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PAPER

PATHOLOGY/BIOLOGY; CRIMINALISTICS

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Alteration of Expirated Bloodstain Patterns by *Calliphora vicina* and *Lucilia sericata* (Diptera: Calliphoridae) Through Ingestion and Deposition of Artifacts*,†

ABSTRACT: Bloodstain pattern analysis can provide insight into a sequence of events associated with a violent crime. However, bloodstain pattern analysis can be confounded by the feeding activity of blow flies. We conducted two laboratory experiments to investigate the relationships between *Lucilia sericata* (green bottle fly) and *Calliphora vicina* (blue bottle fly), expirated bloodstains, and pooled bloodstains on a range of surfaces (linoleum, wallpaper, textured paint). *C. vicina* and *L. sericata* changed bloodstain pattern morphology through feeding and defecation. They also deposited artifacts in rooms where blood was not present originally. Chemical presumptive tests (Hemastix®, phenolphthalein, leucocrystal violet, fluorescein) were not able to differentiate between insect artifacts and bloodstains. Thus, *C. vicina* and *L. sericata* can confound bloodstain pattern analysis, crime scene investigation, and reconstruction. Crime scene investigators should be aware of these fundamental behaviors, and the effects that blow flies can have on expirated and pooled bloodstain patterns.

KEYWORDS: forensic science, blow fly, insect stains, fly spots, leucocrystal violet, fluorescein, phenolphthalein, Hemastix®

The analysis of bloodstain patterns can be a crucial component of a criminal investigation. For example, bloodstain patterns can provide important information relevant to the nature of the type of weapon used, the approximate positions of the individuals and objects in space, and the sequence of events associated with the formation of bloodstains (1). This insight is acquired through an analysis of the shape and location of the bloodstain pattern. However, confusion can arise during bloodstain analysis because bloodstains are not static and can be altered after formation. Some alterations, such as a wipe pattern, are part of the overall bloodstain pattern and can be used in analysis to help recreate the crime scene (1,2). In contrast, other alterations such as the feeding of insects on the bloodstain pattern are so poorly understood that they can confound bloodstain pattern analysis (3). Therefore, a need exists to establish a fundamental understanding of the relationships between insects and bloodstains to assist in the reconstruction of a crime scene, or, at the very least, not confound bloodstain pattern analysis.

A relationship between insects, particularly the blow flies (Diptera: Calliphoridae), and bloodstains has been established since the mid-19th century (4). Yet, little is known about the fundamental interactions between blow flies and bloodstain patterns. Blow flies can form insect stains (artifacts) through normal feeding behavior, which includes the consumption of blood with sponging mouthparts and the regurgitation or defecation of blood-like waste products (5). The blow flies ingest the blood using sponging mouthparts, which take up liquid much like a sponge and can change the appearance of the bloodstain. Blow flies often regurgitate the ingested food by partially expelling it as a bubble and then sucking it back in. The blood is not completely digested before defecation, which can cause the fecal matter to resemble blood in both appearance and chemistry. Regurgitated and defecated fly artifacts are generally small (1–2 mm diameter), can be round, symmetrical or asymmetrical, and have a wide range of color that varies from clear to red and brown to gray-green (3). In addition, fly stains will occasionally have a tail ranging from a few millimeters up to 20 mm in length (3,5). As a consequence, these artifacts can resemble some bloodstain patterns such as medium and high impact and expirated bloodstain patterns (2,3,5).

In addition, common presumptive chemical tests like phenolphthalein, Hemastix®, leucocrystal violet, Sangur, luminol, fluorescein, and DNA typing do not differentiate between fly artifacts and human blood (3,5). To date, only alternate light source (ALS) has provided a means to distinguish between bloodstains and insect artifacts (5). However, only some defecatory artifacts (those that were defecated) fluoresced under blue/green light (465 nm) (5).

To date, no experimental work has been conducted to investigate the relationships between blow flies and expirated bloodstain patterns. To address this gap in knowledge, two controlled laboratory experiments were conducted using various common household wall and floor coverings and two species of flies commonly found at scenes of crime. These two experiments investigated the effects of the blow fly, *Calliphora vicina* (Robineau-Desvoidy, 1830) or

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Lucilia sericata (Meigen, 1826) on an expirated bloodstain pattern on white painted wall, wallpapered wall, and white linoleum floor over a period of 72 h. These two species were used because they are commonly found at crime scenes throughout North America (6).

