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Do necrophagous blowflies (Diptera: Calliphoridae) lay their eggs in wounds? Experimental data and implications for forensic entomology

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ABSTRACT

This study was designed to examine the common belief that necrophagous blowflies lay their eggs in wounds.

The egg-laying behaviour of *Lucilia sericata* was observed under controlled conditions on wet, artificially wounded or short-haired areas of rat cadavers. Flies laid significantly more eggs on the wet area and the area with short hair than on the dry area or area with long hair. No eggs were observed inside the wounds in any of the replicates.

The effect of egg immersion (body fluids often exudes in wounds) on the survival rate of larvae was also investigated. In low water condition, an average of 72.7 ± 7.9% of the larvae survived and they reached a mean length of 7.5 ± 0.6 mm. In contrast, submerging eggs under a high volume of water strongly affected their survival rate (25 ± 3.7%) and development. Similar results were observed using unfrozen pig blood instead of water.

These data question the information found in the literature regarding the preferential egg-laying behaviour of Calliphorids flies in wounds.

This study investigate the factors that affect the oviposition sites of *Lucilia sericata* (Diptera: Calliphoridae) (Meigen, 1826), a very common blow fly species of forensic interest. Blowflies (Diptera: Calliphoridae) have a highly developed olfactory system that allows them to detect corpses at a great distance [1–4]. It is frequently reported in forensic entomology manuals that once on the cadaver, blowflies lay their eggs in natural orifices and wounds [5–10]. The presence of other eggs, larvae or adult individuals can also act as an attractive signal to gravid females and can increase the likelihood of oviposition in a given area [1,11–13].

Oviposition in natural openings, especially on the face, is often observed in field conditions. Due to their weak mouth hooks, first instars are unable to attack hard tissues (e.g., muscle, skin, etc.) and consequently feed on soft areas such as mucous membranes or brain [14–16]. Nostrils offer both protection and a suitable place for larvae to feed, and they are often heavily colonized. Byrd and Tomberlin also note that when excrement-soiled clothing is present, flies often deposit their eggs in these areas, a fact that highlights the importance of surface humidity in determining egg-laying behaviour (in [8]). Larvae feeding on wounds are frequently observed in the context of wound myiasis, i.e. infestation of living animals by fly larvae. In Europe, *Lucilia* is a common pest in sheep, responsible for costly flies strike, and is the main genus involved in human myiasis [17–19]. It is also common in forensic pathology to observe deep alterations of wound characteristics due to necrophagous larvae feeding at the injury site [20,21]. But the observation of larvae feeding in wounds does not require that eggs be laid in these areas. Actually, there appears to be no experimental or quantified data in the literature to support the assertion that blowflies lay their eggs in wounds.

To highlight the importance of this question in a forensic context, it is interesting to consider a case that we analyzed some years ago. In 2009, the naked body of a young woman was discovered in a corn field a few hours after her disappearance. Several wounds caused by a sharp object were present, and her hair was soaked with blood. Throttle traces were also observed on the neck. An autopsy concluded that the victim was first throttled but

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did not die of asphyxiation and was subsequently stabbed in the head following an attempted rape. The only forensic entomology evidence was the unhatched fly eggs that were discovered in the eyes and nostrils of the victim, which were identified as belonging to *L. sericata*. Because thousands were sampled and even more were visible, it was clear that the colonization of these flies took place at a time that the body was easily accessible (i.e., when it was in the corn field). Based on what has been published in the literature, the complete lack of eggs on the bloody wounds would appear to suggest that the victim had not already been stabbed when the Calliphorids flies oviposited on the corpse. However, this scenario was inconsistent with subsequent investigations, and it was lastly proven that the victim was stabbed before being deposited in the field where she was discovered. Colonization by blowflies likely occurred at this time, but the flies did not lay any eggs in her wounds.

On the basis of this case and to determine the reliability of the common belief that necrophagous blowflies lay their eggs in wounds, the egg-laying behaviour of *Lucilia sericata* was observed under controlled conditions on wet, artificially wounded or short-haired areas of rat cadavers. The effect of egg immersion (body fluids often exudes in wounds) on the survival rate of larvae was also investigated.

1. Material and methods

Experiments were performed on a laboratory *L. sericata* population raised and maintained in 50 cm × 50 cm × 50 cm gauze-covered cages. Inbreeding was reduced by adding monthly wild type (France) individuals. Adult flies (250 ± 100) from a single emergence pool (hatching = D0) were maintained at 20 ± 2 °C and 60 ± 15% RH with a 12:12 photoperiod for a maximum of 20 days. Minced beef liver was provided during the first 7 days to promote female ovarian development and was then removed. After 4 more days, females were gravid and ready for experiment (D11) [22].

