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Darkness as factor influencing the oviposition delay in *Calliphora vicina* (Diptera: Calliphoridae)

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ABSTRACT

Many environmental and intrinsic factors (e.g. limited access to the body) can disrupt insect activity, causing a delay in the colonization of a corpse. These elements could hinder an accurate estimation of the minimum Post-mortem Interval (minPMI), raising questions about the limits of forensic entomology. Blow fly are considered mainly diurnal and relatively inactive at night, at extreme temperatures and in dark conditions. Data on their ability to lay eggs in darkness and in laboratory conditions are scarce. Oviposition by Calliphoridae during the day but in darkness has been documented in chimneys, cellars and cars. To investigate delays in oviposition in the dark we carried out laboratory experiments using plastic boxes containing *Calliphora vicina* Robineau-Desvoidy specimens placed in a climatic chambers at different temperatures. We found that *C. vicina* laid eggs in complete darkness inside the plastic boxes, but later than the specimens inside the boxes at light condition. We believe that oviposition can occur in dark indoor environments in conditions of optimal air temperature, gravid flies and an accessible corpse. However, when corpses are discovered in dark environments, entomologists should consider a significant delay in oviposition by blow fly in order to reduce errors in PMI estimation.

1. Introduction

Estimation of the minimum post-mortem interval (PMImin) by entomological methods is based on the age of the oldest fly immature stages or on the ecological succession of arthropods on the corpse.[1–3] When immature stage of Diptera are used as forensic indicators, a crucial question is whether the dead body was colonized by flies shortly after death or later.[4,5] Low or high temperatures, weather, reduced accessibility, night and darkness can delay the colonization of a corpse by invertebrate fauna.[4,6–10] As suggested by some authors,[11,12] blow fly oviposition can be affected by many biotic and abiotic factors. Darkness is one of the conditions that can affect the oviposition behaviour of Calliphoridae since the difficulty of finding a bait or a corpse may delay insect arrival and colonization.[7] Indeed, the main problem of the discovery of a corpse in a dark environment is the exact evaluation of the time before the arrival of the first flies (pre-appearance interval)[13] since a basic component of PMImin estimation is assessment of the elapsed time since colonization of the corpse[14] to the discovery of corpse. Therefore, the assessment of blow fly oviposition and a possible delay in egg-laying in darkness are important topics in forensic investigations. Experiments are needed to study the specific conditions that cause changes in Calliphoridae oviposition behaviour.

There is little evidence of nocturnal deposition by blow fly[8,15] or data on oviposition in dark conditions.[7,12,16] Diptera species, and more specifically Calliphoridae, have different environmental preferences and thus different abilities to access corpses indoor or in shady or dark places.[12,17–21] The lack of knowledge about the oviposition behaviour of blow fly in dark conditions[7] prompted us to conduct a study of egg-laying in a cosmopolitan synanthropic species (*Calliphora vicina* Robineau-Desvoidy, 1830), in darkness in laboratory conditions. Correct PMImin estimation could be significantly affected by whether or not this species lay eggs in dark conditions and if there is a delay in colonization of the remains.

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2. Material and methods

The aim of the experiment was to determine if blowfly *C. vicina* is able to lay eggs in a bait (pig liver) in the dark during daylight hours. A modified experimental design[9] was used to verify egg deposition by gravid females in dark conditions. The effects of the circadian rhythm on the blowfly behaviour were not considered because the aim was to study egg-laying in dark conditions and not at night. Therefore, we did not modify the regular rhythm of the flies, but only light condition.

C. vicina specimens were collected in the field using a modified bottle trap[22] for fly trapping. The trap contained two small containers, one with 200 g of pork and the other with saline solution plus 200 g of meat in order to avoid rapid dehydration of the bait. The sampling area was located in the University of Calabria Botanic Garden (216 m a.s.l; 39° 21′ 25,71″ N and 16° 13′ 42,87″ E), in the city of Rende (southern Italy).

They were reared in plastic boxes in a climatic chamber under an artificial cycle of 12 h light and 12 h dark at 20 °C until the next adult generation. In order to facilitate reproduction we used 20 females and 20 males per cage. The sex ratio was determined on the basis of differences in the eyes[23] selecting them one by one using an entomological aspirator. Thus, a week prior to the darkness experiment, specimens were divided into five clear plastic cages (C1, C2, C3, C4, C5; size: 50 × 12 × 15 cm) and fed with water and powdered milk.

All cages were kept under a Light/Darkness (L/D): 12 h/12 h cycle and at Experimental Temperatures (ET) of 12 ± 0.5 °C for seven days.

According with the optimal thermal range for *Calliphora vicina*,[24] each trial was carried out at the other temperatures (14, 16, 18, 20, 22 or 24 ± 0.5 °C) following the same experimental procedure. In order to induce eggs maturation and before starting with the experiments, we provided liver to the specimens for 4 days ad libitum and after 3 days of break-time to assess egg laying in each experimental cage. Only after observing oviposition we started with the darkness test.

