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Responses of *Lucilia sericata* (Diptera: Calliphoridae) to compounds from microbial decomposition of larval resources

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ABSTRACT Decomposition of vertebrate carrion is partially due to microbes, which release a series of volatile organic compounds (VOCs) at various concentrations. These VOCs are part of ecologically relevant public information that serve as cues attracting blow flies (Diptera: Calliphoridae), such as *Lucilia sericata*, to remains as a resource both for themselves and their offspring. However, these responses are partially governed by sex and physiological state (e.g., gravid and non-gravid) of the fly and concentration of the VOCs. The responses of 7-9-d-old *L. sericata* adults to dimethyldisulfide, indole, isobutylamine, and phenylacetic acid were examined in a Y-tube olfactometer. These VOCs are associated with vertebrate decomposition, carrion-mimicking flowers, as well as fly-attracting bacteria. The results demonstrate a relationship between sex and physiological state with regards to dose-dependent attraction to VOCs that occur during decomposition suggesting that specific decomposition molecules provide distinct types of information to the flies with differing foraging interests. Understanding this dynamic relationship provides insight into the mechanisms regulating arthropod colonization, competition and resulting succession in association with such ephemeral resources.

Keywords: carrion, chemical ecology, microbiology, sexual dimorphism, physiology, interkingdom communication, public information

1 HISTORICALLY, RESEARCH ON CARRION as an ephemeral resource has predominately
2 focused on the ecological interactions occurring on the resource after its death as related to
3 nutrient recycling, as well as the associated population dynamics of those organisms responsible
4 for this ecosystem service (Barton, Weaver, & Manning, 2014; Barton et al., 2013).
5 Consequently, little is known about the mechanisms associated with the ephemeral resource
6 regulating attraction, colonization and utilization of these resources (Tomberlin, Mohr, Benbow,
7 Tarone, & VanLaerhoven, 2011). Identifying and deciphering these mechanisms provide greater
8 insight to the role public information (*sensu lato*- ‘information regarding the quality of a resource
9 gleaned from observing the foraging success of other individuals associated with the resource’
10 (Valone, 1989) associated with these resources plays towards regulating animals competing for
11 them to maximize their fitness (Benbow, Tomberlin, & Tarone, 2015; Tomberlin, Benbow,
12 Tarone, & Mohr, 2011). Indeed, recent efforts suggest microbes serve as a conduit through
13 which many of these cues are released into the environment.

14 Microbes play a pronounced role in the decomposition processes of carrion (Benbow,
15 Tomberlin, & Tarone, 2015; Brodie, Wong, VanLaerhoven, & Gries, 2015; Carter, Metcalf,
16 Bibat, & Knight, 2015; Embriette R. Hyde, Haarmann, Lynne, Bucheli, & Petrosino, 2013;
17 Hyde, Haarmann, Petrosino, Lynne, & Bucheli, 2014; Metcalf et al., 2013; Pechal et al., 2013).
18 Primarily, they serve as a mechanism through which high-molecular-weight nutrients are broken
19 down into simpler carbon, sulphur, nitrogen, and phosphorus-containing compounds (Kasper,
20 Mumm, & Ruther, 2012). In turn, the volatile organic compounds (VOCs) produced by bacteria
21 represent many of the compounds that serve as cues regulating attraction or repellence of insects,
22 in particular blow flies (Diptera: Calliphoridae), to these resources (Brodie, Wong,

23 VanLaerhoven, & Gries, 2015; Frederickx, Dekeirsschieter, Verheggen, & Haubruge, 2012; Ma
24 et al., 2012).

25 The blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) has served as a model for
26 evaluating fly-microbe interactions commonly occurring on carrion and during myiasis (Brodie,
27 Wong, VanLaerhoven, & Gries, 2015; Ma et al., 2012; Tomberlin et al., 2012). The species is
28 known to produce antibiotic molecules and alter microbial communities on its larval resources
29 (Bexfield, Nigam, Thomas, & Ratcliffe, 2004; Cazander, van Veen, Bernards, & Jukema, 2009;
30 Cerovsky et al., 2010; Daeschlein, Mumcuoglu, Assadian, Hoffmeister, & Kramer, 2007; Jaklic,
31 Lapanje, Zupancic, Smrke, & Gunde-Cimerman, 2008; Sherman, Hall, & Thomas, 2000). It is
32 also associated with bacteria that, once introduced to a resource, can alter microbial communities
33 to the benefit of the insect (Erdmann & Khalil, 1986; Sherman et al., 2000; Singh et al., 2015).

