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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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Changes in the Morphology and Presumptive Chemistry of Impact and Pooled Bloodstain Patterns by *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)*

ABSTRACT: Bloodstain pattern analysis can be critical to accurate crime scene reconstruction. However, bloodstain patterns can be altered in the presence of insects and can confound crime scene reconstruction. To address this problem, we conducted a series of controlled laboratory experiments to investigate the effect of *Lucilia sericata* (Meigen) on impact bloodstains and pooled bloodstains in association with three combinations of common surfaces (linoleum/painted drywall, wood floor/wallpaper, and carpet/wood paneling). *L. sericata* fed from the pooled bloodstains and added insect stains through regurgitation and defecation of consumed blood. *L. sericata* formed defecatory trails of insect stains that indicated directionality. Defecatory stains fluoresced when viewed at 465 nm with an orange filter. These observations differed from *Calliphora vicina* insect stains because feeding on blood spatter was not observed and trails of insect stains were formed by *L. sericata*. The fluorescence of defecatory stains can be used as a method to detect insect stains and discriminate them from real bloodstains.

KEYWORDS: forensic science, forensic entomology, bloodstain pattern analysis, insect stains, blow fly, bubbling

Bloodstains are one of the most common forms of physical evidence found at scenes of violent crime (1). Bloodstains are useful because their shape, size, and pattern can provide insight into the events associated with their formation, including the associated sequence of events, number of persons involved, weapons used, and the placement of individuals in space (1,2). Two relatively common bloodstain patterns are impact and pooled patterns. Impact patterns are caused when an object comes into contact with liquid blood and results in the formation of a spatter pattern while pooled patterns are formed when a volume of liquid blood accumulates on a surface (1–3). A pooled pattern indicates that blood was allowed to collect, intentionally or unintentionally, with relatively little disturbance (1–3). While the morphology of a bloodstain is an important source of physical evidence, it is crucial to understand that bloodstains can be altered at a scene where insects are present (4,5), which can lead to confusion in the interpretation of bloodstain patterns (6).

Medical forensic entomology is most often associated with the estimation of postmortem interval (7). Blow flies are regularly used to estimate postmortem interval when they are found feeding on a corpse (7), but they can also use blood as a food source. Fujikawa et al. (5) observed that *Calliphora vicina* (Robineau-Desvoidy) can alter impact stains and pools by the uptake of blood, regurgitation, and defecation. The feeding behavior of *C. vicina* decreased the size of the original bloodstain while regurgitation and defecation added insect stains that reacted positively to presumptive blood tests (phenolphthalein, leucocrystal violet, fluorescein, and Hemastix®; Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) (5). Furthermore, it is unlikely that the behavior of *C. vicina* is representative of all species of blow flies. *C. vicina* is a large (10–14 mm), robust fly that is common in the Northern hemisphere and favors cooler temperatures (4). In contrast, *Lucilia sericata* (Meigen) is a small (6–9 mm) fly that favors warmer temperatures (4). Because of the differences between size and environmental preference of blow flies, the current study was conducted to determine the fundamental interactions between *L. sericata*, impact bloodstains, and pooled bloodstains.

In this study, we tested the null hypothesis that *L. sericata* will not change the morphology or presumptive chemistry of impact and pooled bloodstain patterns. We tested this hypothesis by observing the behavior of groups of 10 *L. sericata* in association with impact and pooled bloodstains in a controlled laboratory setting over a 48-h period. This study was conducted to help investigators visually and chemically differentiate fly insect stains from true impact bloodstain patterns.

