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Source: Journal of Medical Entomology, 47(3):482-486. 2010.

Published By: Entomological Society of America

DOI: 10.1603/ME09052

URL: <http://www.bioone.org/doi/full/10.1603/ME09052>

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Decomposed Flesh as a Vitellogenic Protein Source for the Forensically Important *Lucilia sericata* (Diptera: Calliphoridae)

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J. Med. Entomol. 47(3): 482–486 (2010); DOI: 10.1603/ME09052

ABSTRACT For the carrion-feeding blow flies, a common source of vitellogenic protein are the carcasses that also serve as larval substrates. This attraction continues well after the blow flies have ceased to oviposit on the carrion, and it has been assumed that a decomposed cadaver remains a suitable source for vitellogenic protein; however, this assumption has never been tested. To test this hypothesis, blow flies (*Lucilia sericata* Meigen) were supplied with either fresh or decomposed beef liver as protein sources for vitellogenesis for 5 d. Both treatments produced identical ovarian development, indicating that decomposed flesh remains a suitable protein source for carrion-feeding blow flies. These results support the theory that virgin flies are attracted to cadavers of advanced decomposition as a source of protein.

KEY WORDS vitellogenesis, Calliphoridae, forensic entomology

Vitellogenesis, the process by which yolk is accumulated in insect oocytes, requires nutritional protein intake in many insect groups, including blow flies (*Lucilia sericata* Meigen) (Diptera: Calliphoridae) (Rasso and Fraenkel 1954, Orr 1964, Stoffolano 1974). For the carrion-feeding blow flies the most common source of vitellogenic protein is thought to be the carcasses that also serve as larval substrates, and virgin female flies are assumed to be attracted to carrion along with the gravid females for this reason (Norris 1965, Belzer 1978, Barton Browne and Van Gerwen 1992, Barton Browne 1993, Hayes et al. 1999).

The attraction to a carcass continues well after the blow flies have ceased to oviposit on that carrion, and it has been assumed that a decomposed carcass remains a suitable source for vitellogenic protein (Archer and Elgar 2003), an assumption that, surprisingly, has never been tested. Not only do proteins degrade over time through the actions of autolytic enzymes (particularly protease) (Coe 1974), but the actions of bacteria and fungi that also colonize decomposing cadavers could alter the chemical composition of the corpse to the point that proteins are no longer suitable for vitellogenesis (Carter and Tibbett 2003, Tibbett et al. 2004). From an ecological standpoint, remaining at a carcass after oviposition exposes female flies to greater risk of predation, as the occurrence of insect predators at carcasses increases through time (Smith 1986).

In our previous work, examining the potential for multigenerational colonization of carrion by blow

flies, we observed that although soft tissue remained on the carcasses, no additional colonization occurred (Huntington et al. 2008). One possible explanation for this apparent failure to use resources is that the decomposed carcasses do not in fact possess usable proteins for vitellogenesis. Alternatively, some proteins might be available but not in sufficient quantity to allow maximum egg production. Consequently, we directly examined vitellogenesis by *L. sericata* (as a model blow fly species) on fresh and decomposing liver.

Materials and Methods

Blow flies. Colonies of *L. sericata* were established using eggs collected from human remains in Lincoln, NE in 2007. *L. sericata* is a common carrion fly with nearly worldwide distribution and is an early colonizer of fresh carrion (Hall 1948, Smith 1986). Colonies were divided into 30.5 cm³ insect rearing cages (Bio-Quip Products, Inc., Gardena, CA) and were provided with sucrose and water ad libitum with fresh beef liver for protein and as an ovipositional substrate. When oviposition occurred, ~300 eggs were transferred into foil pouches containing 200 g of beef liver that were then placed into plastic containers lined with 2 cm of vermiculite. Fly colonies were maintained in a Percival growth chamber (Percival Scientific, Boone, IA) set at 24°C and a photoperiod of 16L:8D h.

Experimental Treatments. Following maggot migration and pupariation of a generation of maggots, the surplus decomposed liver was wrapped in foil and placed back into the rearing chamber to be maintained at the same conditions as the puparia themselves. This decomposed liver was tested as a protein source for the resulting flies.

