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Abstract

Caves offer a particular environment where to ask if circadian rhythms still represent an adaptive meaning in an apparent non-cyclic environment. Diversity of animals from caves has been studied in order to detect circadian activity in different behaviors. The present study reports that most of spiders of the families Dipluridae and Ctenidae, found in the deepest and middle zones respectively of the Los Riscos cave, in Querétaro State, México, present circadian rhythms of locomotor activity in free running; and light cycles changes the aforementioned circadian period as it would occur during entrainment process. We discuss about the existence of remnant circadian photoreceptors in a non-photic environment, but also that non-photic zeitgebers may entrain circadian activity of the fauna inside the cave.

Keywords: Ctenidae; Dipluridae; circadian; locomotor activity; cave spiders.

1. Introduction

Caves represent an ecosystem where different habitats are characterized by partial or total darkness; daily physicochemical fluctuations such as temperature, light and humidity are considered damped compared with environment changes at the surface. Caves are, however, a specific habitat for different living organisms that have colonized these subterranean environments, partially or totally along their their life cycle. Organisms from surface that occupy cave environments as a shelter and breeding place frequently are known as troglophiles; while the troglobite fauna presents complete life cycles inside the cave. During many generations troglobites may develop specialized morphological, physiological and ethological adaptations to the specific environment of caves throughout evolution (Trajano et al, 2011).

Caves are habitats in which cyclic environmental signals may not be obvious. At the main entry of the cave, daily cycles in light, temperature and humidity, may play a role in the entraining of the circadian activity for animals that are exposed to these natural signals. Nevertheless, in underground or deepest regions with constant or poorly fluctuant environmental conditions, time signals to entrain endogenously generated circadian rhythms become weak (Poulson & White, 1969; Lamprecht & Weber, 1992). The existence of circadian rhythms in species found in the deepest and dark zones in caves, may be then considered as a residual trait of ancestors living in surface, and when explored in laboratory conditions, damped circadian rhythms may still observed in different species of fish, (Trajano et al, 2005; Pati, 2001, Trajano et al, 2011), crustaceans (De la O-

Martinez et al, 2004); insects as crickets (Hoenen, 2005), millipede (Koilraj et al, 2000), spiders (Hoenen and Gnaspini, 1999) salamanders (Hervant and Durand, 2000), frogs (Espino Del Castillo et al 2009). Even though photic zeitgebers may not occur, non-photic zeitgeber may be of relevance to entrain and feedback the species rhythms inside the cave (Stringer and Rochow, 1997) such as activity of bats (Vanlalnghaka et al, 2005; Joshi and Vanlalnghaka, 2005).

Studying spontaneous circadian activity in species found in caves may give us an idea about the adaptive meaning of having a functional circadian clock in organisms that live in such interesting habitat, and the way in which circadian rhythms would be an advantage in apparently non-cyclic environments.

The Cave "Los Riscos", located in State of Querétaro, México, is a habitat for a limited diversity of animals including arthropods and some vertebrates (Espino-Del Castillo et al, 2009), and an interesting distribution of species may be related with geophysical characteristics of the cave (Espinosa et al, 2012). A particular diversity of spiders were found along this cave, with more than 30 species (Soriano-Morales, 2009; unpublished results); however, it is noteworthy that two frequent species of spider were found in deepest and middle regions of the cave that belong to families Dipluridae (*Euagrus luteus)* and Ctenidae (*Ctenus mitchelli*), respectively. Individuals of these species where collected in the deepest and middle region of the cave. The present work has the aim of studying if is possible to detect circadian locomotor activity rhythms, in laboratory conditions (free running and entrainment to light cycles), from different animals of these two species, collected in deep areas of the cave of Los Riscos.

2. Materials and Methods

2.1. Cave Locality and animals sampling

The cave “Los Riscos” is located at the Sierra Gorda, Queretaro, which is part of the Sierra Madre Oriental in Mexico. It is located at the coordinates 21° 11' 38" N, 99° 30' 50" W, and at 1,122 m above sea level. It represents a mixed underground system that include tunnels and galleries (Espino- Del Castillo et al 2009; Lazcano-Sahagún, 1986a, b; Espinosa et al, 2012). In deepest zone “D” (Figure 1) humidity is above 90% and temperature varies from 18-20 ºC, it is a place in complete darkness. In middle zone of the cave (zone B and C) humidity is the same, but temperature varies from 17-18 ºC, also in complete darkness, however, zone B is near to the main entrance of the cave. Spiders of two arachnid species (*Euagrus luteus* and *Ctenus mitchelli*) were collected and transported to the laboratory of circadian studies. Specimens were transported to laboratory facilities at Facultad de Ciencias, by maintaining spiders in complete darkness and in a cold recipient, as indicated elsewhere (Espino et al, 2009). As soon they arrived to the laboratory, they were located in special aquaria detailed further. After recordings of locomotor activity, specimens were donated to the collection of cave fauna, at the Laboratory of Speleobiology and Acarology UMDI, Facultad de Ciencias, UNAM. Juriquilla. Querétaro, México.