Materials and Methods

Scenes

Experiments were conducted in scaled-down room analogs, referred to hereinafter as "microscenes" (Fig. 1). Microscenes were

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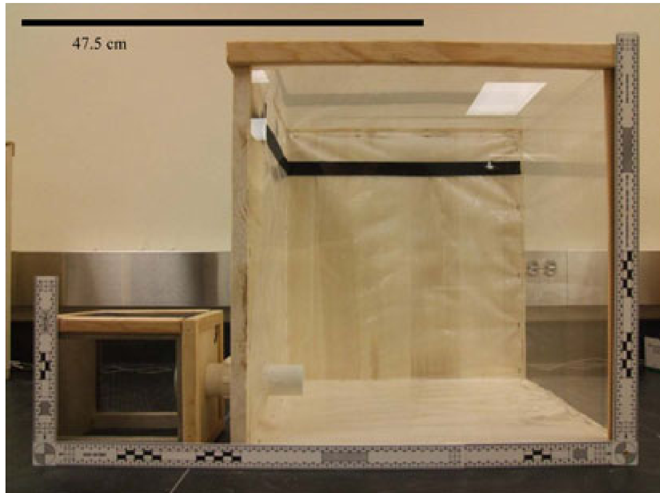

FIG. 1—*Microscene with holding cage attached. Ten blow flies (*L. sericata*) were given access to a range of wall and floor surfaces (linoleum/painted wall, wood laminate/wallpaper, and carpet/paneling) for 48 h at a temperature of 22 ± 2°C following the addition of impact and pooled bloodstains. Black bar indicates 47.5 cm.*

0.47 m^3 wooden boxes with two glass walls with a plexiglass ceiling to facilitate observation and photography. Attached to the microscenes were fly holding cages. Holding cages were 15 × 15 × 10 cm constructed with a wooden base and enclosed by screen (mesh size: 1 mm^2). A 3.8-cm-diameter PVC pipe provided access to the microscene. This allowed the insects to enter the microscene from the holding cage. Slides (42 cm wide × 43 cm tall) were inserted inside the boxes to allow for changes of the two walls and the floor. For evaluation, we distinguished between floor and wall (which we refer to as surfaces) and the components within floors and walls (which we refer to as substrates). We assumed that gravitaxic or phototaxic behaviors might influence surface preferences, while tactile or chemical cues might influence substrate preferences. Floor and wall combinations were based on materials commonly found at crime scenes. Substrates for floor were carpet, wood laminate, and linoleum; substrates for wall were drywall, wallpaper, or wood paneling. Combination 1 consisted of linoleum and drywall; combination 2 consisted of wood laminate and wallpaper; combination 3 consisted of carpet and wood paneling. All substrates except wood paneling were white to avoid potential confounding between surface texture and color. The wood paneling used was "light oak," which was the closest available approximation to white coloration.

Blood

Human blood was drawn intravenously by a certified medical practitioner. Blood (AB+: 6 mL) was drawn into eight blood tubes with no preservatives or anticoagulants. The blood was used to form impact and pooled bloodstains within 5 min of withdrawal.

Insects

L. sericata were maintained in 30.5-cm by 30.5-cm screened boxes in rearing chambers with constant temperatures of 24 ± 1.5°C. Larvae were reared on beef liver and had access to vermiculite for pupation. Adults had access to water and sugar *ad libitum*.

Presumptive Testing

Four chemicals and an alternate light source were used in this study to determine whether the flies changed the chemical composition of blood enough for the tests to differentiate between blood and insect stains. The tests were chosen because they are commonly used in the field by investigators and have variable specificity when locating latent blood. The four chemical tests were phenolphthalein, leucocrystal violet, Hemastix®, and fluorescein. Sterile cotton swabs moistened with two drops of distilled water were used to swab the sample areas (blood, insect stain, and control) before the testing of each chemical. The alternate light source (SPEX Handscope Halogen HS-100; Horiba Jobin Yvon, Edison, NJ) was tested at all available wavelengths (400–700 nm) with red (Marumi, 58 mm, R2), orange (Marumi, 58 mm, YA2), and yellow (Bower, 58 mm, Y2) filters.