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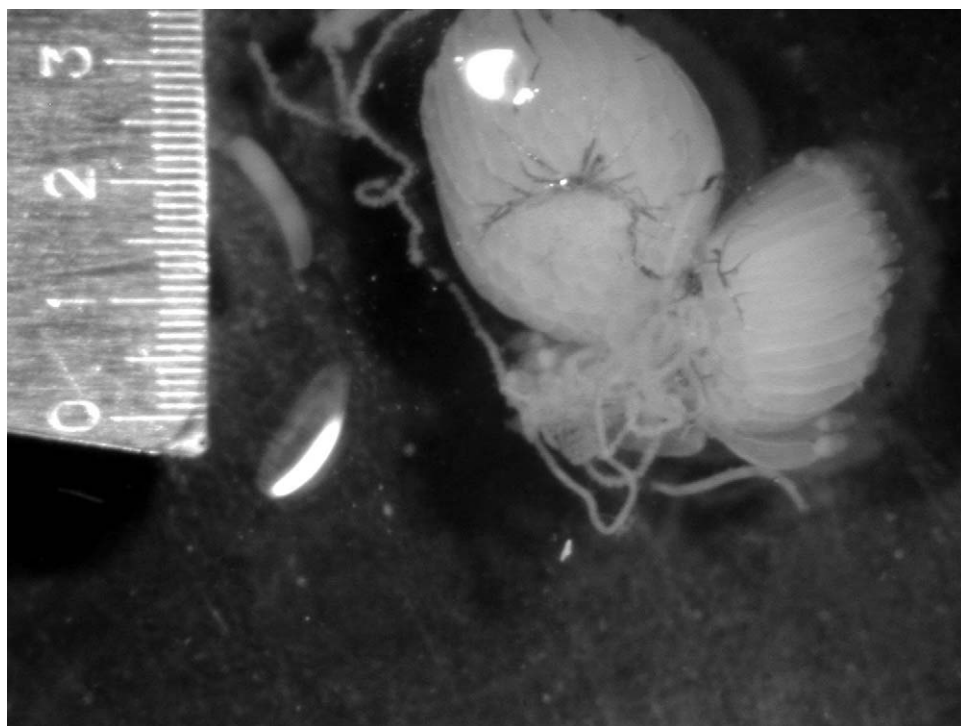


Fig. 1. Stage 10 ovaries from *L. sericata* fed on fresh liver. Scale shows size in millimeters.

Before adult eclosion, 20 puparia were placed into dishes of dry vermiculite in each of 12 separate cages to allow for adult emergence. These cages were placed into the same rearing chamber as the parent colony and were maintained at 24°C and 16L:8D. Each cage was provided with sucrose and water ad libitum. Six cages were used as controls, with no protein sources given to the flies.

Three days after adult emergence, protein sources were introduced to each of the experimental cages for 3 h daily for 6 d. Three cages were provided with fresh beef liver and the remaining three cages were provided with decomposed liver (from the maggots' development). After 3 h of availability, the protein source was removed from the cages and discarded. After 6 d of providing protein to the experimental replicates, the cages were left undisturbed for an additional 9 d. After this period (14 d posteclosion), cages were placed into a freezer for a short time to anesthetize the flies before examination.

Flies in each cage were counted, sexed, and the females were dissected in 10% saline under a stereomicroscope to determine the stage of ovarian development using the scheme of Adams and Reinecke (1979). Criteria for determining stages of ovarian development were summarized by length and shape:

- Stage 1: Ovarian follicle pear-shaped, $\approx 50 \times 27 \mu\text{m}$.
- Stage 2: Ovarian follicle spherical, $\approx 50 \times 38 \mu\text{m}$.
- Stage 3: Follicle $< 100 \mu\text{m}$ in length.
- Stage 4: Follicle $\approx 171 \mu\text{m}$ in length.
- Stage 5: Follicle $\approx 254 \mu\text{m}$ in length.

Stage 6: Follicle $\approx 409 \mu\text{m}$ in length.

Stage 7: Follicle $\approx 544 \mu\text{m}$ in length.

Stage 8: Follicle $\approx 705 \mu\text{m}$ in length.

Stage 9: Follicle $\approx 772 \mu\text{m}$ in length.

Stage 10: Follicle $\approx 910 \mu\text{m}$ in length, a mature egg.

Results and Discussion

Female flies from the control cages lacked any appreciable development in the ovaries. Follicular development in these flies all scored three or less on the Adams and Reinecke scale, with lengths $< 100 \mu\text{m}$. This outcome is not surprising as a protein source is required for vitellogenesis (Belzer 1978, Barton Browne and Van Gerwen 1992, Barton Browne 1993).