2.2. Circadian locomotor activity studies

A total of 20 diplurids and 5 ctenids were collected in different times during three years and used for this study. A group of spiders of both species were initially studied, at least 10 days on continuous dim red light (~1 lx, indicated further also as DD) and then to Light Dark cycles (LD 12:12; 150 lx). A second group of ctenids were recorded first in LD, and then exposed to constant darkness (DD) in order to observe free running rhythms after entraining.

Spiders fed juvenile leg-less krickets once every week, in such way that any locomotor activity from the insects did not interfere with locomotor activity. Special recording aquaria built with acrylic plastic, were equipped with infrared light crossings. Locomotor activity was monitored above a surface of humid soil (Peat Moss) and inside a high humidity environment (fig 2). Recording aquaria were kept in light-tight wooden boxes, continuously ventilated and maintained at 23 ± 2 °C in environment controlled rooms (Miranda-Anaya et al, 2003; Espino-Del Castillo, 2009). Animals were individually recorded and its locomotor activity summarized every 10 min. Data were stored by a data acquisition board and software ACTIBIO (Designed by URIDES, Fac. Psicología, UNAM, México) until further analysis.

2.3. Data Analysis of locomotor activity

Data were analyzed in double plotted actograms. The respective circadian periods (□) of at least five consecutive days were calculated using periodogram intervals using the software CHRONOSFIT (Zuther et al, 2009). Circadian period values with spikes above the confidence interval ($P<0.05$) were considered rhythmic. Comparisons of period length were performed with a non-paired students' t-test, of the software program Statistica (Stat-software, Jandel Scientific, San Rafael, CA. USA). Significant differences were considered when $P<0.05$. Average values are presented as means ± SE.

3. Results

3.1 Locomotor activity rhythms and photic entrainment

Figure 3 shows four representative examples in double plotted actograms of two spiders of family dipluridae (A) and two of family ctenidae (B), held in DD during at least 9 days; correspondent periodograms are shown on the right for DD. The actograms show diversity in structure of activity, going from unimodal to disperse, while circadian periods were still detected. From 20 diplurids explored in this condition, 15 organisms displayed statistically significant circadian activity, and from 5 ctenids studied, three spiders presented significant circadian periods.

When exposed to LD cycles, 10 animals displayed shortening in free running, as shown in Figure 4A. It was, however, not possible to record further locomotor activity because animals died. A different group of diplurids were set in the opposite protocol (Fig. 4B), initiating in LD after being collected and then released in DD. During photoperiod, three of them presented a bout of activity close to lights off during 9 days. When released in DD, a short bout of activity still free ran from the previous phase in LD, with a period longer than 24h indicating a true entrainment. Average free running period during DD and in LD is shown in Fig 4D, even though no significant differences were found, a tendency to shortens the free running period indicates a possible the entraining effect that light had in some organisms, as shown by the SEM.