Materials and Methods

Microscenes

Experiments were conducted in wooden boxes (0.46 m^3) with two glass sides and a plexiglass sliding top, hereafter referred to as "microscenes" (5). One wall was finished with white, textured paint and the other wallpaper. The floor was white laminate tile. The clear sides of the microscene allowed for easy observation and documentation and simulated the windows present in indoor crime scenes (5). Flies were allowed into the microscene via a PVC pipe (4 cm diameter) connected to the "holding cage," a wooden frame box (15 × 15 × 10 cm) enclosed by a screen (mesh size 1 mm^2). With this design, flies had the choice to move in and out of the microscene.

Blood

Human blood was used in this study and was drawn intravenously by a certified medical practitioner. Six milliliters of blood (AB+) was drawn into a vial without preservatives or anticoagulants and used to form two bloodstain patterns within 10 min of being drawn. First, a pool of blood was formed by pouring 3 mL of blood onto the laminate floor. To form the second pattern, an expirated stain, the blood donor placed the remaining 3 mL of blood in his mouth and expirated blood onto the corner between the two nonglass walls. These procedures were repeated for each microscene.

Insects

Two experiments were conducted, each with a different species of blow fly: *C. vicina* and *L. sericata* (Diptera: Calliphoridae). *C. vicina*, commonly known as the blue bottle fly, has a cosmopolitan distribution (7). It is common from Mexico City to Alaska, although it is most abundant in the Midwest from Oklahoma to southern Canada (7). *C. vicina* is one of the first blow flies to become active in spring and one of the last to disappear in the fall, but is relatively scarce in warmer weather, typically May through early October (7). This fly prefers shade and urban environments, although it can be found in rural locations (7). *C. vicina* adults are 10–14 mm long, covered in bristles, with a blue-gray thorax and metallic abdomen.

L. sericata is smaller and more fragile, measuring 6–9 mm in length (7) and is commonly known as the green bottle fly or the sheep blow fly. Adults range in color from blue-green to bronze and are very metallic. *L. sericata* is currently found throughout the world, but is most common in the western regions of the temperate United States and southern regions of Canada. *L. sericata*, like *C. vicina*, is one of the first insects that colonize cadavers, with gravid females ovipositing within hours after death (7). Unlike *C. vicina*, this species prefers bright sunshine and open habitats (7).

Experimental Design

Ten flies were placed in the holding cage *c.* 45 min prior to the formation of the bloodstains. A sugar cube and wet cotton ball were then placed in the microscene via the PVC pipe to provide sources of carbohydrate and water. Bloodstains were formed as described earlier, and the holding cage was then connected to the microscene. The flies had access to the scene for 72 h. Placement of the microscenes was randomized to negate bias of lighting and temperature. The temperature in the room was 21 ± 1°C. The flies were subject to a photoperiod of 10 h light:14 h dark. New surface inserts were created for each experiment. Each experiment was replicated four times, and controls (no flies) were used. This resulted in a total of eight microscenes used per experiment.

At the conclusion of each experiment, flies were removed from the scene using a vacuum cleaner. Four presumptive chemical tests were then used to determine their ability to differentiate fly artifacts from the unaltered bloodstain. The four tests used in this experiment were phenolphthalein, Hemastix®, leucocrystal violet, and fluorescein. Phenolphthalein, Hemastix®, and leucocrystal violet were swabbed on an area without bloodstains or artifacts (control), on bloodstain, and on fly artifact. Fluorescein was sprayed onto the wall or floor panel and then observed with the ALS. Photographs were taken throughout the study. Photographs were taken before and after fly introduction with a Fujifilm IS-1 digital infrared camera. Photographs were reviewed and compared with Corel Paint Shop Pro X.

Results

Both *C. vicina* and *L. sericata* altered the original bloodstain patterns through the consumption of blood and the deposition of regurgitory and defecatory artifacts (Fig. 1*a*). In addition, flies were often observed bubbling blood for several hours (Fig. 1*b*). No evidence of tracking was observed, as the tarsi could not break the surface tension of the blood pools (Fig. 2*a*). Both species consumed wet and dry blood (Fig. 2). When feeding upon dry blood, small "wells" (3) were created in the blood. These marks looked similar to the patterns left by air bubbles in unaltered blood. The flies also consumed artifacts, sometimes within seconds of deposition. Small blood droplets and artifacts could be completely consumed, leaving either no trace, a faint outline, or small imprints left by the proboscis (Fig. 2*b*). More often, only a portion of the bloodstain or artifact was consumed, leaving a significant amount of the original stain.