2. Oviposition on wet, wounded or short-haired cadavers

In this experiment, we investigated the egg-laying behaviour of *L. sericata* gravid females on male laboratory rat cadavers (*Ratus norvegicus)*. The setup was designed to test the likelihood that females would lay eggs in wet, wounded or short-haired areas of the cadaver compared to untreated control areas. Experiments were conducted from May until July between the hours of 10 a.m. and 3 p.m. (a 5 h period) in a laboratory room with artificial neon lighting. The room temperature was kept at 24 ± 2 °C and 60 ± 15% RH.

For each tested condition, 20 gravid females and 10 males from the same pool were sorted and placed in 30 cm × 30 cm × 30 cm gauze cages; caster sugar and water were provided *ad libitum*. A CO_2-killed, freshly unfrozen white rat cadaver (stored in the freezer during 3–5 month, 326 ± 22 g) was placed on a piece of cardboard in the cage on its ventral or dorsal side (the side was alternated between replicates). Four experimental conditions were tested, each of which was performed with 8 replicates (7 for Control).

3. Control: untreated rat cadavers

Wet vs Dry: one half (longitudinally) of the rat cadaver was submerged in water for 15 s. The wet side was alternated between experimental replicates.

Wounded vs Healthy: a 4 cm long transcutaneous wound was made with a surgical scalpel horizontally on the flank of the rat. The entrails inside abdominal cavity were visible but not transected. The side of the wound (i.e., the right or left side of the rat) was alternated between replicates.

Bare vs Haired: one half (longitudinally) of the rat cadaver was shaved using an electric shaver. The hair was cut to a length of 1 mm, and the skin was carefully inspected to exclude any possible skin lesions. The shaved side was alternated between replicates.

After a 5 h period with the cadaver, all flies were removed and killed. Their size was measured using the length of the posterior cross vein (dm-cu) [23]. The eggs were removed using fine forceps and a louse comb and placed in separate vials. These vials were kept at 3 ± 1 °C until counting (24–36 h after the start of the experiment).

The location of the eggs was noted as follows:

Control/tested side. For the wound condition, the location relative to the wound (in or outside) was also reported.

Natural orifices (eggs located in the natural orifices were counted separately from those in the corresponding body areas): face orifices (ears, nostrils, eyes, mouth) and anus.

Body area: 1. head, 2. fore legs, 3. abdomen, 4. hind legs and 5. tail and location: beyond or underneath the cadaver.

4. Effects of egg immersion on the survival rate of blow fly larvae

This experiment was designed to assess the effect of body fluids, which can flow or accumulate in wounds, on the survival rate off eggs and development of larvae. Fresh beef liver (25 ± 5 g) was introduced into fly-rearing cages and checked hourly until the first oviposition was observed [24]. Consequently, the oviposition time (T0) was known with an accuracy of ±30 min. The eggs were immediately divided into 4 batches of approximately 50 eggs (determined gravimetrically). The eggs were then placed on a piece of filter paper in a sterile petri dish (5.5 cm in diameter) to which either a low (1.5 mL, control) or a high (7.5 mL) volume of water or freshly thawed pig blood (no additives) was added (Fig. 1). At low

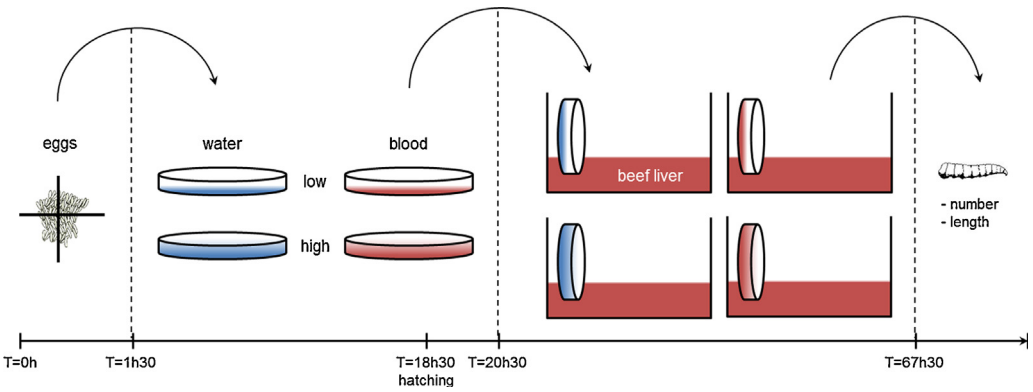

Fig. 1. Experimental setup used to analyze the effects of egg immersion on the survival rate of *L. sericata* larvae.