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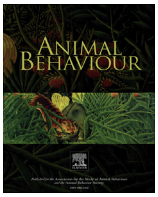

Responses of *Lucilia sericata* (Diptera: Calliphoridae) to compounds from microbial decomposition of larval resources

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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 for their offspring. However, these responses are partially governed by sex and physiological state (e.g. gravid and nongravid) of the fly and concentration of the VOCs. We examined the responses of 7–9-day-old *L. sericata* adults to dimethyldisulphide, indole, isobutylamine and phenylacetic acid in a Y-tube olfactometer. These VOCs are associated with vertebrate decomposition, carrion-mimicking flowers as well as fly-attracting bacteria. Our results demonstrate a relationship between sex and physiological state with regard 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.

Historically, research on carrion as an ephemeral resource has predominately focused on the ecological interactions occurring on the resource after its death as related to nutrient recycling, as well as the associated population dynamics of those organisms responsible for this ecosystem service (Barton et al., 2013; Barton, Weaver, & Manning, 2014). Consequently, little is known about the mechanisms associated with ephemeral resource-regulating attraction, colonization and utilization of these resources (Tomberlin, Mohr, Benbow, Tarone, & VanLaerhoven, 2011). Identifying these mechanisms will provide greater insight into how public information (sensu lato, ‘information about the quality of a patch that can be obtained by observing the foraging success of other individuals in that patch’; Valone, 1989, page 357) influences the behaviour of individuals competing for these resources (Benbow, Tomberlin, & Tarone, 2015; Tomberlin, Benbow, Tarone, & Mohr, 2011). Indeed, recent efforts suggest that microbes serve as a conduit through which many of these cues are released into the environment.

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

The blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) has served as a model for evaluating fly–microbe interactions commonly occurring on carrion and during myiasis (Brodie, Wong, et al., 2015; Ma et al., 2012; Tomberlin et al., 2012). The species is known to produce antibiotic molecules and alter microbial communities on its larval resources (Bexfield, Nigam, Thomas, & Ratcliffe, 2004; Cazander, van Veen, Bernards, & Jukema, 2009; Cerovsky et al., 2010; Daeschlein, Mumcuoglu, Assadian, Hoffmeister, & Kramer, 2007; Jaklic, Lapanje, Zupancic, Smrke, &

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Gunde-Cimerman, 2008; Sherman, Hall, & Thomas, 2000). It is also associated with bacteria that, once introduced to a resource, can alter microbial communities to the benefit of the insect (Erdmann & Khalil, 1986; Sherman et al., 2000; Singh et al., 2015).

Recently, it has been proposed that *L. sericata* relies on bacterial cues to guide decisions regarding attraction to and colonization of larval resources, such that a mutant strain of the commensal bacteria *Proteus mirabilis* (Singh et al., 2015), which is deficient in the ability to swarm (a quorum sensing response) and is rescued by putrescine (a quorum-sensing molecule), is less attractive to *L. sericata* (Ma et al., 2012). This mutant strain differentially produces several known fly attractants compared to its parent strain, and a fly's response is regulated by its sex and physiological state (i.e. gravid versus nongravid) (Tomberlin et al., 2012). This observation is not surprising, given that gravid females are more attracted to fresh carrion, while nongravid adults are more attracted to decomposed vertebrate carrion (Mohr & Tomberlin, 2014) and that carrion is not a necessary resource for males. Taken together, these observations suggest that microbial metabolites can provide information to blow flies evaluating 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, dimethyldisulphide (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 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, et al., 2015; Frederickx, Dekeirsschieter, Verheggen, et al., 2012) and are released from decomposing remains (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; Statheropoulos, Agapiou, Spiliopoulou, Pallis, & Sianos, 2007). Typically, blow flies colonize resources quickly, such that some sulphur compounds (e.g. DMDS, which is more pronounced late in the decomposition process) would likely be a signal that the resource is unsuitable for colonization as it has probably 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 nongravid 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 from flies.