Bloodstains and artifacts were consumed, and artifacts were deposited, during both the day and night. The greatest quantity of artifacts with tails (artifacts with a sperm-like tail connected to a round or tear-shaped body shown in Fig. 3*a*) was produced in the dark, which might indicate an increased frequency of walking rather than flying in the dark. Fly artifacts were found on all surfaces of the microscene, including areas of blood spatter (Fig. 3*a*) and on the framework of the holding cage. Some artifacts were deposited in a group with other artifacts, while some artifacts were deposited alone. None of the four presumptive chemical tests were able to distinguish between bloodstains and fly artifacts. All four tested positive for blood when tested on fly artifacts, which indicates that certain properties from the blood remained unchanged.

The first *C. vicina* entered the microscene within 10 min. These flies fed on the blood within 5 h and displayed a high level of activity throughout the experiment, which included feeding on blood and artifacts and deposition of artifacts. Although many defecatory artifacts were formed, only two processes of formation were observed. In one case, a fly lowered its abdomen toward the ground and released a stream of liquid from the anus. In the other case, a fly defecated and then moved, dragging clear waste behind it. The fly spread the waste with its legs, leaving a fly spot with a

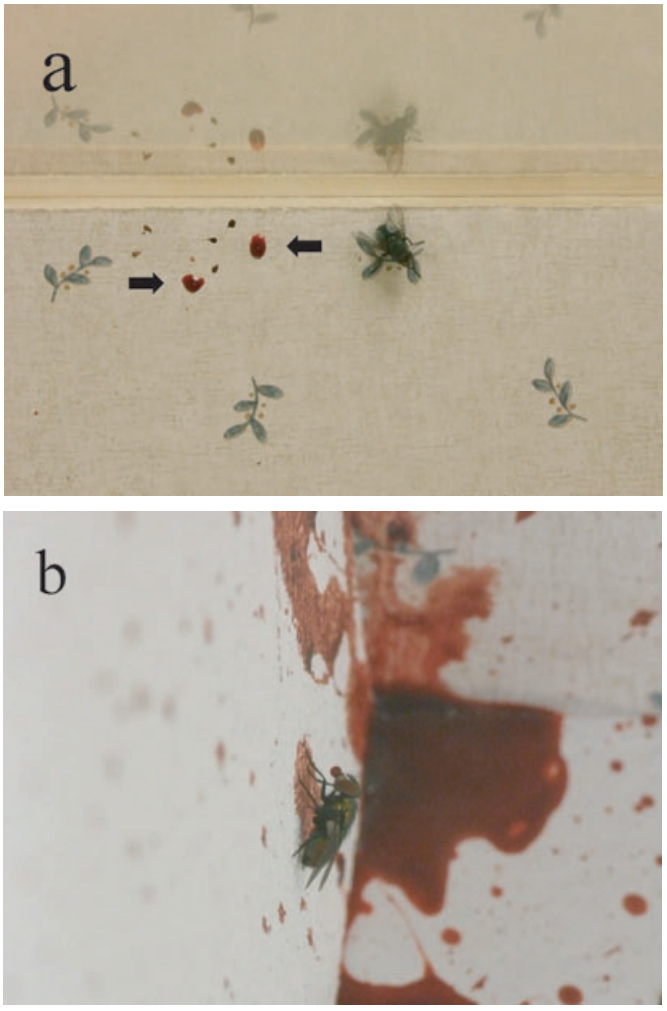

FIG. 1—*Regurgitory and defecatory artifacts (a: regurgitory artifacts indicated by arrows) and the bubbling of blood (b) associated with* Lucilia sericata *and* Calliphora vicina *following the expiration and pooling of fresh human blood into a microscene (0.46 m^3 wooden box with glass sides, plexiglass sliding top and wallpaper at 21 ± 1°C).*