34 Recently, it has been proposed that *L. sericata* relies on bacterial cues to guide decisions
35 regarding attraction to and colonization of larval resources, such that a mutant strain of the
36 commensal bacteria, *Proteus mirabilis* (Singh et al., 2015), which is deficient in the ability to
37 swarm (a quorum sensing response) and that is rescued by putrescine (a quorum sensing
38 molecule), was less attractive to *L. sericata* (Ma et al., 2012). This mutant strain differentially
39 produced several known fly attractants compared to its parent strain, and fly response was
40 regulated by its sex and physiological state (i.e., gravid versus non-gravid) (Tomberlin et al.,
41 2012). This observation is not surprising, given that gravid females are more attracted to fresh
42 carrion, while non-gravid adults attracted to more decomposed vertebrate carrion (Mohr &
43 Tomberlin, 2014) and that carrion is not a necessary resource for males. Taken together, these
44 observations suggest that microbial metabolites can provide information to blow flies evaluating
45 a potential resource regarding the nutritional value of that particular resource and that different

classes of *L. sericata* may process the same microbial cues within the contexts of their particular needs and priorities (i.e., finding a mate versus acquiring vitellogenic nutritional resources).

When considering how different flies may perceive bacterial odours, it is important to consider where the odours originate. One major nutritional component of blow fly resources is protein. Thus it is not surprising that the target molecules chosen for this study, dimethyldisulfide (DMDS), phenylacetic acid (PAA), indole (IND) and isobutylamine (IBA), are by-products derived from the essential amino acids of methionine (Hayward, Jeavons, & Nicholson, 1977), phenylalanine (Erdmann & Khalil, 1986), tryptophan (Sasaki-Imamura, Yano, & Yoshida, 2010), and valine (Richardson, 1966), respectively. However, while these amino acids are required for immature development and vitellogenesis (Huntington & Higley, 2010) they may not be necessary for mating. This means that different sexes or physiologically distinct *L. sericata* may interpret the public information derived from those amino acids in distinct ways commensurate with their particular needs. For instance, sulphur compounds are known fly attractants (Brodie, Wong, VanLaerhoven, & Gries, 2015; Frederickx, Dekeirsschieter, Verheggen, et al., 2012) and are released from decomposing remains (M. Statheropoulos, Spiliopoulou, & Agapiou, 2005). However, these molecules exhibit temporally variable patterns of release over the first few days of decomposition (Forbes & Perrault, 2014; Stadler, Stefanuto, Brokl, Forbes, & Focant, 2013; M. Statheropoulos, Agapiou, Spiliopoulou, Pallis, & Sianos, 2007). Typically, blow flies colonize remains quickly, such that some sulphur compounds (e.g., DMDS is more pronounced late in the decomposition process) would likely be a signal that remains are unsuitable for colonization as they have likely already been colonized by competitors. However, these molecules would still be a good predictor of a nutritional resource that contains essential amino acids, which would be desirable to non-gravid females, and could

also serve as a signal for males seeking unmated females. This logic can be extended to all classes of decomposition odours, yielding the prediction that potential blow fly resources of differing ages and value to a fly will release different VOC profiles that elicit different behavioural responses, like those previously discussed, from flies.

The hypothesis of this study was that *L. sericata* response to carrion associated VOCs at different doses will differ based on their sex and ovarian status. We predicted that, while all are cues of decomposition, each molecule would yield a distinct behavioural impact on different sexes of *L. sericata* and on females with different ovarian states. There are numerous lines of evidence that the compounds tested are, or could be, ecologically active. DMDS, IND, and phenolic compounds are known blow fly attractants and components of fly traps (Ashworth & Wall, 1994; Urech et al., 2004). Furthermore, bacteria commonly associated with decomposing vertebrate remains produce three of these compounds (DMDS, IND, and IBA) (Paczkowski & Schütz, 2011) that differentially impact fly attraction and oviposition (Tomberlin et al., 2012). Interestingly, IBA is also produced by carrion mimicking *Arum* lilies, which depend on blow flies for pollination (Smith & Meeuse, 1966). A fourth molecule, PAA, which is produced by the fly commensal, *P. mirabilis*, was also tested. This compound is associated with blow fly larvae (Bromel, Duh, & Erdmann, 1983), is a known antibiotic molecule that benefits fly larvae by suppressing pathogenic bacteria (Erdmann, 1987), and is structurally similar to phenylethyl alcohol; a molecule that differed between mutant and wild type strains of *P. mirabilis* that impacted fly attraction (Ma et al., 2012). In addition, DMDS, skatole (by-product of IND metabolism), IBA and other amines, and phenolic compounds (like PAA) are all noted as scent compounds in carrion and dung-mimicking flowers (Jürgens & Shuttlesworth, 2015; Smith &

Meeuse, 1966). And, as previously stated, DMDS, IND, and PAA are commonly associated with decomposing vertebrate remains that serve as larval and vitellogenic resources for this species.

Methods and Materials

Colony maintenance. *Lucilia sericata* adults from a strain serving as a model population (Sze et al., 2012) were maintained in 30 x 30 x 30 cm BioQuip bug dorms (Bioquip Products, Rancho Dominguez, CA, USA) maintained in a rearing room at 27°C, 60-70 RH, 14:10 L:D. Adult flies were fed bovine liver blood *ad libitum* for the first five days post emergence in order to stimulate ovarian development. Flies were then provided sugar and water until testing at 7-9-d-old.

