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Chronobiological studies on body search, oviposition and emergence of *Megaselia scalaris* (Diptera, Phoridae) in controlled conditions

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ABSTRACT

Circadian clocks have evolved to synchronize physiology, metabolism and behaviour to the 24-h geophysical cycles of the Earth. Understanding the circadian clock mechanism could play an important role in forensic entomology because it temporally gates behaviour such as locomotor activities, feeding, mating, egg laying and adult emergence which could provide useful information for crime reconstruction. The scuttle fly *Megaselia scalaris* colonises both exposed and buried bodies, whether indoors or outdoors. Locomotor activity, oviposition and adult emergence of this species have been investigated using technologies employed in many previous *Drosophila* circadian studies. The results reported here clearly highlight the underlying role of the circadian clock in regulating the behaviour of males and females of *M. scalaris*, and show the role of light as a "zeitgeber" for clock resetting. In contrast to Calliphoridae, *M. scalaris* can reach the oviposition site and lay eggs in darkness both during the day and the night, although the number of ovipositing females is lower under subjective darkness. The number of eggs laid shows a clear circadian rhythm with much higher numbers laid during the day than during night or subjective night. In conclusion, locomotor activity and oviposition rate of *M. scalaris* is under circadian clock control with significant forensic implications.

1. Introduction

Circadian clocks have evolved to synchronize physiology, metabolism and behaviour to the 24-h geophysical cycles of the Earth [1]. Though the chronobiology of diverse organisms from bacteria to humans has been studied, the preeminent circadian model both in the laboratory and under natural and semi-natural conditions is the fruit fly *Drosophila melanogaster* (e.g. [1–3]). Understanding the circadian clock mechanism could play an important role in forensic entomology (where insects are used to obtain useful information for crime reconstruction) because it temporally gates behaviour such as locomotor activities, feeding, mating, egg laying and adult emergence.

Colonisation of human cadavers by insects (mainly flies and beetles) allows a minimum post mortem interval (mPMI) to be estimated. The prevailing opinion is that flies – the first colonizers of a cadaver – are not active during the night time period, and therefore do not oviposit during this time, despite some sporadic observations of nocturnal oviposition or larviposition among Calliphoridae and Sarcophagidae [4–7]. Determining flies' ability to reach a body in dark conditions and the prevalence – if any – of nocturnal oviposition therefore becomes fundamental for the accurate estimation of the mPMI.

In addition to blow (Calliphoridae), house (Muscidae) and flesh flies (Sarcophagidae), scuttle flies (Phoridae) are also reported in cadavers. In particular, *Megaselia scalaris* Loew, 1866 (Diptera, Phoridae), is known to have an important role in forensic entomology due to its ability to colonise both exposed and buried bodies, whether indoors or outdoors [8–12]. The species is widely distributed in warmer regions of the world [8], and it has been reported to be shifting its distribution to Northern regions, possibly as a result of global warming [13,14]. Although there is a wealth of information regarding the biology of this species, including non-forensic contexts [8], there is only a single field observation [15] and indoor experiment [16] reporting nocturnal activity and oviposition in *M. scalaris*. Lacking are data concerning the night-time behaviour and chronobiological profile of this species.

In contrast to previous studies which employed subjective, observational data, we have used the same infrared activity monitors (TriKinetics Inc, MA) employed in many previous *Drosophila* studies to quantify circadian locomotor profiles and

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pupal emergence time. The activity rhythms of *M. scalaris* were monitored over several days in LD 12:12 cycles (12 h of light followed by 12 h of dark) at 21 °C — conditions corresponding to those found in the species' original distribution area of the Mediterranean and North America during spring and summer [8]. These observations were complemented with experiments performed for over a week in constant darkness (DD), conditions which may occur in the forensic context, and which assay the endogenous free-running period of the *M. scalaris* clock.

2. Material and methods

Megaselia scalaris flies were collected from a wild population in Pisa (Italy) in 2011 and bred in an incubator (LMS 410XAL) under LD 12:12 and a constant temperature of 21 ± 0.5 °C.

All experiments were recorded in an incubator at 21 ± 0.5 °C. Experiments were illuminated either with white cold LEDS under a photoperiod of 12:12 LD, or were recorded under complete darkness (DD) or constant light (LL).

Fly locomotor activity was recorded using a TriKinetics DAM5 unit (*Drosophila* activity monitor) using tubes with a length of 65 mm and a diameter of 5 mm, which allowed the flies free movement without flying (*M. scalaris* being of a size comparable to or smaller than *D. melanogaster*). Individual flies (male and virgin female) were introduced into tubes. Food was positioned at one end and the other was sealed with cotton allowing some air flux. Temperature, humidity and light intensity were recorded by a TriKinetics DEM (*Drosophila* environmental monitor). Flies were entrained to a 12:12 LD cycle for 3 days and then subjected to free running DD conditions for 7 days.