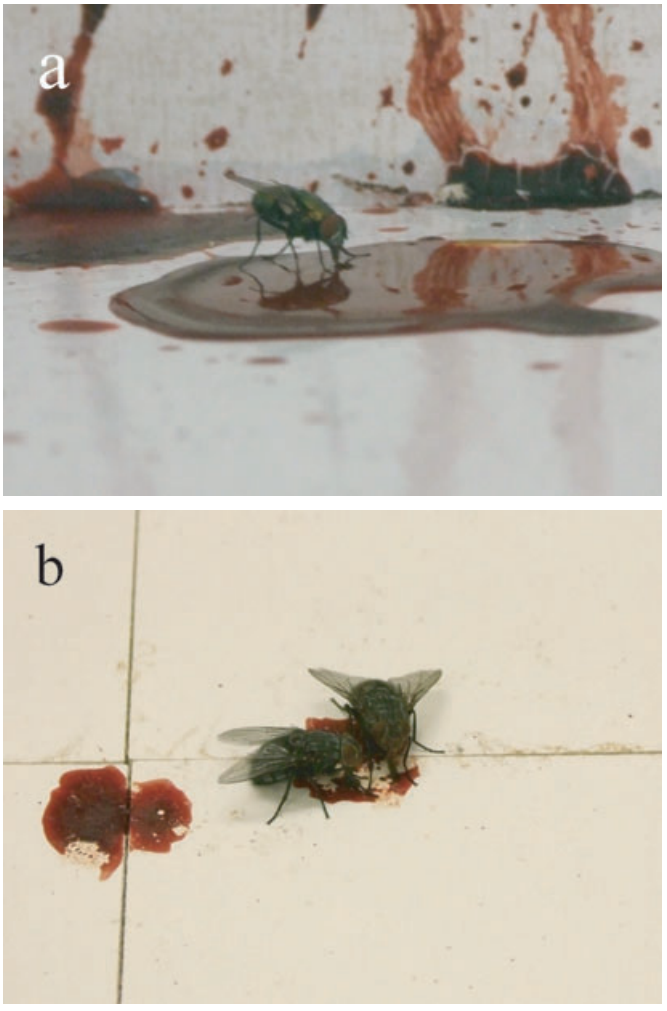

FIG. 2—*Feeding on wet blood (a) and dry blood (b) by* Lucilia sericata *and* Calliphora vicina *following the expiration and pooling of fresh human blood into a microscene (0.46 m^3 wooden box with glass sides, plexiglass sliding top and wallpaper at 21 ± 1°C). Tarsi (feet) of both species did not break the surface tension of the pooled bloodstain (a).*

thick trail. Immediately after defecating, the fly began feeding on its own artifact. Artifacts with tails were observed on all surfaces: paint, wallpaper, tile, and glass. *C. vicina* changed the shape of a wet artifact, through feeding, from round to tear-shaped. Translocation, or movement of a portion of artifact to a new location without leaving a trail (Fig. 3*b*), was observed on the wallpaper insert. By the end of the experiment, 2–3 *C. vicina* had died per microscene, with the exception of one scene in which no flies died. All of the dead *C. vicina* were found in the holding cage.

The first *L. sericata* entered the microscene within 1 h. These flies were less active than *C. vicina*: they fed less and deposited fewer artifacts. *L. sericata* were observed primarily feeding on sugar or artifacts, although they were occasionally observed feeding on both wet and dry blood. These flies would feed individually or communally, typically in groups of three or more flies. Several large clusters of artifacts were consumed, either by an individual fly or through communal feeding. The deposition of an artifact with a tail was observed. The tail was formed when some of the waste remained stuck to the anus, creating a string of defecatory material going from the wall to the abdomen. As the fly walked toward the ceiling, it pushed at the string with its legs until the string fell in a direction not consistent with the direction the fly was moving, thus creating the tail of the artifact (Fig. 4*a,b*). However, few artifacts with tails were observed. Rather, *L. sericata* deposited several small, round artifacts in a trail, or line. Another method of bloodstain alteration was observed when one *L. sericata* moved a dry flake of blood *c.* 1 cm across the floor while walking. By the end of the experiment, three *L. sericata* had died in each of the experimental microscenes, with the exception of one scene in which only one fly died. A little less than half of the dead *L. sericata* were found in the microscenes (four dead in the microscenes: six dead in the holding cage).