Experimental Design

A combination of 10 male and female flies were randomly chosen and placed in holding cages 30 min prior to the formation of bloodstains. Impact bloodstains were constructed by pooling 3 mL of blood on the floor surface and striking it with a flyswatter. Three milliliters of blood was added to the floor surface as a pool after the impact stain was made. Microscenes were then connected to the holding cages to allow flies to migrate to the scene. Observations were recorded every hour for 9 h. After 9 h, the lights were shut off and remained off until the following morning. The flies remained in the microscene for 48 h at a temperature of 22 ± 2°C. After 48 h, the flies were removed and placed in a separate cage so they could not be used in subsequent experiments. Alternate light source testing was conducted first, followed by Hemastix®, phenolphthalein, and leucocrystal violet swabs. Fluorescein was the last chemical applied to test for insect stains. New surface inserts were constructed for each experiment. Four microscenes with blood and flies and four microscenes with blood and no flies were used for each combination. This resulted in a total of eight microscenes per experiment. Photographs were taken throughout the study to document morphological changes in the stain patterns. Photographs were taken with a Fujifilm IS-1 Digital Infrared Camera (Fujifilm, Valhalla, NY), in standard mode (hot mirror/cut filter attached), mounted on a tripod. Photographs were reviewed and compared using Adobe Photoshop CS3 (Adobe, San Jose, CA).

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) procedures in Microsoft Excel® 2007 (Redmond, WA). To meet assumptions of homogeneity of treatment variances required for ANOVA, all raw data were log transformed, $\ln(x + 1)$ before analysis. Surfaces (floor and wall) were evaluated by ANOVA. The effect of substrate texture and location was evaluated by ANOVA, and potential differences were evaluated by unprotected paired *t*-tests (with 3 df for all tests). Unprotected *t*-tests were used to increase discrimination of any potential substrate differences. As no insect stains occurred in any controls, control data were not included in any of the analyses.

Results

No fly type insect stains were seen in the four control microscenes. In the experimental microscenes, *L. sericata* migrated into combinations 1 (linoleum floor/painted walls) and 2 (wood floor

laminate/wallpapered wall/white painted wall) within 2 h of bloodstain formation. Less than 50% of the flies used in combination 3 (carpet/paneling/painted wall) migrated, but flies that did migrate required 3–5 h. There was a high mortality rate of *L. sericata*, with over 50% dying within the first 24 h of each experiment.

L. sericata fed at intervals of less than 5 min. The flies fed from the pools of all three combinations and feeding continued after the blood had dried (*c.* 24 h). The feeding activity at the pool left little physical evidence behind, with no noticeable smudging or smearing of the parent stain. Blood tracks (tarsi prints) were not observed although tarsi contacted blood (Fig. 2). Feeding on insect stains was observed, but feeding on spatter was not observed. Insect stains resulting from defecation were shaped like a teardrop with a long tail or round and domed with no tail (Fig. 3). Regurgitated insect stains were generally round with little or no tail. No insect stains were deposited in or on bloodstains. During defecation, trails of insect stains that showed directionality were observed (Fig. 4).

When deposited, insect stains were caused by regurgitation and defecation of ingested blood, but no insect stains were deposited on the carpet substrate (combination 3). Seventy percent of the insect stains were formed in late afternoon and continued through the night (*c.* 15 h). No significant differences were noted between defecation and regurgitation deposition on floor and wall surfaces (defecation $F_{1,22} = 0.30$; $p = 0.59$; regurgitation $F_{1,22} = 1.3$; $p = 0.27$). Among floor and wall substrates, no differences (floor defecation $F_{2,9} = 1.6$; $p = 0.25$; floor regurgitation $F_{2,9} = 1.6$; $p = 0.25$; wall defecation $F_{2,9} = 1.4$; $p = 0.30$; wall regurgitation $F_{2,9} = 0.92$; $p = 0.43$) were observed for defecation or regurgitation.

A difference in the results of the presumptive tests was not observed. The reaction times of the blood and insect stains when tested with phenolphthalein, Hemastix®, leucocrystal violet, and fluorescein were all under 2 sec. Blood did not fluoresce; however, defecatory insect stains fluoresced under light at 465 nm when viewed through an orange filter (Marumi, 58 mm, YA2) with no added chemicals.

Discussion

The current results show that the adult blow fly *L. sericata* has little effect on the shape and chemistry of pooled bloodstain patterns through feeding. However, they can alter impact bloodstain patterns by depositing insect stains in the form of trails that show directionality. These results differ from the behavior of the blow fly *C. vicina*, which altered the shape of bloodstains through feeding and deposited insect stains randomly, seldom showing directionality (5). The high mortality rate associated with *L. sericata* meant that fewer flies migrated into the scene to feed and deposit insect stains. Thus, we conclude that (i) different blow flies can have contrasting effects on impact and pooled bloodstain patterns and (ii) insect stains differ with different substrates.

