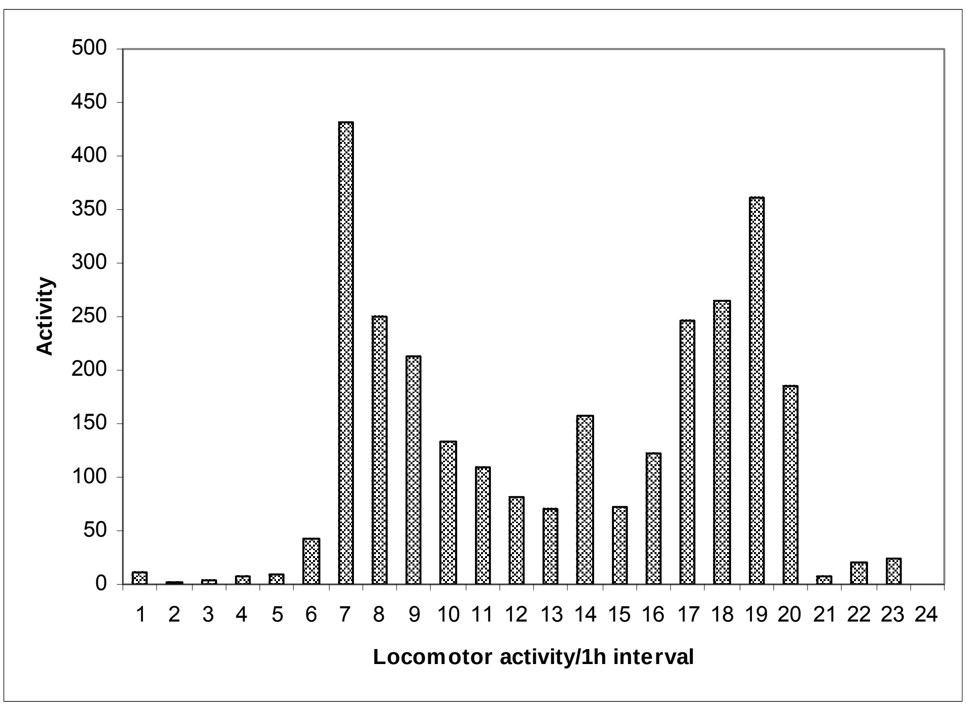

Figure 2. Average locomotor activity of *D. rajasekari* from Sakleshpur at 910m altitude.

Discussion

Circadian rhythm helps the organism to adapt to daily light/dark cycles and environmental cues such as light, moisture, temperature and can entrain the clock cells that affect rhythmic behavior

(Klarsfeld and Rouyer, 1998; Zheng and Sehgal, 2008; Cong *et al*., 2010). The wild type *D. melanogaster* flies are extensively studied and exhibit regular locomotor rhythm under 12L/12D light cycles. It had two peaks, one at dawn and the other at dusk (Wheeler *et al*., 1993; Forster, 2001; Sharma, 2003). Organisms that live in an environment in which they experience alternating times of constant conditions and diurnally changing physical and social conditions are useful as they enable researchers to examine the plasticity of circadian rhythms in response to the change in the environment (Sharma, 2003). Flies from Sakleshpur populations, such as *D. agumbensis* and *D. rajasekari*, showed unimodal activity. Activity was observed throughout the day in both the species. Simunovic and Jaenike (2006) have reported locomotor behavior of a few species of *Drosophila* from higher latitude active throughout the day and species from lower latitude activity restricted to a particular time of the day. Locomotor behavior of a high altitude Himalayan strain of *D. helvetica* showed unimodal activity pattern with a single delayed peak that commenced in the forenoon and continued till evening, while the low altitude Himalayan strain of *D. helvetica* had bimodal activity pattern with an early morning peak that was separated from the evening peak (Vanlalhriatpuia *et al*., 2007). Altitudinal strains of *D. bipectinata* exhibited bimodal activity with morning and evening peaks, and *D. malerkotliana* showed unimodal activity with evening peak (Palaksha *et al.,* 2011).

In the present study both the species of *Drosophila* showed activity throughout the day. This might be due to the flies experience of low desiccation stress in high altitude regions. They inhabit in the higher altitude where temperature is much less and relative humidity is very much higher than the lower altitude. Thus, in a physically permissive environment, selection may favor those individuals that continue their activities, mating, and egg laying throughout the day.

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