Flight activity of the blowflies, *Calliphora vomitoria* and *Lucilia sericata*, in the dark

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Abstract

Many species of insects are able to fly at night or in very low light intensities. The question of whether calliphorid blowflies are also able to do this to locate a corpse and oviposit nocturnally is of considerable forensic importance. However, to date studies of this behaviour have been contradictory. Here, the activity and number of *Calliphora vomitoria* L. and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) caught on sticky-traps were examined in a slow-speed wind tunnel, at different intensities of artificial light. The traps were either unbaited or baited-with liver. The number of both species caught, decreased incrementally as light intensity was reduced. While the responses of the two species were broadly similar, *L. sericata* were significantly more active than *C. vomitoria*, especially at higher light intensities. The number of flies of both species that were caught was higher in the presence of liver bait, but the presence of the liver bait did not change the shape of the relationship between catch and light intensity. Hence, light intensity acts as an independent exogenous stimulus for activity and although liver volatiles increase activity levels, they are not necessary as an activation stimulus. Comparison of the numbers caught in small or large enclosures suggests that any flies caught in darkness probably alighted on the trap by chance and that in darkness, while flies may be activated by carrion odours, they do not appear to be able to navigate effectively to the source of that odour. The results presented here suggest that in darkness, the probability of oriented flight leading to oviposition on a corpse by either species, is relatively low.

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1. Introduction

In forensic entomology, when the age of insect larvae is used to estimate the minimum post-mortem interval (PMI), knowledge of the factors that are likely to delay the time between death and oviposition will clearly be of critical importance. One of the key questions relates to the effects of periods of darkness. Most blowflies are usually considered to be largely diurnal and relatively inactive at night [1]. Hence a body left exposed at night would not attract flies until the next morning [2,3]. However, a number of anecdotal observations suggest that nocturnal oviposition may occur, particularly with species of *Calliphora*. For example, *Calliphora vicina* R.-D. were observed laying eggs in a slaughterhouse at night [4] and on a cadaver in a dark cave [5]. However, experimental studies which have attempted to test these observations have given contradictory results. Nocturnal oviposition was observed by both *C. vicina* and *Lucilia sericata* (Meigen) on carrion both placed directly on the ground [6] and on carrion raised off the ground to ensure that only flying insects were likely to reach it [7]. However, studies in the dark and artificially illuminated areas during the night found no evidence of nocturnal oviposition [8], neither did 2-year study of carrion colonisation in Indiana, USA [9]. Oviposition was found to be synchronised with the onset of the photophase following a period of darkness [3].

Clearly whether or not flies are active at night is an important factor which may significantly affect the estimate of PMI. The aim of the present work therefore was to undertake a comparative investigation of the question of whether blowflies are active in darkness, using two species of forensic importance, *Calliphora vomitoria* L. and *L. sericata*.

2. Methods

Fly behaviour was investigated in the laboratory using a slow-speed wind tunnel, constructed of aluminium with clear reinforced-glass sides and ceiling
The wind-tunnel had a working section length of 2 m, width of 1.3 m and height of 1 m and was illuminated by one 1.5 m, 32 kHz, diffused fluorescent light. Air was drawn into the wind tunnel through a vertical bed of activated charcoal by a centrifugal fan and blown upwards into a secondary horizontal bed of activated charcoal filter. The airflow was then rotated through 90° by a series of aluminium vanes and straightened by passing through two sheets of steel mesh before entering the working section of the wind tunnel. Air exited the wind tunnel via a sheet of 2 mm diameter nylon mesh covering the downwind end of the working section. The wind tunnel was maintained in a small room at 25 °C (range ±1 °C).

Larval *C. vomitoria* and *L. sericata* to be used in experimental trials were obtained from a local fishing-bait shop and kept in the laboratory to pupate. The adults that emerged were maintained in a cage (30 cm × 30 cm × 30 cm) supplied with granulated sucrose and water ad libitum, and at 24–25 °C and under a 16 h light:8 h dark cycle. Female adults only were used in experiments, when 6 days-of-age. Prior to use they were briefly cooled, to allow them to be counted and sexed.

Sticky-traps were constructed from a square of cardboard (10 cm × 10 cm) covered by a white plastic sheet coated with polybutene-based, high-tack, non-setting adhesive (AgriSense BCS Ltd., Pontypridd). The cardboard base and sticky plastic had a hole in its centre, under which was placed a plastic beaker (4 cm × 6 cm) covered with mesh. The beakers were either empty or contained 50 g of liver. The liver used was bought from a local retailer, cut into pieces the size of liver around (4 cm × 6 cm) and placed on the bottom of the beaker. A greater number of flies was caught when traps were baited with liver than when unbaited (*P* < 0.001; Fig. 1). There was no significant interaction between light intensity and the presence or absence of liver bait (*F* = 2.70, *P* = 0.05), indicating that the responses of flies to light intensity followed the same pattern in both liver and control treatment groups. Tukey multiple range tests showed a significant difference between the 100% (345 W m⁻²) and 32% (110 W m⁻²) treatments and between the two higher and the two lower light intensities, but no significant difference between the number caught at the lowest light intensity or in darkness.