4. Discussion

Caves are environments that have a diversity of microenvironments according to their topography. Light may be scattered at different areas from diverse entries, but most of the caves present environments in complete darkness. "Los Riscos" Cave in Querétaro, present both kind of characteristics and a diversity of fauna have been studied, indicating that either troglobitic and troglophile organisms are found. The zones where spiders were collected are totally dark at midday (Espino Del Castillo, et al, 2009). It is uncertain if this species of arachnids have constant mobility to areas where light may act as zeitgeber; Ctenids were collected in the deepest region of zone B (Fig 1) where, from few positions, it is possible to observe light from the main entry. However the zone where Diplurids were collected, no daylight can be seen. In our recordings of locomotor activity, circadian photoreceptors in most spiders seem to be functional, even though light cycle may not be the main zeitgeber for these organisms in their natural habitat. It is well known that diverse cave insects may display circadian rhythms of activity (Hoenen, 2005; Koilraj et al, 2000; Oda et al, 2000; Hoenen and Gnaspini, 1999; Stringer and Rochow, 1997). In the present work, we noted that most of the organisms collected of Dipluridae and Ctenidae presented statistically significant circadian rhythms. Despite of the fact that light entraining occurs, in an environment where no light cycles were detected, we could consider this characteristic as a remnant photosensitivity able to feed circadian oscillators in these species. Also, it is important to note that a high number of Diplurids displayed significant circadian rhythms, with a diversity of activity structure from unimodal to bimodal and arrhythmic; indicating that activity profile of inhabitants in the cave may be diverse, and non-photic zeitgebers may exist where daylight does not reach (Stringer and Rochow, 1997). Particularly, the location where Diplurid spiders were collected is a place where a colony of vampire bats *Dermodus rotundus* and *Diphylla ecaudata* can be located (Espinosa et al, 2012). Bats enter to the cave during morning and leaving near dusk. It is possible that this activity would work as a non-photic zeitgeber, since bats are hosts of parasitic flies such as streblids (Guerrero & Morales-Malacara 1996). And, in deepest zone of this cave, activity of flies was seen where these bats species have their roosting place (zone D): therefore, it is possible that those streblids flies may be preys for these arachnids, along with the fact that the soil of the cave present fresh stools of bats, that may carry other fauna. It has been discussed that circadian clocks provide fitness advantage even to organisms living under constant conditions. However, studies carried out on free-living animals under field conditions may give a better idea about the cyclic environment (Paranjpe y Sharma, 2005), considering more than just physicochemical variables, and understanding the adaptive meaning of circadian rhythms when non-photic zeitgebers may occur.

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Figure 1. An overview of the Los Riscos Cave, indicating sampling area (section D: *E. luteus*; section B: *C. mitchelli*) where diplurids and ctenids were collected.

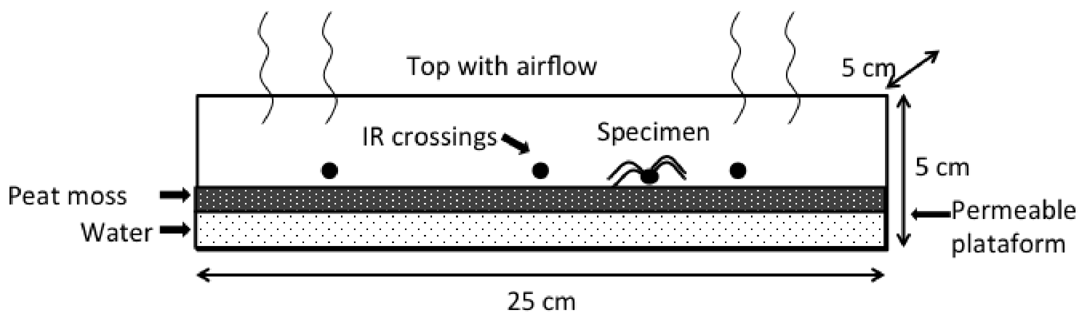

Figure 2. Aquarium used for recording locomotor activity in spiders. In order to ensure high humidity, a section of water underneath a permeable floor of moss, allowed saturate humidity of substrate.