Beam crossing events were recorded in 30 min bins, and DD data were trimmed such that the final 24 h of activity from any individual not surviving the experiment were excluded. DD data were analysed using cosinor analysis [17] to scan the best fitting period with a length between 8 and 32 h in 0.2 h steps. Significance was assessed by F-ratio where a significant F-value means the amplitude was significantly >0. This was achieved by randomising activity data 1000 times using a Monte Carlo approach, and accepting as significant any period that had an F-value within the upper 5% of the distribution of maximum F-values. F-value plots and double plotted actograms for each fly were also manually inspected.

Emergence was investigated using the same DAM5 monitors but with shorter tubes (5 mm width, 30 mm long) in which individual pupae were placed at the midpoint of the tubes underneath the IR beams. When the fly emerged it would activate the beam.

To determine the time of day during which *M. scalaris* oviposits, 120 mated females were placed individually into glass tubes containing food at one end and left for 12 h, either during the day, night, or subjective day under DD (i.e. when the lights would normally be on). The experiment was repeated three times.

Oviposition experiments were performed in light boxes where flies were introduced following the LD phases. Males and females flies were maintained for one day in a breeding jar before females being selected for the experiments. Commercial cat food, previously tested as a suitable substratum for oviposition and larval growth, was used in all experiments. Eggs were counted on the deposition medium under a Leica M60 stereomicroscope. Percentage of deposition was calculated counting the number of females laying eggs divided by the total number of females. All of the experiments were triplicated.

Statistical analysis was performed using SPSS statistical software V.20. Data reported on plots are presented as average ± standard error of the mean (SEM).

3. Results

To understand the potential for flies to colonise a body, it is fundamental to know when individuals of either sex are active. The general pattern of activity for both sexes indicates a persistent nocturnal activity with an average of 11.08 ± 0.73 counts 30 min^{-1} for males and 10.75 ± 0.64 counts 30 min^{-1} for females (Fig. 1). During the light phase, activity increased significantly in both sexes (17.17 ± 0.44 for males and 17.04 ± 0.46 for females) but with no significant sex differences (ANOVA Time $F_{1,1004} = 62.4$, $p = 0.000$; Sex $F_{1,1004} = 0.08$, $p = 0.77$). In addition the locomotor activity profiles for males and females showed bimodal peaks: a well-defined Evening (E) peak in both sexes, and a Morning (M) peak that was clearer in males than in females. Both sexes responded to the light transitions with a clear burst of activity termed 'environmental masking' that reflects a startle response.

In both sexes, the anticipatory increase in activity before lights on was observed for males rather more than females, and both sexes show a gentle anticipation of lights off, and certainly not as dramatic as that usually observed for *D. melanogaster* (eg. [18]). As this anticipation of light transitions is driven by the circadian clock in *Drosophila*, we sought to evaluate whether the locomotor rhythms in LD cycles are under (circadian) clock regulation. Flies that had been entrained in 12:12 LD for 3 days, were subsequently maintained in constant darkness (DD) for several days (Fig. 2a, b). The activity pattern under DD revealed the presence of M and E peaks without the masking burst of activity associated with the light transition. 86.3% of male flies and 73.5% of females demonstrated a statistically significant ~24 h rhythmic pattern of activity (Fig. 3).

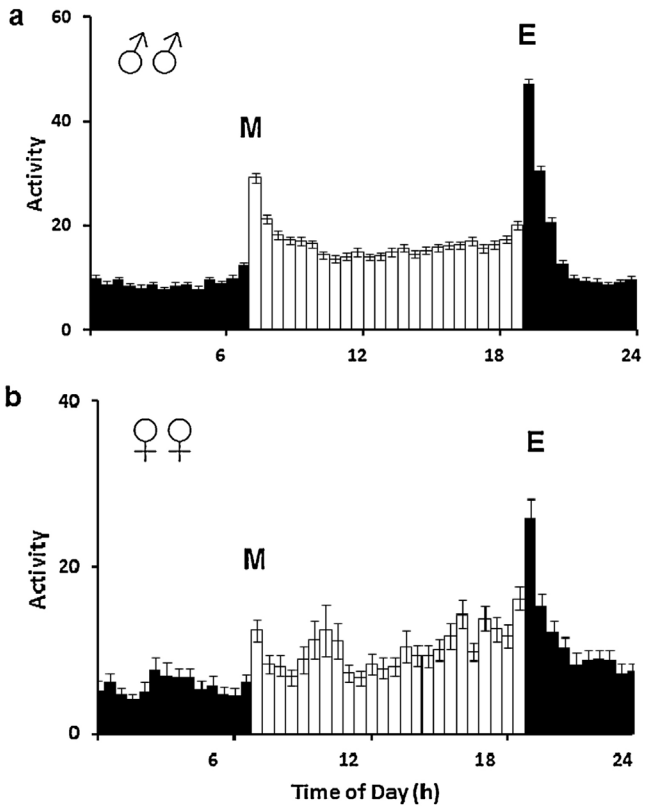

Fig. 1. Average activity profiles (±SEM) of male (N = 593) (a) and female (N = 205) (b) flies under 12:12 LD photoperiod and constant temperature (20 ± 0.5 °C). The activity profiles (white bars white during day and black during the night) showed bimodal peaks at morning (M) and at evening (E). The bimodal pattern is more evident in males. A persistent nocturnal activity is present in both sexes.