In order to maintain the colonies, flies remaining in the cages after day nine were provided bovine liver in 88.7 ml plastic bath cups (Walmart, Inc., Bentonville, AR, USA) for 2-3 days to stimulate oviposition on 50 g beef liver placed in another bath cup. Resulting eggs and liver were placed into a 900 ml glass mason jar. The bottom 5 cm of the jar was filled with vermiculite. Larvae dispersing from the liver were allowed to pupate in the vermiculite. Resulting adults were managed using methods as described previously.

Y-tube olfactometer assay. Because *L. sericata* is a rare fly in central Texas, USA (Mohr & Tomberlin, 2014), and our desire to work with a model species in decomposition ecology research, a dual choice olfactometer was used to evaluate the behavioural response of *L. sericata* adults exposed to treatments in the laboratory (Brundage, 2012; Tomberlin, Rains, Allan, Sanford, & Lewis, 2006). Furthermore, using this approach allowed us to control biotic (e.g., fly age, avoid infections, source population of flies) and abiotic factors (e.g., temperature, humidity, lighting) that are known to influence blow fly behaviour.

The olfactometer was covered with a removable glass sheet. A 50 mm USB powered computer cooling fan (5VDC Fan, Dc Fans, Thermal Management NMB Technologies

Corporation, Chatsworth, CA, USA) was used to pull air through the olfactometer. Airflow through the olfactometer at the access port was measured using an anemometer (Testo 435-1, Testo, Inc., Sparta, NJ, USA) to be 0.5 m/sec (based on a 90 s average) (Brundage, 2012). Two 15 mm diameter, 14.5 cm long glass tubes containing activated charcoal (Aqua-Tech, Marineland Aquarium Products, Moorpark, CA, USA) were attached to the Ziploc containers, which were used to hold the treatments for all experiments. The charcoal cleaned the air flowing through the olfactometer. Containers were attached to olfactometer arms with size 14 Tygon tubing, and the containers were rotated after the completion of each experiment. Fluorescent lights provided overhead illumination. Olfactometer assays were carried out with temperature between 20°C and 23°C. The Y-tube was cleaned after each test with 80% ethanol and allowed to air dry for 2 min subsequent to the following experiment. Ten μ L of each tested solution was applied to a filter paper ($\theta = 22.5^\circ$, radius 4.5 cm) (Fisher Scientific, Waltham MA, USA) and allowed 5 min for the solvent to evaporate in the ventilation hood before putting it into a container. Treatment location in the arms of the Y-tube was rotated between replicates to rule out any bias for either side of the arm. Newly treated filter papers were used for each insect. Each fly was introduced into Y-tube olfactometer for three minutes (Brundage, 2012).

DMDS (Sigma Aldrich, Basic materials, St. Louis, MO, USA, Purity $\geq 99.0\%$) was diluted with acetone to concentrations of 0.0005 μ g, 0.005 μ g, 0.05 μ g, and 0.25 μ g. Using the same methods, IND (Sigma Aldrich, Basic materials, St. Louis, MO, USA, purity $\geq 99.0\%$) was prepared in a series of doses at 0.05 μ g, 0.50 μ g and 5.00 μ g; PAA (Sigma Aldrich, Basic materials, St. Louis, MO, USA, purity $\geq 99.0\%$) was prepared to make a series of doses at 0.1 μ g, 1.0 μ g and 10.0 μ g; and, IBA (Sigma Aldrich, Basic materials, St. Louis, MO, USA, purity $\geq 99.0\%$) was prepared to make a series of doses at 0.01 μ g, 0.10 μ g and 1.00 μ g. These doses

were selected due to their biological relevance in association with carrion decomposition and carrion insect responses (Dekeirsschieter et al., 2013) including *L. sericata* adults (Frederickx, Dekeirsschieter, Verheggen, & Haubruge, 2012).

Twenty male, twenty gravid, and twenty non-gravid females were tested for each treatment. Each individual fly was introduced into the Y-tube at the entrance of the main branch and had a choice between the treatment and control (acetone). If a fly remained within the stem of the olfactometer 30 s after being introduced, it was replaced. No more than three flies failed to respond for any of the compounds or associated concentrations examined and therefore were excluded from the analysis. Total residence time in each arm was recorded for each fly.

Statistical analysis. The residence time data were analysed with PROC MIX (SAS 2011) using a full factorial design. The statistical model tested fixed factors that included the physiology (indicates the types of the flies based on their sexes and ovarian development), response to treatment (response to chemical dose versus control), and dose (indicates different doses of the compounds) as well as interactions. Least square means were used to evaluate statistical differences among treatments ($P < 0.05$). In addition, flies were categorized as gravid females (GF), non-gravid females (NG) and males (M) as defined in Mohr & Tomberlin (2014).

Ethical note. Fly colonies are carefully managed. Flies were maintained in a controlled environment within our laboratory. They were provided with essentials (e.g., food and water) to reduce stress. No IACUC permit was required to conduct this research.

Results

The results of the experiments indicate that each molecule exhibits a distinct impact on behaviours exhibited by flies. Behavioural responses of flies to each compound were dependent

on concentrations of the tested compounds and fly physiological state (gravidity and sex). Accordingly, it is useful to evaluate the results per molecule.