For the experiment, cages C1, C2 and C3 were placed in climatic chambers in darkness for 7 h (09:00–16:00 p.m.) and exposed to the ET. Concurrently, cages C4 and C5 were placed in another climatic chamber at the same temperature and at the same period of time (09:00–16:00 p.m.), but in lighted conditions.

To exclude the effect of previous L/D conditions on oviposition, a new sample of flies was tested for each ET. In the trials, the powdered milk was replaced with 50 g of liver inside a Petri dish placed directly on the floor of the cage.

Every hour, the liver pieces in the 5 plastic boxes (C1–C5) were inspected for egg deposition. Five replicates were performed for each set of cages C1–C5 (i.e. 25 trials for each temperature). At the end of the trial, the laid eggs were counted (N_{dark}, Number of eggs in dark vs N_{light}, Number of eggs in light) and the time to first oviposition (egg deposition latency) was evaluated in the **dark (C1, C2, C3)** vs **light (C4 and C5)** cages. In order to eliminate lighting effects, we always kept the flies under dark condition. Thus, we removed the bait from the cages in dark conditions and in a short period of time of just a few seconds. The cages were kept inside the climate chamber until the end of the daily experiment, but we always checked the eggs in the petri dish in light condition.

3. Statistical analysis

The difference in the time of first oviposition between females laying in dark and light conditions was assessed with an independent-samples *t*-test. For all ET the same test was used to compare the mean number of eggs laid in light vs dark cages. Means and standard errors of the means (SEM) are reported throughout the text. We performed Pearson's product moment correlation analysis[25] to evaluate the association between ET values and the total number of eggs laid in both experimental conditions (L/D). Statistical analyses were carried out with SPSS 21.0 (Statistical Package for Social Sciences, © Copyright 1989, 2013 SPSS, Inc., an IBM Company).

4. Results

Egg-laying by *C. vicina* females occurred in darkness at all ET and we did not observe zero values under any conditions, furthermore, even if with different results (Table 1), oviposition occurred in all trials (100% of cases, Fig. 1).

Pooling data for both experimental conditionswe did not found significant difference (independent-samples *t*-test P = 0.228; df = 12) in the mean number of eggs laid in light versus dark conditions (N_{light} = 3084.92 ± 868.93 SEM eggs; N_{dark} = 1813.57 ± 494.24 SEM eggs, Fig. 2). Furthermore, the ET did not significantly affect the total number of eggs laid neither the dark nor the light ($N_{dark} = 7$, $r_{dark} = 0.077$, $P_{dark} = 0.435$; $N_{light} = 7$, $r_{light} = 0.332$, $P_{light} = 0.233$). However, considering the total number of eggs laid in dark vs light conditions within each ET, we found a significant difference between both 16 °C–24 °C towards remaining temperatures. In fact, 22,289 eggs (N = 11,052 in Dark, N = 11,237 in Light) were laid at 16 °C while 24,587 eggs (N = 10,762 in Dark, N = 13,825 in Light) were laid at 24 °C (Table 1). Hence, the eggs laid at these two ET account for 57.67% (46,876 of 81,274 total; Fig. 1, Table 1) of the pool of eggs laid. Besides that, we found a greater number of eggs laid in the range 12–18 °C (*Nsum* = 47,114; *Nmin* = 233, *Nmax* = 6952); than in 20–24 °C range (*Nsum* = 34,160; *Nmin* = 199, *Nmax* = 7400). Pooling data for both experimental conditions and considering the time of first oviposition we found a significant delay under dark conditions (N_{dark} = 4.56 ± 0.15 SEM hours, N_{light} = 1.75 ± 0.59 SEM hours; independent-samples *t*-test P < 0.001; df = 12, Fig. 3). This significant difference is clearly evident even if we consider the association between time of first oviposition at different ET and conditions (L/D; Fig. 4).

5. Discussion

Despite their inability to fly in dark conditions, female blow fly found the food source probably guided by gravity and olfactory stimulus. Even though the bait was easily found by the flies due to the experimental design, we recorded a significant delay in egg-laying in the dark cages with respect to those placed in the light

Table 1
Mean, maximum and minimum number of eggs laid by females of *C. vicina* at different experimental conditions and temperatures.

Exp. Cond.	ET (°C)	Mean	Max	Min	SE	Sum
Dark	12	1656	1868	1307	176	4969
Light	12	1324	1485	1162	162	2647
Dark	14	1388	1937	974	286	4165
Light	14	2375	2425	2325	50	4750
Dark	16	3684	4334	2808	455	11052
Light	16	5619	6952	4285	1334	11237
Dark	18	814	1191	233	295	2441
Light	18	2927	3700	2153	774	5853
Dark	20	1160	1325	1027	88	3479
Light	20	1376	1528	1224	152	2752
Dark	22	406	592	199	114	1217
Light	22	1063	1140	985	78	2125
Dark	24	3587	4964	2413	743	10762
Light	24	6913	7400	6425	488	13825