ECOLOGICAL AND PHYSIOLOGICAL LIMITATIONS OF CARRION FLY COLONIZATION OF CADAVERS IN TERRESTRIAL ECOSYSTEMS

by

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Carrion flies, particularly blow flies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae), play a vital role in the cycling of organic material by aiding in the decomposition of vertebrate cadavers in terrestrial ecosystems. Because of this close association with dead bodies, these flies also serve as the primary indicators of the postmortem interval for forensic entomologists based on the rapid nature of fly colonization. I addressed some of the assumptions made with regard to the colonization of terrestrial cadavers by carrion flies. This starting point was ideal because the whole of insect-driven decomposition begins with the colonization of the cadaver, usually by flies.

By first conducting experiments on the potential for blow flies to produce multiple generations on single cadavers, I was able to demonstrate through a choice/ no choice scenario that blow flies do not in fact produce a second generation on carrion. This has confirmed assumptions in the literature which had previously not been tested. As a follow-up to this study, I examined the effects of decomposition on vitellogenic protein sources for blow flies to determine whether or not flies visiting decomposed bodies are indeed gaining dietary protein as has been assumed. My data show that flies successfully complete vitellogenesis on aged carrion at the same rate as they do on fresh liver.

Finally, I tested the assumption that flies do not colonize bodies at night, an assumption that has been tested several times before by various authors using inappropriate experimental procedures. By killing pigs (23-32 kg each) after dark, and placing them in field conditions of varying light intensities, I was unable to show any signs of carrion fly colonization. This reinforces the assumption that flies do not colonize bodies after dark, and indicates that the potential for nocturnal colonization of a body by flies has been overstated by some authors.

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Table of Contents

List of Tables

List of Images

In Text

Chapter Two

Chapter Three

Introduction and Literature Review

The role of flies with respect to the decomposition of animal carrion is a natural phenomenon that has been written about by playwrights and studied by naturalists for thousands of years (Greenberg and Kunich 2002). Yet there are many aspects of this vital part of organic cycling that we do not understand. For example, what are the detailed mechanisms of attraction of carrion flies, primarily blow flies (Diptera: Calliphoridae) and to a lesser extent flesh flies (Diptera: Sarcophagidae),) to decomposing animals? More broadly, what are the key behavioral and physiological determinants of oviposition among carrion flies, and how do these differ among species?

While the specific chemical compounds involved in the attraction of flies to carrion are largely unknown (Ashworth and Wall 1994), though we know that the attraction is rapid. Many studies have shown that blow flies will alight on a dead animal within a very short time after death, often within minutes, and will begin to oviposit shortly thereafter (Payne 1965; Johnson 1975; Lane 1975; Goddard and Lago 1985; Tullis and Goff 1987; Anderson and VanLaerhoven 1996; Tabor et al. 2004). The succession of various species of blow flies in relation to the decomposition of carrion has been studied (e.g., Denno and Cothran 1975), as have the chemical compounds that emit from cadavers over time (Vass et al. 2004), though the two have not yet been linked. In the absence of reliable research related to cadaver colonization by carrion flies, assumptions about fly behavior and physiology abound in the literature (e.g., Keh 1985; Smith 1986; Catts 1992; Amendt et al. 2004).

In addition to their role in nutrient cycling, carrion flies also play a vital role in medicocriminal entomology, the use of insects as evidence in cases of human death or

neglect. Estimations of the time since death, or postmortem interval (PMI), are made by estimating the age of the insects on a body and correlating that to the time of colonization (Keh 1985; Smith 1986; Catts and Haskell 1990; Catts 1992). The nature of medicocriminal entomology and its relationship with the legal system require that we know everything we can about carrion flies and decomposition (Hall and Huntington 2008). Therefore, whenever possible, nothing should be taken for granted and no assumptions should be made.

It is my goal, then, to address some of the assumptions made with regard to the colonization of terrestrial cadavers by carrion flies. This starting point is ideal because the whole of insect-driven decomposition begins with the colonization of the cadaver, usually by flies. Moreover, terrestrial cadavers, bodies found on the surface of land (not buried or in water), are those most frequently encountered by carrion flies and by medicocriminal investigators (Norris 1965; Erzinçlioğlu 1996; Spitz 2006).

Multigenerational Colonization

Carrion flies are attracted to cadavers by volatile chemicals, particularly sulfurous compounds, being emitted from them during the process of decomposition (Ashworth and Wall 1994). Hydrogen sulfide gases originate from the breakdown of sulfur-containing amino acids, and this process occurs primarily during putrefaction, while the flesh of the body still remains intact and relatively unchanged (Gill-King 1997). While there may be other attractive chemicals in the mélange of compounds emitted from a decomposing corpse (Vass et al. 2004), flies can tell the difference between a fresh corpse and a decomposed corpse.