2.2. Large scale trial

In the dark in the small cages, it was possible that if flies were spontaneously active they might have alighted on the sticky-traps at random rather than having demonstrated any specific orientation towards the liver bait. To allow this possibility to be considered further, in a second trial 200 female *C. vomitoria* or *L. sericata* were released directly into the entire wind tunnel with a larger sticky-trap (30 cm × 30 cm) and 100 g liver as bait. Flies were again left for 3 h before the numbers caught were counted.

2.3. Data analysis

All data were tested for normality and, where required, log₁₀ transformation was used to normalise the residuals. The log₁₀ (+1) of the number of flies caught was then subjected to analysis of variance with the light intensity and bait presence or absence as fixed factors. Tukey pairwise comparisons were used to compare the means of within treatment groups.

3. Results

3.1. *L. sericata*

A greater number of flies was caught when traps were baited with liver than when unbaited (*F* = 20.19, *P* < 0.001; Fig. 1) and more flies were caught at higher light intensities (*F* = 43.08, *P* < 0.001; Fig. 1). No significant interaction was found between light intensity and the presence or absence of liver bait. Tukey pairwise comparisons showed a significant difference between the 100% (345 W m⁻²) and 32% (110 W m⁻²) treatments and between the two higher and the two lower light intensities, but no significant difference between the number caught at the lowest light intensity or in darkness.
Fewer *C. vomitoria* were caught than *L. sericata* overall ($P < 0.05$) and in both control and liver-bait treatment groups (Fig. 3).

### 3.4. Large scale trial

When flies were released into the entire wind tunnel in the dark, rather than the small mesh cages, a significantly lower mean percentage of flies was caught when they were released in the dark into the smaller cages ($\chi^2 = 30.01$; $P < 0.005$). Means of 1.04% and 24.22% of the *L. sericata* were caught and 3.0% and 14.72% of the *C. vomitoria* were caught, in the wind tunnel and cages, respectively.

### 4. Discussion

Many insects, such as moths, flies, beetles and bees, are well known to be able to fly at night in very low light intensities [11,12]. Nocturnal flight activity is often strongly related to ambient temperature. However, to date, the debate over the ability of calliphorids to locate a corpse and oviposit nocturnally has been inconclusive. Here, the number of *L. sericata* and *C. vomitoria* caught, which it is assumed reflects underlying levels of flight activity, decreased as light intensity was reduced. It is notable however, that in the small cages used, captures were not reduced to zero even in, what was assumed to be total darkness. The room in which the wind tunnel was placed had no windows and no other form of illumination such as infra-red lighting; however, the possibility of some low level illumination, for example, leaking from under the laboratory door, can not be completely ruled out. If this was the case it was certainly at a level that was too low to be measured by the light intensity meter used here. Nevertheless, the graded response to light intensity, particularly when the liver bait was present, is surprising. From an adaptive perspective, it might have been anticipated that flies would remain active down to a threshold below which their eyes could not function effectively, and that an all-or-nothing relationship between activity and light intensity would have been observed. The ommatidia of compound eyes are capable of complex photoreceptor dark adaptation, although resolution may decrease as the signal to noise ratio increases at lower light intensities [13,14].

The responses of the two species were broadly similar, but *L. sericata* were significantly more active than *C. vomitoria*, especially at the higher light intensities and particularly at 110 W m$^{-2}$. As a result, the decline in activity of *L. sericata* appeared to show something of a step response, with a substantive decline in activity between 110 and 40 W m$^{-2}$ whereas the decline in activity seen in *C. vomitoria* appeared to be more gradual. This difference may be related to their different behaviours in the field; *C. vomitoria* occur more commonly in shaded and edge-habitats found in woodland and hedgerows. These environments have a lower level of illumination than the open, field habitats where *L. sericata* are more commonly caught [15–17].

The numbers of flies of both species that were caught was higher in the presence of liver, but the presence of the liver did not change the shape of the relationship between activity and light intensity. This shows that light intensity acts as an exogenous independent stimulus for activity and that although liver odours increase the level of activity of the flies, they are not necessary as an activating stimulus. Hence fly activity can be modulated directly by light intensity, without the need for volatile odours to trigger activation. The fact that *L. sericata* were more active than *C. vomitoria* both in the presence and absence of the liver bait also suggests that the difference in activity level is not causally related to any difference in the way these two species respond to odour cues.

In the small cages, the fact that flies continued to be caught in the dark, particularly on the liver-baited sticky-trap, suggested that either that there was undirected flight and that flies, moving at random, eventually alighted by chance on the sticky surface, or that the flies were capable of oriented flight towards the odour source in darkness. To attempt to distinguish between these alternative possibilities, trials were carried out in the much larger volume of the entire wind tunnel. In these trials, captures were reduced to almost zero, suggesting that the former alternative was most likely; in darkness, while flies may be activated by carrion odours, they do not appear to be capable of the oriented flight necessary to bring them to the source of...
that odour. Captures in darkness, were probably therefore the result of random movements.

The results presented here do not exclude the possibility that these species can locate an oviposition site and oviposit nocturnally. An especially full moon or artificial lighting might provide a strong enough source of illumination, particularly in urban areas. Nevertheless, the probability of oriented flight leading to oviposition in the absence of such illumination would appear to be relatively low.

References