DMDS

This study identified a three-way interaction between dose of DMDS, response to test (treatment vs control), and physiology (male, gravid and non-gravid) (Table 1). Fly responses to DMDS were marginally different in response to control and among doses of DMDS and physiological classes (different sexes and ovarian status) ($df = 6, 480$; $F = 2.08$; $P = 0.0537$) (Table 1 and 2). Per cent response $\pm$ SEM of 7-9-d-old *L. sericata* adults based on physiology (i.e., sex and ovarian status) to different doses of DMDS are presented in Table 3. In response to DMDS at $0.0050 \mu\text{g}$ ($df = 6, 480$; $F = 2.92$; $P = 0.0037$), gravid females were repelled (73%) while males were attracted (64%) to the compound (Figure 1, Tables 2 and 3). However, response to $0.0500 \mu\text{g}$ of DMDS did not show significant attraction across sex or ovarian status. Generally, DMDS appeared to be repellent or unimportant to fly decision-making. At a few concentrations, DMDS appeared to be attractive, and this attraction differed between sexes ($0.2500 \mu\text{g}$, non-gravid; $0.0050 \mu\text{g}$, male) with females preferring to orient toward the compound at the highest dose tested, while males only preferred to do so at a much lower concentration.

IND

All fly categories exhibited a significant response to different doses of IND ($df = 2, 342$; $F = 7.17$; $P = 0.0009$) (Table 2). Per cent response $\pm$ SEM of 7-9-d-old *L. sericata* based on physiology (both sex and ovarian status) to different doses of IND can be found in Table 4 and Figure 2. Generally, the compound was attractive to both sexes at the highest dose (64%-66%) tested and repellent (53%-65%) at the lowest dose, with only non-gravid females preferring (57%) the intermediate dose.

PAA

Flies exhibited a sexually dimorphic and physiologically dependent response to PAA. Sex and ovarian status impacted fly response to PAA ($df = 2, 358$; $F = 4.4$; $P = 0.0130$) (Table 2). Per cent response $\pm$ SEM of 7-9-d-old *L. sericata* based on fly physiology (i.e., sex and ovarian status) to different doses of PAA can be found in Table 5. Generally, females appeared to be attracted to this compound while males did not. Non-gravid (62% to 0.1 μ g) and gravid females (72% to 10 μ g) all showed significant attraction to PAA within the tested doses range (Table 2 and Figure 3).

IBA

Flies exhibited a complex response to IBA. Responses to IBA significantly differed between control and treatments ($df = 2, 342$; $F = 2.45$, $P = 0.0459$) (Table 2). Gravid (25% greater) and non-gravid females (35% greater) showed greater responses to the lower doses of IBA when compared with males; non-gravid females were attracted (~70%) to IBA at 0.10 μ g (Figure 4), and males showed significant repellence (~70%) to IBA at 0.01 μ g (Figure 4). Per cent response $\pm$ SEM of 7-9-d-old *L. sericata* of different sexes to different IBA doses are presented in Table 6. Generally, both types of female appeared to orient toward the compound, but with different dose preferences, while males were both attracted and repelled by the compound depending on concentration.

Discussion

In previous work, the Gram-negative bacterium *P. mirabilis*, which is common on decomposing remains (Barnes, Gennard, & Dixon, 2010), was isolated from *L. sericata* salivary glands and a series of *P. mirabilis* mutants with decreased swarming ability (i.e., quorum sensing response) were constructed (Ma et al., 2012). One of these mutant strains deficient in swarming

was rescued by putrescine (Ma et al., 2012), which is a known quorum sensing compound (Sturgill & Rather, 2004), and was used to test its effect on attraction and oviposition of *L. sericata* (Tomberlin et al., 2012). The study determined that this *P. mirabilis* mutant was less attractive to *L. sericata* and induced lower levels of oviposition compared to the wild type strain (Tomberlin et al., 2012). Gas chromatography-mass spectrometry profiles of the mutant and wild type strains determined that compounds emitted from the wild type *Proteus* and this mutant strain were quite different with respect to DMDS, a PAA-related molecule, IND, and IBA (Tomberlin et al., 2012). More recently, *P. mirabilis* was determined to be highly attractive to the secondary screwworm *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae), which commonly occurs on vertebrate carrion as well (Chaudhury, Zhu, & Skoda, 2016). These data suggest *P. mirabilis* could be serving as a general source of cues indicating the presence of nutrients essential for adult and larval development.

Our study expands upon current knowledge regarding the role of sex (i.e., male and female) and physiological state (i.e., gravid and non-gravid) on fly response to select compounds associated with decomposition of vertebrate carrion. For each compound, fly response was regulated by one or more of the factors measured in this study, demonstrating interactions among several of them. These data indicate fly appearance and utilization of carrion as observed in a previous study (Mohr & Tomberlin, 2014) are dependent on olfaction responses to key compounds associated with the decomposition process. Such information is critical as it could be indicative of resource quality or the presence of predators (Brundage, Benbow, & Tomberlin, 2014; Shorrocks & Bingley, 1994) and could be used for enhancing fly traps, as our data suggest that different types of flies of the same species (male, gravid female, non-gravid female) would be attracted to different concentrations of fly trap components. These data could also be useful

when assessing VOCs profiles of human remains for estimating time of death (Dekeirsschieter et al., 2009; Paczkowski, Nicke, Ziegenhagen, & Schütz, 2015; Statheropoulos et al., 2007). It is clear that different compounds convey different types of information to flies and it will be useful to dissect the information conveyed by each of these molecules in further detail.