Figure 0.1. The blue bottle fly (*Calliphora vicina*) is a blow fly most frequently seen in urban areas during the fall and spring. Here it is seen feeding on liver that also serves as an egg-laying substrate in a laboratory colony. Photo by Tim Huntington

Archer and Elgar (2003) examined fly differentiation of carrion age by successively trapping the flies attracted to carrion over the course of decomposition to assess how the nature of decomposition affects blow fly use of a corpse. They found that there are clear interactions between the gender of the flies and the stage of decomposition (early or late). Additionally there were interactions between the ovarian status of the females (gravid, non-gravid, early egg development, or late egg development) and the stage of decomposition. Those flies that were ready to oviposit were attracted immediately after death, and those with undeveloped ovaries made up higher proportions of the populations later in the decompositional stages.

This behavior is not unexpected, as Hayes et al. (1999) showed that flies of different genders and reproductive status had different resource requirements when

attracted to carrion. Aside from the obvious attraction to carrion as an oviposition medium (Norris 1965), males are apparently attracted to the carrion as a place to find virgin females (Erzinçlioğlu 1996), and virgin females are thought to be attracted to carrion as a protein source to complete vitellogenesis (Belzer 1978; Barton Browne and Van Gerwen 1992; Barton Browne 1993). This protein requirement does not appear to be "source-dependent", and is has been assumed that females can obtain their protein from decomposed carcasses as easily as fresh remains (Archer and Elgar 2003).

While blow flies continue to be attracted to the carrion well into the later stages of decomposition, the carcass is no longer attractive as an oviposition medium after some point, and it is widely held that the maggots which fed on a corpse will not normally eclose as adults and oviposit on the same corpse (Nuorteva 1977).

If adult blow flies eclose into a situation where there is no carrion source other than their larval host, will they oviposit on this carcass or die without reproducing? I propose to test this question by conducting a choice/ no choice study. This research is not strictly an academic or hypothetical exercise, as human or animal remains may be found under such circumstances somewhat regularly. A rat which dies in a box, for example, may be colonized by blow flies easily enough, but those adult flies that originate from the rat carcass may not have stimuli strong enough to lure them from within the box (compared to the strong stimuli to enter the box and oviposit). There are a number of medicocriminal cases involving human corpses being found in closets and car trunks, but if left undiscovered long enough will the blow flies re-colonize their parent's carrion?

Vitellogenesis

Vitellogenesis, the process by which yolk is accumulated in insect oocytes, requires nutritional protein intake in many insect groups, including blow flies (Rasso and Fraenkel 1954; Orr 1964; Stoffolano 1974). For carrion-feeding blow flies the most common source of vitellogenic protein are the carcasses which also serve as larval substrates, and virgin female flies are thought 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). This attraction continues well after the blow flies have ceased to oviposit on the carrion, and it has been presumed that a decomposed cadaver remains a suitable source for vitellogenic protein (Archer and Elgar 2003).

Previous studies have examined the relationship between various diets and the success of vitellogenesis in blow flies. Evans (1935) reported that females of *Lucilia sericata* failed to develop their ovaries when maintained on a diet of only sugar and water, but when fed meat quickly succeeded in ovarian development. *Lucilia cuprina* has been shown to obtain dietary protein from sheep dung (Webber 1958). The ovarian development of *Chrysomya putoria* and *C. megacephala* was tested against diets of beef liver and chicken, human, and cattle feces by Linhares and Avancini (1989), which showed significant differences in the onset and completion of vitellogenesis depending on food source. Although these differences in ovarian development were associated with food source, no direct comparison between protein contents of the diets were made, and the authors assumed that chicken feces contained the highest amount of protein and cattle feces the least. Studies conducted on primary screwworm flies, *Cochliomyia*

hominivorax, demonstrated that females fed on dung were not successful in completing vitellogenesis while those fed on spent larval medium (incubated blood-milk-egg mixture) were successful, indicating that attraction to dung is more likely linked to host finding rather than as a protein source, as was previously thought (Mackley and Snow 1982). *Phormia regina* has been demonstrated to obtain suitable protein for vitellogenesis from both the glebae of fungi (Stoffolano et al. 1989) and from various types of feces (Stoffolano et al. 1995), although both of these sources require extended feeding durations when compared to fresh liver.

Although it is generally assumed that blow flies continue to feed on corpses that are no longer attractive for oviposition to obtain vitellogenic protein (Archer and Elgar 2003), this assumption is based on field observations and has never been confirmed by . Indeed, the studies of Mackley and Snow (1982) demonstrate that assumptions based on field observations regarding blow flies and protein consumption are not always accurate.

As this review indicates, the question of whether or not a decomposed cadaver can provide sufficient nutritive value for ovarian development remains unanswered.

Nocturnal Colonization

One of the primary factors in medicocriminal entomology that affects the estimation of a PMI is the time that carrion flies oviposited/larviposited on the deceased. Many species of carrion flies oviposit on a body within a short time after death, sometimes within minutes, so the age of fly immatures will relate closely to the time of death (Smith 1986; Anderson and VanLaerhoven 1996; Haskell et al. 1997). While estimating the age of the immatures of various fly species may be done through degree-