Discussion

The results show that *C. vicina* and *L. sericata* can alter expirated and pooled bloodstain patterns through feeding and deposition of artifacts. These results are similar to those from the previous experimental study on *C. vicina* by Fujikawa et al. (5). Not previously observed was that *C. vicina* constructed artifacts via translocation of expirated bloodstains. In addition, differences in fly behavior and the nature of alterations were observed between species. Thus, we conclude that *C. vicina* and *L. sericata* have different interactions with pooled and expirated bloodstains. In

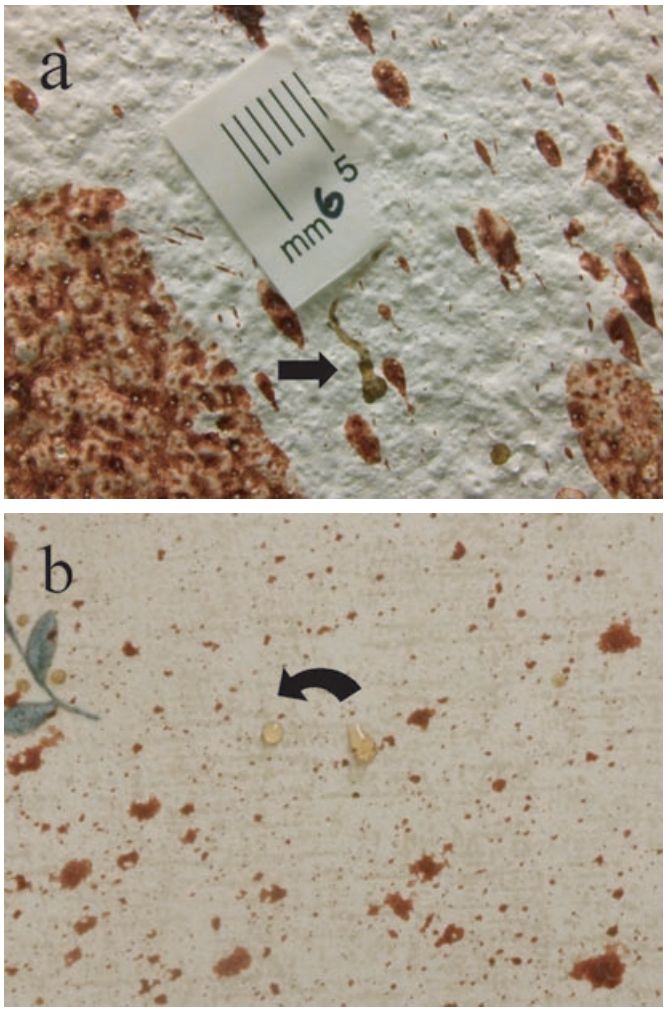

FIG. 3—*Insect stain within expirated bloodstain (a: indicated by arrow) and translocation of artifact (b: direction of movement indicated by arrow) associated with* Calliphora vicina *following the expiration and pooling of fresh human blood into a microscene (0.46 m^3 wooden box with glass sides, plexiglass sliding top and wallpaper at 21 ± 1°C).*