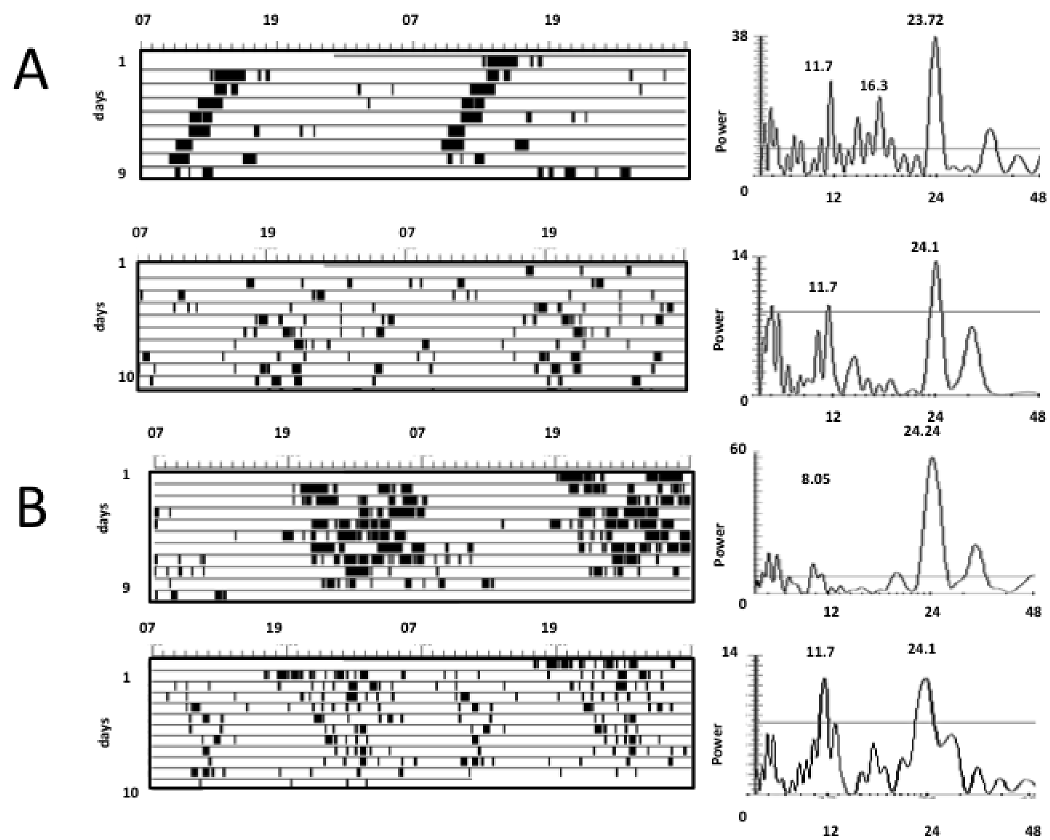

Figure 3. Actograms of locomotor activity from spiders Dipluridae (A) and Ctenids (B), showing circadian rhythms in DD, correspondent periodograms are shown at the right.

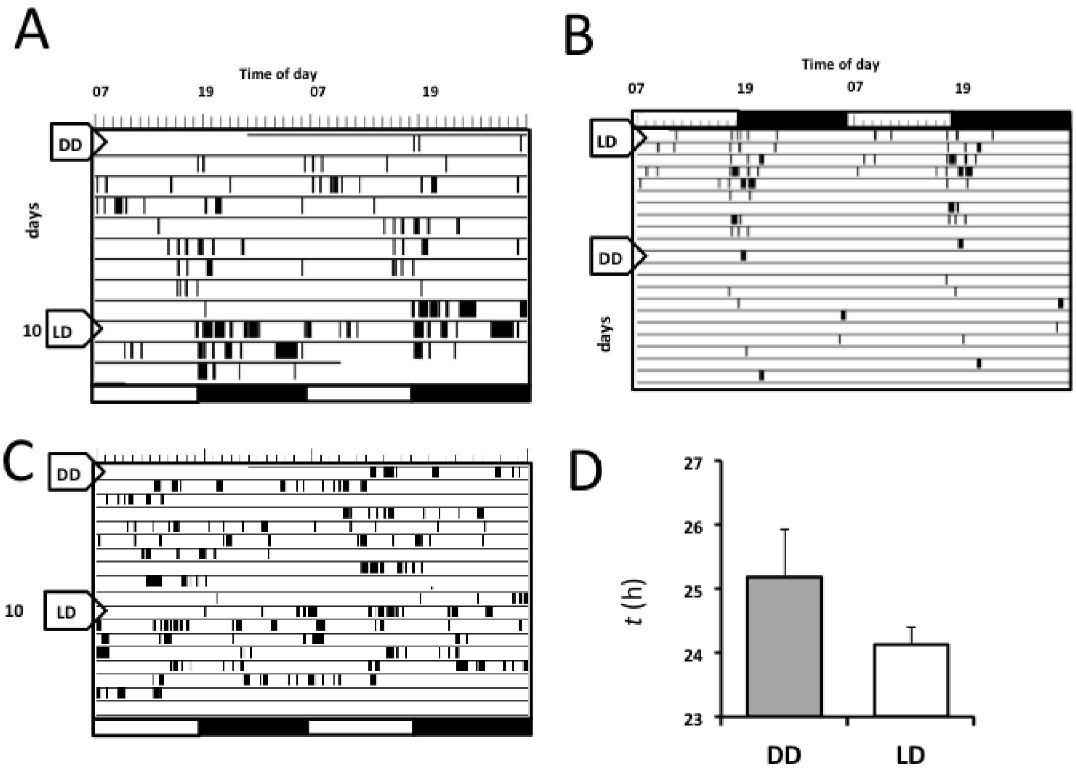

Figure 4. Actograms of free running rhythms and entraining of Diplurids (A and B) wen first in LD and then in DD, free running rhythms are noted, and of a Ctenid in C. In D, average of free running period in DD (25.18± 0.75 h) gets shorter in LD (24.12± 0.29 h).