DMDS, as stated in the introduction, is a key compound released during the active decay state (Dekeirsschieter et al., 2009) and has previously been demonstrated to attract *L. sericata* (Frederickx, Dekeirsschieter, Verheggen, et al., 2012). However their dose responses and associated preferences to the tested compounds as related to physiological status were not reported. We determined here that flies responses to DMDS were significantly different based on dose, sex and ovarian status. In response to DMDS at 0.005 µg gravid females were repelled while males were attracted. This differential response may be explained by the fact that DMDS does not begin to accumulate until decomposition has advanced to the point where flies have already colonized (Dekeirsschieter et al., 2009). Accordingly, it may serve as a cue to gravid females that a particular odour source is likely already colonized and a poor resource for her offspring, while also serving as a cue to males that unmated females are likely to be associated with remains as a vitellogenic resource.

IND was generally attractive to all flies at high concentrations and repellent at low concentrations. IND, as stated in the introduction, is a common volatile produced by bacteria associated with decomposing vertebrate remains (Paczkowski & Schütz, 2011). Furthermore, this compound is a well-known fly attractant (Urech et al., 2004) and plays an important role in bacterial quorum sensing (Lee et al., 2008). Interestingly, the behavioural responses of bacteria to IND are temperature dependent. At human body temperature, IND has less of an effect on *Escherichia coli* (e.g., whole-transcriptome gene expression, antibiotic resistance, promoter

activity, and cell division) than at lower temperatures (25°C and 30°C) (Lee et al., 2008) (e.g., after defecation or death of the host) when bacteria are stressed due to instability in homeostasis. IND produced by *E. coli* also suppresses biofilm production by competing bacteria, such as *Pseudomonas aeruginosa* (Lee, Jayaraman, & Wood, 2007). We hypothesize this plastic bacterial response to IND allows *E. coli* to persist in an unstable environment (e.g., faeces or vertebrate carrion). As IND is a chief fly attractant, this molecular cue (which is derived from catalysis of the essential amino acid tryptophan) could allow for flies to detect and locate valuable resources for larval development, while simultaneously recruiting flies to transfer *E. coli* to more suitable habitats.

Similarly, PAA is also a common volatile produced by bacteria associated with vertebrate decomposition (Paczkowski & Schütz, 2011). From a behavioural perspective, PAA is a potential microbial cue that would indicate the presence of a selective antibiotic on a resource that could indicate the presence or absence of beneficial microbiota on remains. PAA is a molecule produced by *P. mirabilis*, which previously was discussed as commensal with *L. sericata* (Erdmann, 1987). This compound produced by *P. mirabilis* functions as an antibiotic (Erdmann, 1987) and potentially aids with *L. sericata* survivorship on decomposing tissue such as carrion or necrotic wounds (i.e., wound debridement therapy). Furthermore, PAA is structurally similar to a molecule that is differentially produced by mutant and wild type *P. mirabilis* (Tomberlin et al., 2012) that impact *L. sericata* behaviour. Similar compounds are known to be present as VOCs produced by blow fly larval feeding (Frederickx, Dekeirsschieter, Brostaux, et al., 2012). While it is not known at this time, we suspect that PAA could be important for detecting the presence of conspecific and heterospecific larvae or commensal bacteria on carrion rather than the carrion itself. Such abilities are crucial for assessing resource

quality and availability. This information is critical for determining if heterospecifics or conspecifics could have a negative impact on resulting offspring survivorship due to predation (Brundage et al., 2014) or cannibalism (Flores, Longnecker, & Tomberlin, 2014). In line with this hypothesis, there was clear sexual dimorphism in the response to this compound, with females preferring to orient toward the compound, while males did not respond to it.

The compound IBA is related to two common classes of decomposition products, short chain amines and short chain acids. Butyric acid is a known fly attractant and a compound produced during decomposition of vertebrate remains (Dekeirsschieter et al., 2009). This molecule is also highly differentially produced between mutant and wild-type bacteria that impact *L. sericata* behaviour (Ma et al., 2012). The presence of butyric acid most likely serves as an indicator of resource quality. Butyric acid is produced by bacteria, such as *Lactobacillus*, that commonly occur in all life stages of *L. sericata* (Singh et al., 2015) as well on decomposing remains where they originate from the digestive tract (Metcalf et al., 2013). Butyric acid functions as a means to reduce environmental hazards to these bacteria themselves, such as carcinogens generated by commensal bacteria (Kailasapathy & Chin, 2000), as well as competitors like *Salmonella* (Mikkelsen, Naughton, Hedemann, & Jensen, 2004). Such molecules may be a cue to flies of the microbial community quality and composition on a resource.

