JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, November 2009, Vol. 54, No. 6
doi: 10.1111/j.1556-4029.2009.01198.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

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The Nocturnal Ovipositing Behavior of Carrion Flies in Cincinnati, Ohio

ABSTRACT: The behavioral patterns of nocturnal oviposition represent a window of time that potentially has a large impact on postmortem interval estimations. We investigated the behavioral patterns of carrion flies at night by exposing euthanized rats between sunset and sunrise to see if carrion flies oviposited upon the carrion over two consecutive summers. We investigated urban and rural locations, in both lit and unlit conditions with $n = 125$. We found that nocturnal ovipositing did not occur in the Cincinnati metropolitan area. We conclude that nocturnal oviposition is an unlikely event in the Cincinnati metropolitan area.

KEYWORDS: forensic science, forensic entomology, diptera, nocturnal oviposition, Calliphoridae, Sarcophagidae

It has long been known that postmortem interval (PMI) estimates based on flies rely upon species-specific knowledge (1). One aspect of this species-specific knowledge is ovipositing/larvaposition behavior (hereafter just "ovipositing," for clarity). It is currently debated whether or not "carrion" flies are exclusively diurnal ovipositers or if they also oviposit at night (2–6). Understanding of this behavior impacts the estimates provided by the forensic entomologist in a direct manner, because PMI estimates that include the possibility of nocturnal ovipositing can be as much as 12 h different from estimates that exclude the possibility (2). Therefore, investigating this behavior and resolving whether or not it occurs directly impacts our ability to provide more accurate PMI estimates.

Several field studies have established the arrival and departure patterns of flies attracted to carrion sources (5,7–10). A clear pattern emerges from this research: carrion flies begin their activity in late morning, become most active in early-late afternoon with a sharp decline at or just before sunset. Based on these data, mathematical models currently used to infer PMI from carrion fly larvae developmental stage assume that adult flies will not arrive at a corpse until daybreak (5). However, some literature has specifically identified flies not only actively flying at night, but ovipositing as well (2,3,11). If flies are active at night, then it is critical to establish the frequency with which that activity occurs. Both Greenberg (2), and Singh and Bharti (3) report nocturnal ovipositing levels near 30% for blow flies (family Calliphoridae) and Singh and Bharti (11) report nocturnal ovipositing levels of 20% for flesh flies (family Sarcophagidae). In contrast, several studies have failed to find nocturnal ovipositing behavior at all (4–6,12–14). The problem with several of these subsequent projects is that they have low sample size, with only Amendt et al. (14) and Stamper and DeBry (6) having above 20 samples. At such small sample sizes, it becomes hard to notice any "rare" nocturnal activity, let alone refine how common or rare it truly is. Because of this, our present study doubles the number of sites used and substantially increases our total sample size, compared to our previous research (6). Together the 2006 and 2007 seasons represent 125 samples from four locations each with lit and unlit conditions.

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Received 16 Sept. 2008; and in revised form 17 Dec. 2008; accepted 23 Dec. 2008.

Materials and Methods

We carried out nocturnal ovipositing studies from late July through September of 2006 and 2007 at four sites in and near the metropolitan area of Cincinnati, Ohio. Site one was an urban location in Mount Washington Township. Lot size in this area is about 0.15 acres, with single- or small multi-family (2–5 family) buildings on most lots in the surrounding area. The front yard contained a streetlight that operated constantly throughout the study, while the back yard contained a motion-activated light but was otherwise unlit. Site 1 was sampled in both 2006 and 2007.

Site 2 was a more rural location, utilizing the front and back yard of a house in a sparsely populated portion of Batavia Township, east of Cincinnati. Lots in this area averaged several (3+) acres in size, each contained only a single-family home, and were largely zoned agricultural. The front yard of this property was within 10 meters of two streetlights while the back yard was unlit. Site 2 was sampled in both 2006 and 2007.

Site 3 was another urban location, located in the west side of Cincinnati. Lot size in this area is about 0.08 acres, with single-family buildings in the surrounding area. The front yard of this property was directly under a streetlight while the back yard was only lit by a motion-activated light. Site 3 was sampled in 2007.

Site 4 was another urban location, located in the north central area of Cincinnati. Lot size in this area is about 0.06 acres, with single-family buildings in the surrounding area. The front and east side yard of this property was within 30 feet of a streetlight. The west side yard was in darkness due to the close proximity of an adjacent house (<5 meters between the two houses) that shaded the west yard from streetlights further down the street. Rats were placed in the west yard (unlit) and the east yard (lit). Site 4 was sampled in 2007.

