SHORT COMMUNICATION

Human Wound Colonization by *Lucilia eximia* and *Chrysomya rufifacies* (Diptera: Calliphoridae): Myiasis, Perimortem, or Postmortem Colonization?

MICHELLE R. SANFORD,[1,2] TERRY L. WHITWORTH,[3] AND DARSHAN R. PHATAK[1]

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ABSTRACT The infestation of human or animal tissues by fly larvae has been given distinctive terminology depending on the timing and location of colonization. Wounds and orifices colonized by Diptera in a living human or animal are typically referred to as myiasis. When the colonization occurs after death, it is referred to as postmortem colonization and can be used to estimate the minimum postmortem interval. What happens when the human, as in the case presented here, has a necrotic limb while the human remains alive, at least for a short period of time? The case presented here documents perimortem wound colonization by *Lucilia eximia* (Wiedemann) and *Chrysomya rufifacies* (Macquart) and the considerations for approximating development temperatures and estimating the time of colonization (TOC). This represents the first record of *L. eximia* in human myiasis in the United States and the first record of the co-occurrence of *L. eximia* and *C. rufifacies* in human myiasis in the United States. The TOC was estimated using both ambient and body temperature. Insect colonization before death complicates the estimation of TOC and minimum postmortem interval and illustrates the problem of temperature approximation in forensic entomology casework.

KEY WORDS forensic entomology, myiasis, time of colonization, casework

Myiasis, the infestation of living tissues by flies, is well documented in the tropics and neotropics (Francesconi and Lupi 2012). Before the successful eradication of the primary screwworm fly, *Cochliomyia hominivorax* (*americana*) (Coquerel) (Diptera: Calliphoridae), in the United States, myiasis of wildlife and domestic animals was common in Texas (Lindquist 1937, Knipling 1939) with reports in humans as well (Causey 1937, James 1947, Scott 1964, Macias et al. 1973). After the eradication program, more reports of human myiasis caused by *Lucilia* (*Phaenicia*) *sericata* (Meigen) (Diptera: Calliphoridae) (Rice and Nelson 1956, Horen 1967, Greenberg 1984, Sherman 2000), *Chrysomya* spp. (Diptera: Calliphoridae) (Richard and Gerrish 1983), and *Phormia regina* (Meigen) (Diptera: Calliphoridae) (Hall et al. 1986) were reported.

Lucilia (*Phaenicia*) *eximia* (Wiedemann) has been reported as causing myiasis in animals (Diptera: Calliphoridae) (Madeira et al. 1989, Azeredo-Espin and Madeira 1996). This species has not been previously documented in association with human myiasis in the United States and is considered an uncommon species, observed most often in Texas and Florida (Whitworth 2010). It is widespread throughout the tropics occurring in Central and South American countries (Baumgartner and Greenberg 1985, Vianna et al. 1998, Batista-da-Silva et al. 2011). It has been proposed for use in wound debridement in Colombia (Echverri et al. 2010), suggesting that it is capable of feeding on necrotic wound tissue, but whether it prefers to lay eggs in wounds is unknown. *L. eximia* is a known carrion breeding fly species (Hall 1948).

Four members of *Chrysomya* (Diptera: Calliphoridae) have been introduced to the Americas, including *Chrysomya albiceps* (Wiedemann), *Chrysomya putoria* (Wiedemann), *Chrysomya megacephala* (F.), and *Chrysomya rufifacies* (Macquart) (Baumgartner and Greenberg 1984). Two of these species are currently present in North America. *C. megacephala*, introduced from Africa (Baumgartner and Greenberg 1984), and *C. rufifacies* from the Australasian region (Wells et al. 1999). In parts of their native range, Thailand for example, they have been recorded simultaneously in human myiasis (Sukontason et al. 2005). In wet and warm climatic zones of the Hawaiian Islands, these two species have also been collected in cases of cattle myiasis (Zimmerman 1944, Shishido and Hardy 1969). However, to our knowledge *L. eximia* and *C. rufifacies* have yet to be recorded together in human myiasis.

Colonization by flies after death is useful in estimating the minimum postmortem interval (PMI_{MIN}), a key task of a forensic entomologist. Species-specific developmental data and local temperature data are used to work backwards to estimate the time of fly egg

[1] Pathology Division, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054.
[2] Corresponding author, e-mail: michelle.sanford@ifs.hctx.net.
[3] Department of Entomology, Washington State University, WA 99164.