Short chain amines like IBA, putrescine (a known fly attractant and bacterial QS molecule), and cadaverine are products of amino acid degradation. The mutant tested in Ma et al., (2012) was rescued by putrescine, suggesting some functional redundancy between IBA and putrescine in *P. mirabilis*. It is not surprising that flies would use a cue of amino acid decomposition and bacterial growth to assess resource quality. Interestingly, this compound

seemed to elicit different responses from each type of fly tested. Gravid females, which would be seeking an oviposition site, appeared to prefer low doses of IBA. However, non-gravid females, which would be searching for a vitellogenic resource, were universally attracted to the concentrations tested in this experiment. Males, which would most likely be looking for mates on carrion, appeared repelled by the molecule unless at the highest dose tested, which would mean they were only attracted to a concentration that would put them in the proximity of unmated females. Accordingly, IBA seems to play an important role in guiding the decisions of blow flies of any class.

Interestingly, IBA may also play a role in a less appreciated aspect of blow fly biology – pollination (Heath, 1982). There are several publications that demonstrate a role of blow flies in pollination in plants as diverse as onions (Currah & Ockendon, 1983), carrot (Howlett, 2012), and oxeye daisies (Brodie, Smith, Lawrence, & Gries, 2015). This function is thought to be an outcome of adult fly attendance at flowers to consume nectar (Gardener & Gillman, 2002). Interestingly, IBA and other amines are also known components of floral odour for at least some *Arum* lilies (Smith & Meeuse, 1966) and belong to one general class of plant-produced fly attractants in carrion- and dung-mimicking flowers (Jürgens & Shuttleworth, 2015). The potential connection between IBA and blow fly pollination, especially in plants that are not obvious carrion or faeces mimicking plants, warrants further study.

In addition to the revelations related to the impact of specific compounds on fly behaviour, the statistical approach taken here represents an advance in the approach to studying blow fly behaviour. The statistical approach employed for analysing the data generated in this study allowed for the integration of all variables into a single model for a given compound to determine the impact of concentration as well as sex and physiological state on fly response. This

approach revealed important aspects of the biological system of interest. Taking such factors into account is critical, as physiological status can profoundly affect the ability of an insect to detect and respond to odorous cues (Libert et al., 2007). While previous work in this area has been extremely valuable in understanding fly behaviour, future studies should attempt to employ similar approaches in order to gain greater interpretive value from the data generated when appropriate.

The response of *L. sericata* to carrion resources is associated with a suite of biotic and abiotic factors, including a suite of volatile organic compound profiles, which may differ across carrion types and the associated microbes found on remains. The current research advanced our understanding of the factors impacting fly attraction to such a resource. The next step will be to measure the response of adult flies to mixed VOCs associated with a carrion resource. In addition, it will be important to track the origins of these compounds (essential amino acids) and their relevance to larval and microbial fitness. And, it will be helpful to conduct additional studies in the field, in order to determine the degree to which the sex and physiological specificity of the fly responses in laboratory studies can be replicated. Such steps could be beneficial for producing a clearer understanding of the attraction, colonization and succession of arthropods on vertebrate carrion, which could be useful for application in a number of areas including medical and veterinary entomology. These and similar findings have implications for the design and implementation of fly traps (Dethier, 1947, 1956; Hobson, 1936), interpretations of evidence in forensic entomology (Tomberlin, Benbow, et al., 2011; Tomberlin, Mohr, et al., 2011), the role of filth-feeding flies in pollination biology (Gardener & Gillman, 2002), and can advance our understanding of how flies impact the proliferation and distribution of pathogens into the environment.

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Table 1. Type 3 tests of fixed effects for impact of fly physiology on response to different VOCs at different concentrations were compared in a Y-tube olfactometer.

Tested VOCs	Effect	Number of degree of	Denominator degree of freedom	F Value	Pr > F
DMDS	Dose ¹	3	480	1.44	0.2299
	Physiology ²	2	480	0.89	0.4116
	Response to Treatment ³	1	480	0.91	0.3407
	Physiology*Response to Treatment	2	480	0.25	0.7813
	Dose*Physiology	3	480	0.69	0.5605
	Dose*Response to Treatment	6	480	0.31	0.9341
	Dose*Physiology*Response to Treatment	6	480	2.08	0.0537
IND	Dose	2	324	0.09	0.9172
	Physiology	2	324	0.31	0.7323
	Response to Treatment	1	324	1.28	0.2596
	Physiology*Response to Treatment	2	324	0.14	0.8709
	Dose*Response to Treatment	2	324	7.17	0.0009
	Dose*Physiology	4	324	0.09	0.9865
	Dose*Physiology*Response to Treatment	4	324	0.94	0.4381
PAA	Dose	2	358	0.37	0.6903
	Physiology	2	358	0.12	0.8836
	Response to Test	1	358	6.33	0.0123
	Physiology*Response to Treatment	2	358	4.40	0.0130
	Dose*Response to Treatment	2	358	0.14	0.8726
	Dose*Physiology	4	358	0.14	0.9685
	Dose*Physiology*Response to Treatment	4	358	1.43	0.2232
IBA	Dose	2	342	0.14	0.8673

Physiology	2	342	0.87	0.4184
Response to Treatment	1	342	1.08	0.2991
Physiology*Response to Treatment	2	342	7.23	0.0008
Dose*Response to Treatment	2	342	1.50	0.2252
Dose*Physiology	4	342	0.77	0.5479
Dose*Physiology*Response to Treatment	4	342	2.45	0.0459

¹Dose = response by flies to different doses; ²Physiology = sex and ovarian status of flies; ³ Response to Treatment = response to chemical dose versus control.