It was difficult to distinguish between regurgitated and defecated insect stains because of their similar shape and size. However, defecatory insect stains with tails fluoresced under light at 465 nm (SPEX Handscope Halogen HS-100) with an orange filter (Marumi, 58 mm, YA2), which indicated some change in the chemistry, but this could not be detected with commonly used presumptive chemical tests. *L. sericata* deposited twice as many regurgitory insect stains than defecatory insect stains with 159 regurgitated and 78 defecated insect stains. This was based on the number of insect stains that had tails versus those that did not, because it was difficult to observe the difference between the regurgitated and defecated insect stains that had a similar shape.

The lack of insect stains on the carpet and the paucity of insect stains on the wall substrates associated with the carpet may be because the blood in the carpet was more difficult to extract with sponging mouthparts. Thus, there was little blood for them to deposit. The carpet avoidance behavior is similar to what was observed with *C. vicina* in similar experiments (5). As no insect

FIG. 2—L. sericata *attempting to feed from a blood pool after 10 blow flies (*L. sericata*) were given access to a range of wall and floor surfaces (linoleum/painted wall, wood laminate/wallpaper, and carpet/paneling) within an 0.475 m^3 wooden microscene for 48 h at a temperature of 22 ± 2°C following the addition of impact and pooled bloodstains.*

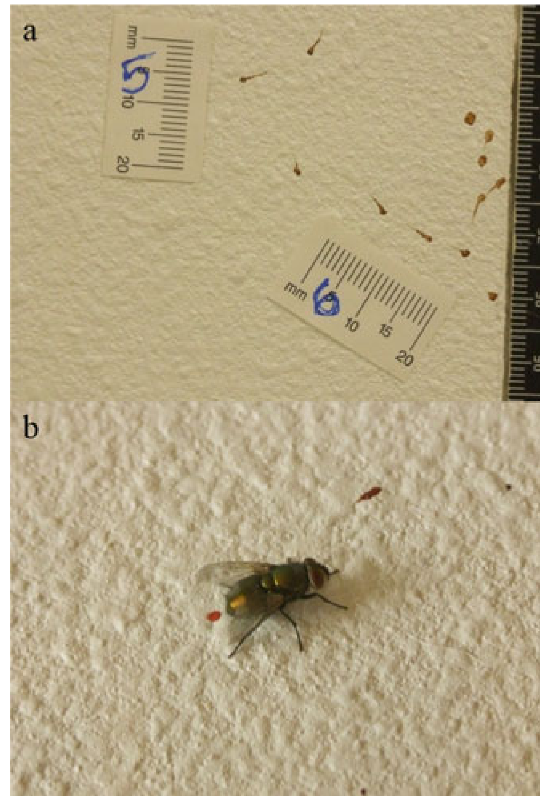

FIG. 3—*Shapes of defecatory insect stains: (a) tailed insect stain and (b) domed insect stain with no tail after 10 blow flies (*L. sericata*) were given access to a range of wall and floor surfaces (linoleum/painted wall, wood laminate/wallpaper, and carpet/paneling) within an 0.475 m^3 wooden microscene for 48 h at a temperature of 22 ± 2°C following the addition of impact and pooled bloodstains.*