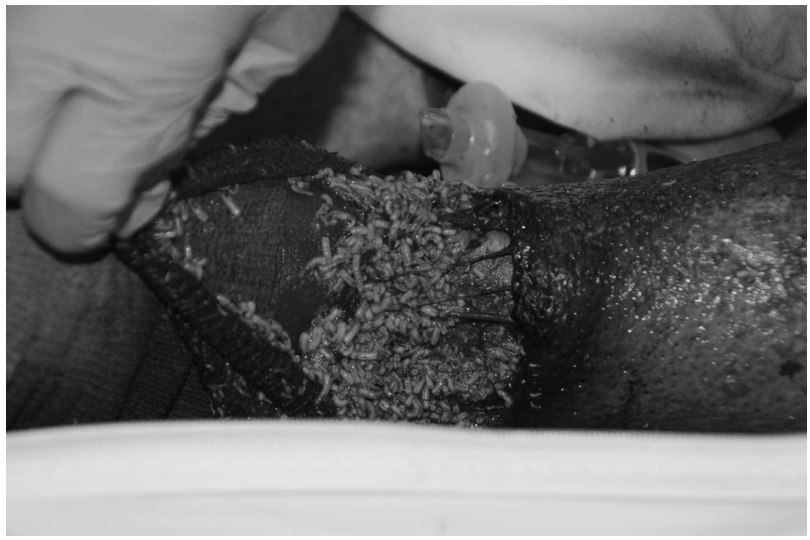

Fig. 1. Autopsy photograph illustrating the extent of the larval fly infestation of the lower left leg of the decedent.

deposition (Catts and Goff 1992). The temperatures experienced by the developing larvae influence the rate of development and the estimated age of the larvae. When the larvae develop on dead tissue, ambient air temperature is used to approximate development temperature, as the body is expected to cool to ambient conditions. However, in cases of myiasis the temperature used to approximate development time is body temperature. Thus, the differentiation of myiasis from postmortem colonization becomes critical to accurately estimate the PMI_{MIN} or the time when flies may have first deposited eggs, the time of colonization (TOC; Byrd and Castner 2000).

The case presented here describes the apparent infestation of a wound on a necrotic limb around the time of death (perimortem) of the individual and the difficulties present in determining how much time had passed since the wound was colonized. This report also documents the first record of human myiasis caused by *L. eximia* and the first published record of the co-occurrence of *L. eximia* and *C. rufifacies* involved in human myiasis in the United States.

Materials and Methods

Case Details. A homeless, 60-yr-old male was found nearly unresponsive outdoors behind a local business in Houston, TX, pronounced dead shortly after arrival to the emergency room and was transported to the Harris County Institute of Forensic Sciences on 4 June 13. Larval fly activity was noted around the decedent's scrotum and penis, and extensive larval fly activity was observed on the lower left leg near the ankle (Fig. 1); medical records noted that there was no pulse in this leg. The body was stored in the morgue cooler at ≈7.2°C from the time of check-in until autopsy the following day, when specimens were collected. Bacterial samples were taken at the hospital for culturing and identification by the hospital laboratory. The cultures identified *Proteus mirabilis* (Enterobacteriales: Enterobacteriaceae) as a bacterial species in the left leg wound, left lung, spleen, and blood, and sepsis due to this bacterium was a contributing factor in the decedent's cause of death. Additional autopsy findings related to the decedent's cause of death included hypertensive and atherosclerotic cardiovascular disease, chronic obstructive pulmonary disease, and cirrhosis.

Specimens. A portion of the larval specimens collected from the leg wound during autopsy were processed by hot water killing (Adams and Hall 2003), followed by placement in 70% ethanol, and a portion were prepared for rearing on defrosted beef liver at warm room temperature for confirmation of identification. Because a large number of specimens were collected, the remaining larvae were killed and preserved by direct placement into 70% ethanol. Larval measurements and microscope photographs were made using a Keyence VHX-600 digital microscope (Keyence Corporation of America, Itasca, IL).