Table 2. Differences of Least Squares Means for impact of fly physiology on response to different VOCs at different concentrations were compared in a Y-tube olfactometer (GF: gravid females, NG: non-gravid females, M: males).

VOCs	Effect	Dose (µg)	Physiology	Response to Treatment	vs	Dose (µg)	Physiology	Response to Treatment	Pr > t
DMDS	Dose ¹ *Physiology ² *Response to Treatment ³	0.005	GF	Compound	vs	0.005	M	Compound	0.0037
IND	Dose*Response to Treatment	0.05	ASC ⁴	Control	vs	5.00	ASC	Control	0.0091
	Dose*Response to Treatment	0.05	ASC	Compound	vs	5.00	ASC	Compound	0.0067
	Dose*Response to Treatment	5.00	ASC	Control	vs	5.00	ASC	Compound	0.0012
PAA	Response to Treatment	ADC ⁵	ASC	Control	vs	ADC	ASC	Compound	0.0123
	Sex*Response to Treatment	ADC	GF	Control	vs	ADC	GF	Compound	0.0022
	Sex*Response to Treatment	ADC	NG	Control	vs	ADC	NG	Compound	0.0169
	Dose*Physiology*Response to Treatment	10	GF	Control	vs	10.0	GF	Compound	0.0014
IBA	Physiology*Response to Treatment	ADC	GF	Compound	vs	ADC	M	Compound	0.0121
	Physiology*Response to Treatment	ADC	M	Control	vs	ADC	M	Compound	0.0188
	Physiology*Response to Treatment	ADC	M	Control	vs	ADC	NG	Control	0.0053
	Physiology*Response to Treatment	ADC	M	Compound	vs	ADC	NG	Compound	0.0151
	Physiology*Response to Treatment	ADC	NG	Control	vs	ADC	NG	Compound	0.0041
	Dose*Physiology*Response to Treatment	0.01	M	Control	vs	1.00	M	Control	0.0027
	Dose*Physiology*Response to Treatment	0.10	NG	Control	vs	0.10	NG	Compound	0.0059
	Dose*Physiology*Response to Treatment	0.01	M	Control	vs	0.01	M	Compound	0.0025

¹Dose = response by flies to different doses; ²Physiology = sex and ovarian status of flies; ³Response to Treatment = response to chemical dose versus control; ⁴ASC = data for all sex categories combined; ⁵ADC = data for all doses combined.

Table 3. Per cent response $\pm$ SEM of 7-9 days old *L. sericata* adults based on physiology (i.e., sex and ovarian status) to different doses of DMDS when paired against a control in a Y-tube olfactometer.

Doses (μ g)	Physiology (n ¹)	Percentage response to DMDS $\pm$ SEM	Percentage response to Control $\pm$ SEM
0.2500	GF (20)	55.88 $\pm$ 8.96	45.12 $\pm$ 8.96
	NG (20)	66.18 $\pm$ 16.40	33.82 $\pm$ 16.40
	M (20)	45.42 $\pm$ 10.10	54.58 $\pm$ 10.10
0.0500	GF (20)	46.41 $\pm$ 9.69	53.59 $\pm$ 9.69
	NG (20)	44.67 $\pm$ 9.32	55.33 $\pm$ 9.32
	M (20)	46.72 $\pm$ 10.32	53.28 $\pm$ 10.32
0.0050	GF (20)	26.38 $\pm$ 8.61	73.62 $\pm$ 8.61
	NG (20)	47.67 $\pm$ 8.32	62.33 $\pm$ 8.32
	M (20)	64.57 $\pm$ 9.42	35.43 $\pm$ 9.42
0.0005	GF (20)	49.81 $\pm$ 8.26	50.19 $\pm$ 8.26
	NG (20)	43.41 $\pm$ 8.10	56.59 $\pm$ 8.10
	M (20)	49.34 $\pm$ 10.04	50.66 $\pm$ 10.04

¹n = replicates

Table 4. Per cent response $\pm$ SEM of 7-9 days old *L. sericata* adults based on physiology (i.e., sex and ovarian status) to different doses of IND when paired against a control in a Y-tube olfactometer.