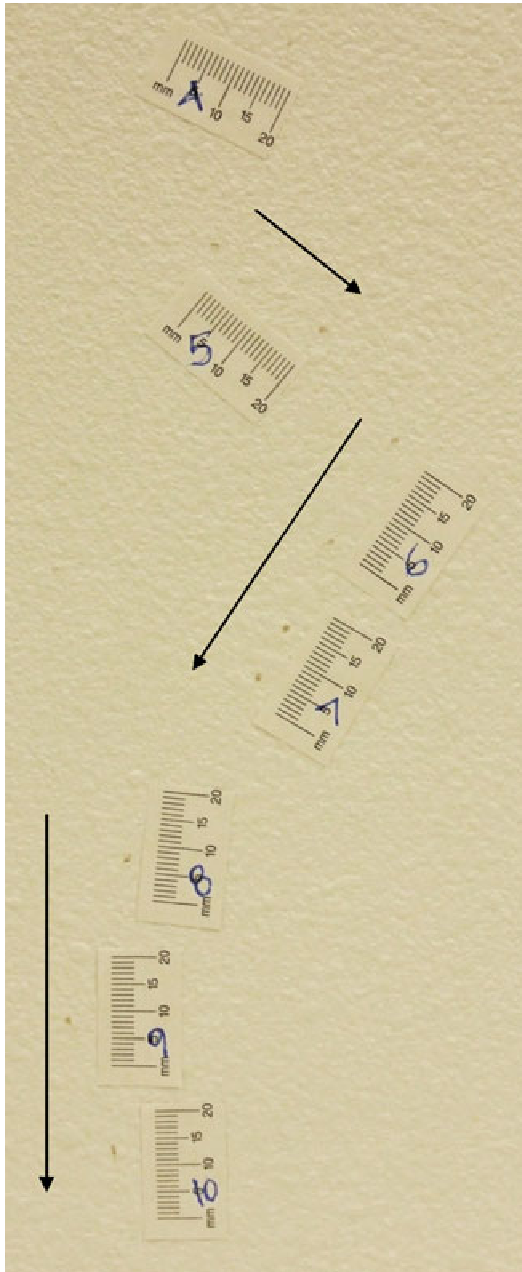

FIG. 4—*Trail of defecatory insect stains made by fly walking during defecation after 10 blow flies (*L. sericata*) were given access to a range of wall and floor surfaces (linoleum/painted wall, wood laminate/wallpaper, and carpet/paneling) within an 0.43 m*^{3} *wooden microscene for 48 h at a temperature of 22 ± 2°C following the addition of impact and pooled bloodstains. Arrows indicate direction of travel.*

stains occurred on carpet, and because our *a priori* expectation was that insect stains would be less common on carpet (fly tarsi can become entrapped in carpet fibers), evidence supports the idea that flies avoid carpet after blood feeding. As a practical implication of this finding, we expect fewer fly stains in crime scenes where blood pools or spatter occurred primarily on carpeting and that any fly stains that did occur would be smaller and fewer.

The cause of the high mortality rate of *L. sericata* remains unknown. The adult flies used in each experiment were *c.* 2 weeks old. It is unlikely that the mortality rate was related to nutrition because the flies had access to water and sugar in the microscene throughout the experiment. However, on average, only about 50% of the flies migrated to the microscene. Although the flies did not have access to protein prior to the experiment, the blood did not appear to be a strong attractant nor was it sufficient to sustain the activity of half of the blow fly population.

We chose *L. sericata* as an experimental subject because of its association with crime scenes and as a good species to compare with our other experimental subject, *C. vicina* (5), which is much larger. With both species, blood spatter on carpet reduced the occurrence and size of insect stains, and (fortuitously) chemical responses to insect stains are similar. However, *L. sericata* and *C. vicina* exhibit differences in both the size and geometry of their insect stains. Moreover, *C. vicina* showed significant differences in the number of insect stains on floor and wall substrates, which is a much greater discrimination than observed with *L. sericata*. Given these differences, similar studies with other key blow fly species are essential for building a comprehensive, reliable understanding of bloodstain and insect interactions.

More broadly, how can we, or need we, distinguish insect stains from other impact stains? Objective, reliable characteristics for discriminating blood spatter are crucial in aiding law enforcement and crime scene personnel in reconstructing crime scenes when bloodstains are present. The combination of our findings here, fluorescence of fly defecatory stains (5), and techniques outlined in Benecke and Barksdale (6) [especially, a tail (L_{tl})-to-body (L_B) ratio greater than one ($L_{tl}/L_B > 1$), flies (dead or alive) in vicinity of body or bodily fluids, and linear and/or random directionality] are building this set of objective, reliable criteria.

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