Total sample size for this experiment was 128, with 48 samples taken in the 2006 season (reported in [6]) and 80 samples taken in the 2007 season. Three of these samples were removed due to

contamination (described below) resulting in a final sample size of 125. In 2006, 12 samplings occurred with both sites 1 and 2 visited on the same night (12 nights, 2 sites, 2 rats per site). In 2007, 10 samplings occurred with all four sites being visited on the same night (10 nights, 4 sites, 2 rats per site). For both seasons, two rats were placed at each location on the same night, with one rat in lit and one rat in unlit conditions at each location. "Lit" conditions were continual ambient light from streetlights, while "unlit" conditions lacked a continual light source.

Dead rats were carefully prepared, exposed, and allowed to "mature" as previously described (6). In summary, adult rats were individually bagged and frozen when they were donated for our research after euthanization by the Genome Research Institute. Donated rats were "negative control" rats, meaning they were not genetically modified nor were they subject to any nutrition regimes ranging outside normal maintanence diets. Rats were euthanized according to IACUC-approved protocols prior to their donation to our laboratory. Forty-eight hours prior to night-time exposure, rats were placed inside sealed containers and allowed to thaw to ambient outside temperatures. Rats remained inside sealed containers and were transported to each site for exposure. At the site, rats were placed inside unlidded Gladware® containers with a sand substrate and placed on the ground under a mesh (1 cm × 1 cm) exclosure cage to prevent vertebrate contamination. Placement occurred within 45 min to 1 h from the onset of sunset, as determined by local weather reporters. Rats were then collected within 45 min to 1 h of sunrise, again as determined by local weather reporters. Since sites were spread out over the Cincinnati metropolitan area, two field workers were used to lay out and collect specimens. These field workers then met and all specimens were transported back to the wooden shed used for bait maturation.

Our previous work on nocturnal ovipositing indicated that postexposure contamination was a significant problem if not carefully controlled for (6). Thus, we continued to utilize a methodology that provided multiple barriers to postexposure contaminants. First, bait was stored in Gladware® containers that featured lids modified with screening for ventilation. Second, the plastic containers were then slid inside Delnet® pollinator exclusion bags that were tied closed. Finally, postexposure bait was housed in a wooden shed that was modified to allow for maximum ventilation but prevent adult fly access (fully detailed in [6]). To check for postexposure contamination, a negative control rat was placed inside the maturation shed each night of sampling, then removed when that night's samples were removed from the shed at the end of their maturation period.

Diurnal samplings were not carried out during this study, because of serious cross-sample contamination issues during pilot experiments (6). However, in 2004 researchers at Northern Kentucky University carried out a series of diurnal oviposition studies within 5 miles of our study sites 1 and 2, and identified the following species as diurnally ovipositing on rotting chicken: *Cochliomyia macellaria*, *Phormia regina*, *Calliphora vicina*, *Calliphora vomitoria*, *Lucilia illustris*, *Lucilia sericata*, *Lucilia coeruleiviridis*, *Bufolucilia silvarum*, and *Pollenia* sp. (Greg Dahlem, personal communication).

Results and Discussion

During none of the 128 samplings was nocturnal ovipositing activity observed. On two occasions, larvae appeared on the negative control rat, but no flies were reared from the baited samples from those nights. These samples were retained in the study. On three occasions, baited samples showed maggot activity. All three of these instances showed clear signs that the activity was due to postexposure contamination. Postexposure contamination evidence was evident in the form of egg casings being present on the screened lid or on the Delnet® netting. The three contaminated rats were removed from the study, providing a final total sample size of 125. The diurnal positive controls from the 2006 season adequately demonstrate that our rats are suitable bait (6).

Environmental conditions for the 2006–2007 seasons are as follows. Temperature ranged from 13°C to 31.6°C. Relative humidity ranged from 20% to 97%. Light level ranges for unlit conditions were 0–34 LUX and lit conditions were 12–100 LUX. These environmental parameters all fall within the ranges reported in previous studies that found (2,3,11) and did not find (4,5,12–14) nocturnal ovipositing activity.

Our findings do not support the view that there is any relationship between light intensity and nocturnal oviposition behavior as proposed by Singh and Bharti (11). Nor do we find compelling evidence that nocturnal ovipositing behavior might be different for urban and rural locations as suggested by Greenberg (2). Given these results, we feel that the nocturnal ovipositing rates indicated by Greenberg (2) and Singh and Bharti (3,11) cannot be applied to the Cincinnati metropolitan area. Further research will be needed to ascertain the true prevalence of nocturnal oviposition, as well as the factors that might make such behavior more or less likely. At a minimum, future field research will need to have increased sample sizes from the historical norm for these experiments to allow for a proper assessment of the true rarity of this behavior, if it is found to occur at all.

Acknowledgment

Funded by Wiemen/Wendell/Benedict research fellowships for both 2006 and 2007 (TS).

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