Dose (μ g)	Physiology (n ¹)	Percentage response to IND $\pm$ SEM	Percentage response to control $\pm$ SEM
5.00	GF (20)	64.67 $\pm$ 7.93	35.33 $\pm$ 7.93
	NG (20)	64.11 $\pm$ 9.36	35.99 $\pm$ 9.36
	M (20)	66.98 $\pm$ 6.39	33.02 $\pm$ 6.39
0.50	GF (20)	42.83 $\pm$ 8.05	57.17 $\pm$ 8.05
	NG (20)	57.97 $\pm$ 9.89	42.03 $\pm$ 9.89
	M (20)	49.71 $\pm$ 10.95	50.29 $\pm$ 10.95
0.05	GF (20)	47.88 $\pm$ 9.84	53.12 $\pm$ 9.84
	NG (20)	38.19 $\pm$ 9.36	61.81 $\pm$ 9.36
	M (20)	34.55 $\pm$ 9.75	65.46 $\pm$ 9.75

¹n = replicates

Table 5. Per cent response $\pm$ SEM of 7-9 days old *L. sericata* adults based on physiology (i.e., sex and ovarian status) to different doses of PAA when paired against a control in a Y-tube olfactometer.

Doses (μ g)	Physiology (n ¹)	Percentage response to PAA $\pm$ SEM	Percentage response to control $\pm$ SEM
10.0	GF (20)	72.08 $\pm$ 9.41	28.92 $\pm$ 9.41
	NG (20)	58.13 $\pm$ 7.96	41.87 $\pm$ 7.96
	M (20)	41.12 $\pm$ 10.13	58.88 $\pm$ 10.13
1.0	GF ((20)	55.88 $\pm$ 9.36	44.12 $\pm$ 9.36
	NG (20)	55.35 $\pm$ 8.43	44.65 $\pm$ 8.43
	M (20)	49.82 $\pm$ 9.36	50.18 $\pm$ 9.36
0.1	GF (20)	59.93 $\pm$ 6.53	40.07 $\pm$ 6.53
	NG (20)	62.41 $\pm$ 11.07	37.59 $\pm$ 11.07
	M (20)	42.805 $\pm$ 10.32	57.20 $\pm$ 10.32

¹n = replicates

Table 6. Per cent response $\pm$ SEM of 7-9 days old *L. sericata* adults based on physiology (i.e., sex and ovarian status) to different doses of IBA when paired against a control in a Y-tube olfactometer.

Doses (μ g)	Physiology (n ¹)	Percentage response to IBA $\pm$ SEM	Percentage response to control $\pm$ SEM
1.00	GF (20)	54.98 $\pm$ 8.84	44.02 $\pm$ 8.84
	NG (20)	68.77 $\pm$ 8.08	31.23 $\pm$ 8.08
	M (20)	68.30 $\pm$ 9.63	31.70 $\pm$ 9.63
0.10	GF (20)	50.02 $\pm$ 9.73	49.98 $\pm$ 9.73
	NG (20)	70.91 $\pm$ 8.03	29.09 $\pm$ 8.03
	M (20)	35.46 $\pm$ 10.16	64.54 $\pm$ 10.16
0.01	GF (20)	62.68 $\pm$ 6.95	47.32 $\pm$ 6.95
	NG (20)	65.05 $\pm$ 7.29	34.95 $\pm$ 7.29
	M (20)	30.05 $\pm$ 9.64	69.95 $\pm$ 9.64

¹n = replicates

Figure Captions

Figure 1. Mean probability of response $\pm$ SEM of gravid (n = 20) and male (n = 20) *L. sericata* adults (7-9-d-old) to DMDS at 0.0050 μ g DMDS versus a control in a Y-tube olfactometer. Different letters indicate significant difference ($P < 0.05$). GF = gravid female, M = male.

Figure 2. Mean probability of response $\pm$ SEM of *L. sericata* adults (7-9-d-old) (n = 60 per dose) to different IND doses versus a control in a Y-tube olfactometer. Different letters above same colour columns indicate significant difference ($P < 0.05$).

Figure 3. Mean probability of response $\pm$ SEM of all *L. sericata* adults (7-9-d-old) to different PAA doses versus a control in a Y-tube olfactometer; a) overall mean response for all fly categories (n = 180) and doses combined; b) gravid females (n = 60) to all PAA doses combined; c) non-gravid females (n = 60) to all PAA doses combined; d) gravid females (n = 20) to 10.0 μ g PAA vs control. Different letters over columns in each figure indicate significant difference ($P < 0.05$). NG = non gravid; GF = gravid female.

Figure 4. Mean probability of response $\pm$ SEM for *L. sericata* adults (7-9-d-old) to different IBA doses versus a control in a Y-tube olfactometer; a) comparison of mean probability of male (n = 60) versus gravid female (n = 60) *L. sericata* response across IBA doses vs control; b) comparison of mean probability of male (n = 60) versus non-gravid females (n = 60) across IBA doses vs control; c) mean probability of male (n = 20) *L. sericata* to IBA at 0.01 μ g vs control; d) Probability of non-gravid female (n = 20) *L. sericata* in response to IBA at dose 0.10 μ g vs control. Different letters above the columns in the figure indicate significance ($P < 0.05$). M = males, NG = non-gravid adult, GF = gravid adult.

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Figure 1

Figure 2

Figure 3a

Figure 3b

Figure 3c

Figure 3d

Figure 4a

Figure 4b

Figure 4c

Figure 4d