Female flies from the experimental cages exhibited swollen abdomens, with separated stergites and visible membranes on the ventral surface, a classic indication of being egg-laden (Erzinçlioğlu 1996).

Dissections of the females from experimental cages revealed advanced ovarian development in every sample, with all of the females from both the fresh liver and decomposed liver replicates having stage 10 follicular development on the Adams and Reinecke scale. There were no observable differences between the treatments (Figs. 1–3).

The results of the dissections demonstrate that there are no apparent differences between ovarian developments in *L. sericata* with diets of fresh liver and decomposed liver. This finding supports the assumption of Archer and Elgar (2003) that blow flies visiting



Fig. 2. Stage 10 ovaries from *L. sericata* fed on decomposed liver. Scale shows size in millimeters.

corpses after ovipositional cues have passed are obtaining vitellogenic proteins. Although differences have been seen in blow fly vitellogenesis when fed various protein sources, these differences are likely caused by variances in the percent of protein in the diets rather than the sources themselves (Webber 1958, Cook 1991, Stoffolano et al. 1995). Because protein is still protein, decomposition does not seem to have an impact on ovarian development.

Given that *L. sericata* did not display differences in ovarian development when fed on fresh and decomposed carrion, it seems likely that other species of carrion-feeding blow flies will follow similar trends. Flies in the *Lucilia* genus typically arrive early in the succession of insects on a cadaver (Hall and Doisy 1993), so we speculate that if any of the carrion flies would exhibit vitellogenic variances between fresh and decomposed protein sources it would be the *Luciliinae*.

One hypothesis for the failure of sarcosaprophagous blow flies to produce multiple generations on a single cadaver has been that nutritional suitability of the carcass has changed over the course of decomposition to the point that it is unsuitable to the flies, both as a source of vitellogenic protein and as a developmental substrate for larvae (Huntington et al. 2008). This study demonstrates that multigenerational colonization of carrion is not limited by biochemical or microbial changes (Clark et al. 1997, Gill-King 1997) that would affect ovarian development of blow flies, but is instead limited by some other factor.

Changes in the chemical and microbial composition of decomposed flesh (Clark et al. 1997, Gill-King 1997) may represent a limiting factor of recolonization of parent carrion, but in instances of large carcasses decomposition is not a single simultaneous process, with parts of cadavers being less decomposed than others. In these instances, flesh in a relatively fresh condition would seem like an attractive site for oviposition, though oviposition does not occur (Huntington et al. 2008). This indicates that blow flies ovipositional criteria likely differs from larval substrate criteria. Further study is needed to determine the success of larval development on decomposed tissues.

A second explanation of the failure to produce multiple generations on a single carrion source relates to competition between carrion-feeding insects. Because carrion is an ephemeral and valuable resource to necrophilous organisms (Carter et al. 2007), intense inter- and intra-specific competition exists between sarcosaprophagous insects (e.g., Norris 1965, Lane 1975, Atkinson and Shorrocks 1984, Trumbo 1990, Trumbo and Fiore 1994). It is likely that the evolution of highly sensitive chemoreceptors in blow flies allow for the rapid exploitation of cadavers to avoid much of this competition, allowing for greater larval success and increased fecundity. We hypothesize that gravid blow flies avoid ovipositing on decomposed carrion, not because the cadaver is unsuitable as a larval substrate, but because the larvae would not successfully compete for resources.



Fig. 3. Stage 10 ovaries from *L. sericata* in saline. The six pairs of ovaries on the left are from flies supplied with fresh liver, and the six pairs on the right are from flies supplied with decomposed liver. No obvious differences exist between the two treatments.

This simple question of whether or not female blow flies can use decomposed tissue as a protein source is of importance beyond the need to confirm a long-standing assumption in the literature. Rapid host finding, lack of multiple generations on a single host carcass, and temporal differences in carcass colonization by different species (Smith 1986) strongly suggest that evolution among carrion-colonizing insects has been driven by competition. The confirmation that physiological barriers do not exist for vitellogenesis by a carrion-colonizing fly eliminates a possible alternative hypothesis and thereby, indirectly supports the notion that competition has been the key evolutionary force in carrion-colonizing insects.

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Received 23 February 2009; accepted 8 December 2009.