Estimate of TOC. Quality Controlled Local Climatological Data from the nearest weather station located at George Bush Intercontinental Airport, Houston, TX, (Station #12960) was obtained from The National Oceanic and Atmospheric Administration's National Climatic Data Center (http://cdo.ncdc.noaa.gov/qclcd/QCLCD?prior = N) for May and June 2013. Degree hours were calculated in Microsoft Excel 2007 (Microsoft Corporation, Portland, OR) using a base 10°C developmental threshold by the accumulated degree hour method (ADH; Higley and Haskell 2000).

Results and Discussion

Two types of larvae were collected: a smooth larva and a spiny larva. The smooth type specimens were third instar and tentatively identified as *L. eximia*

(Knipling 1939) or *Lucilia coeruleiviridis* (Macquart) (Diptera: Calliphoridae) (Seago 1953). Additional first- and second-instar smooth larval specimens were collected but these specimens were only identified as Calliphoridae. The spiny specimens were identified as second instar *C. rufifacies* (Wells et al. 1999). Reared adults were confirmed as *L. eximia* and *C. rufifacies* (Whitworth 2010). To our knowledge, this represents the first time that these two species have been implicated together in human myiasis. The decedent was colonized before death; however, the leg was essentially dead and presumably cooler than the rest of the body while the decedent remained alive before death at the hospital. The lack of circulation to the leg would have cooled the temperature of the tissues that the fly larvae fed upon, but it is not clear how long the limb was necrotic and hence how cool the temperature may have been with respect to body temperature when the eggs were initially laid.

The difficulty in approximating the temperatures experienced by the developing larvae directly affects the estimated TOC. The highest possible temperature the larvae may have experienced would have been the decedent's body temperature and the lowest possible temperature was likely ambient air temperature. Therefore, a range encompassing these two extremes was generated and the actual time of fly colonization likely falls within this range. A conservative estimate based on completion of the second instar of *L. eximia* at 21.7–26.0°C (cyclic temperature; Greenberg and Szyska 1984) places colonization of the leg in the late evening of 1 June 2013 or the early morning of 2 June 2013 using the ambient air temperatures obtained from the nearest weather station. Using an estimated body temperature of 37°C, the ADH for completion of second instar *L. eximia* (1121.1 ADH) may have been reached if the eggs had been laid late on 2 June 2013 or early on 3 June 2013. The ADH for the completion of the first-instar *C. rufifacies* larvae at 26.7°C (cyclic temperature; Byrd and Butler 1997) would have been met if eggs had been laid in the afternoon of 3 June 2013 using ambient air temperatures from the nearest weather station. Based on body temperature and development at 32.2°C (Byrd and Butler 1997), the ADH would have been met if the eggs had been laid late in the day of 3 June 2013. These conservative estimates suggest that *L. eximia* likely colonized between the 1 and 3 June 2013 and *C. rufifacies* colonized later on 3 June 2013. We would propose that the colonization was perimortem, occurring around the time of the terminal events leading to the decedent's death. If the decedent had been found without all the details regarding his terminal events, the estimated PMI_{MIN} would have been inaccurate and potentially misleading because of the unknown amount of time between the TOC and the decedent's death.

Another potential source of error in the estimate of TOC is that temperature-based development data sets available for *L. eximia* and *C. rufifacies* were not local. Using data from a different locally adapted population may not accurately reflect the development and temperature response of a local population of the same fly species (Gallagher et al. 2010). The only dataset located for *L. eximia* was from a population derived from Peru (Greenberg and Szyska 1984). Data for *C. rufifacies* were from a Florida population (Byrd and Butler 1997). However, using a more locally derived data set obtained at a different temperature of 28.3°C suggests colonization of *C. rufifacies* (Flores 2013) may have occurred in the afternoon of 2 June 2013 closer to the colonization time of *L. eximia.* The remaining source of error then becomes the difference in the temperatures from which the development data were obtained.

The case presented here documents the first report of human myiasis caused by *L. eximia* and the first reported co-occurrence of *L. eximia* and *C. rufifacies* in human myiasis. It is unusual to have such extensive knowledge of a decedent's terminal events with respect to insect colonization. In most instances where the forensic entomologist is called upon to make an estimate of TOC and PMI_{MIN} knowledge about myiasis and medical history will be unknown, a consideration of premortem colonization may be a significant consideration especially when medical history suggests conditions that may predispose an individual to skin ulcers and difficulties in peripheral circulation. Without the unusual knowledge about this specific case, the estimated TOC would have overestimated the PMI by as much as 2 d.

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