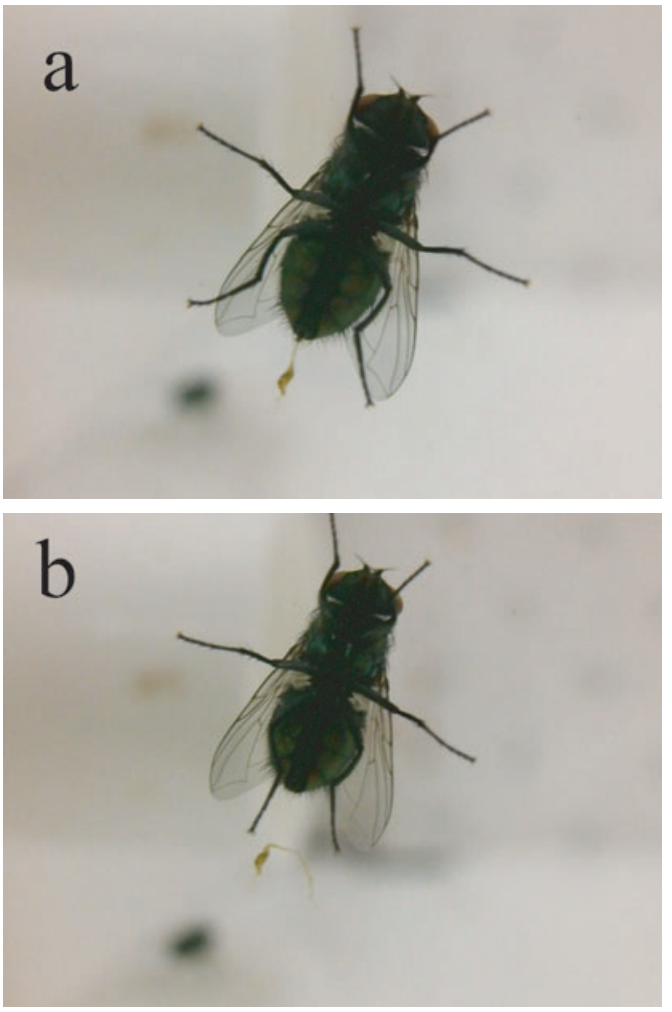

FIG. 4—*Formation of depository artifact, beginning with the waste strung out between the tip of the abdomen and the glass surface (a) and then falling in a direction inconsistent with the direction traveled (b) by* Lucilia sericata *following the expiration and pooling of fresh human blood into a microscene (0.46 m^3 wooden box with glass sides, plexiglass sliding top and wallpaper at 21 ± 1°C).*

addition, we conclude that insect stains can be formed by *C. vicina* in rooms or areas that did not originally contain bloodstains.

It is important to note that insect stains can be found in rooms in which blood was not expirated. This behavior has been documented in multiple case studies (3,8) and is perhaps the blow fly behavior that is most likely to confound bloodstain pattern analysis, particularly when coupled with their tendency to deposit artifacts in a group. A group of round, tail-less artifacts can resemble expirated or medium to high impact bloodstain patterns. When such a group of artifacts is in a room or location without blood, it can lead investigators to the conclusion that a violent event occurred somewhere it did not. Other ways that artifacts and bloodstain patterns can be confused that were observed in this experiment include the observation that fly feeding can resemble dried blood with air bubbles or flakes of dry blood peeling off of a surface. In addition, some of the expirated spatter in control scenes had tails going in directions that were contrary to the majority of drops, which is one of the indicators used to determine fly artifacts from bloodstain patterns. Thus, there are no firm rules (see Ref. [3]) that can be used to differentiate artifacts from bloodstain patterns, and it is important not to include or dismiss a stain simply because it does not follow dogma. The context of the bloodstain pattern and crime scene must be used in analysis.

Although *C. vicina* and *L. sericata* demonstrated some similar behavior in their interactions with blood, several differences were observed. The increased level of activity and artifact formation by *C. vicina* may be because of the fact that it is a more robust fly and requires more nutrients to sustain itself. It may also reflect the difference of temperature preference between the two species: *C. vicina* is noted for preferring cooler temperatures, whereas *L. sericata* prefers warmer temperatures. The temperature experienced by both species was 21°C, which is closer to the temperatures preferred by *C. vicina*. Another difference observed between species was that *C. vicina* deposited more artifacts with tails than *L. sericata*. Although this might indicate that *C. vicina* is less likely to confound bloodstain pattern analysis than *L. sericata*, *C. vicina* also deposited some artifacts that would have been extremely difficult to distinguish from bloodstain patterns if the experimental photographs had not been available to compare the bloodstain pattern pre- and postexperiment. Thus, crime scene investigators need to be alert to the presence of artifacts at the crime scene. The presence of artifacts with tails should serve as an indicator that there

are likely other insect artifacts without tails. Finally, it is apparent that *C. vicina* is more likely to confound bloodstain pattern analysis through feeding. *C. vicina* was observed feeding on blood throughout the experiment, whereas *L. sericata* was rarely observed feeding on blood, particularly after the first 48 h.

In conclusion, *C. vicina* and *L. sericata* affected bloodstain patterns through feeding and defecation. However, *C. vicina* and *L. sericata* interacted with the blood differently. These species can deposit artifacts in rooms or locations in which blood was not present. Because of these behaviors, *C. vicina* and *L. sericata* can confound bloodstain pattern analysis and thus crime scene investigation and reconstruction. Crime scene investigators should be aware of these fundamental behaviors, and the effects that blow flies can have on expirated and pooled bloodstain patterns.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

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