In the present study, we tested the hypothesis that responses of *L. sericata* to carrion-associated VOCs at different doses differ based on the sex and ovarian status of individuals. We predicted that, while all VOCs are cues of decomposition, each molecule would yield a distinct behavioural impact on different sexes of *L. sericata* and on females of different ovarian state. 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 are 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 affect 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. PAA is associated with blow fly larvae (Bromel, Duh, & Erdmann, 1983) and is a known antibiotic molecule that benefits fly larvae by suppressing pathogenic bacteria (Erdmann, 1987). It is structurally similar to phenylethyl alcohol, a molecule that affects fly attraction and that differs between mutant and wild-type strains of *P. mirabilis* (Ma et al., 2012). In addition, DMDS, skatole (a 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 & Shuttleworth, 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 *L. sericata*.

METHODS

Colony Maintenance

Lucilia sericata adults from a strain serving as a model population (Sze et al., 2012) were maintained in 30 × 30 × 30 cm BioQuip bug dorms (Bioquip Products, Rancho Dominguez, CA, U.S.A.) maintained in a rearing room at 27 °C, 60–70% relative humidity, and on a 14:10 h light:dark cycle. Adult flies were fed bovine liver blood ad libitum for the first 5 days postemergence in order to stimulate ovarian development. Flies were then provided sugar and water until testing at 7–9 days old.

To maintain the colonies, flies remaining in the cages after day 9 were provided bovine liver in 88.7 ml plastic bath cups (Walmart, Inc., Bentonvillle, AR, U.S.A.) for 2–3 days to stimulate oviposition on 50 g of 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, U.S.A. (Mohr & Tomberlin, 2014), and because of our desire to work with a model species in decomposition ecology research, we used a dual-choice olfactometer 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 for biotic factors (e.g. fly age, fly source population, infection status) and abiotic factors (e.g. temperature, humidity, lighting) 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, U.S.A.) 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, U.S.A.) to be 0.5 m/s (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, U.S.A.) 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 conducted at 20–23 °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. We applied 10 μl of each tested solution to a filter paper ($\theta = 22.5°$, radius 4.5 cm) (Fisher Scientific, Waltham, MA, U.S.A.) and allowed 5 min for the solvent to evaporate in the ventilation hood before putting it into a container. We rotated the treatment location in the arms of the Y-tube 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 3 min (Brundage, 2012).

We diluted DMDS (Sigma Aldrich, Basic Materials, St Louis, MO, U.S.A., purity ≥99.0%) with acetone to concentrations of 0.0005 μg, 0.005 μg, 0.05 μg and 0.25 μg. Using the same methods, we prepared IND (Sigma Aldrich, purity ≥99.0%) at doses of 0.05 μg, 0.5 μg and 5 μg, PAA (Sigma Aldrich, purity ≥99.0%) at doses of 0.1 μg, 1 μg and 10 μg, and IBA (Sigma Aldrich, purity ≥99.0%) at doses of 0.01 μg, 0.1 μg and 1 μg. 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, et al., 2012).

We tested 20 males, 20 gravid females and 20 nongravid females 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 ($N \leq 1$/replicate of any treatment), it was replaced and excluded from the analysis. Total residence time in each arm was recorded for each fly.

Statistical Analysis

We analysed the residence time data with PROC MIXED v.9.3 (SAS Institute, Cary, NC, U.S.A.) using a full factorial design. The statistical model tested fixed factors that included the physiology (which indicates the type of the fly based on sex and ovarian development), response to treatment (chemical dose versus control) and dose (of compounds) as well as interactions. We used least square means to evaluate statistical differences among treatments ($P < 0.05$). In addition, we categorized flies as gravid females, nongravid females and males, as defined in Mohr and Tomberlin (2014).

Ethical Note

Fly colonies were carefully managed. Flies were maintained in a controlled environment within our laboratory and 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 indicated that each VOC compound exhibited a distinct impact on the flies' behaviour. Behavioural responses of flies to each compound were dependent on concentrations of the tested compounds and fly physiological state (gravidity and sex). Accordingly, we evaluate the results separately for each molecule.

DMDS

This study identified a three-way interaction between dose of DMDS, response to test (treatment versus control) and physiology (male, gravid and nongravid) (Table 1). Fly responses to DMDS were marginally different in response to control and among doses of DMDS and physiological classes (sex and ovarian status) ($F_{6,480} = 2.08$, $P = 0.0537$; Tables 1,2). Percentage response ± SEM of 7–9-day-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 0.005 μg ($F_{6,480} = 2.92$, $P = 0.0037$), gravid females were repelled (73%) while males were attracted (64%) to the compound (Fig. 1, Tables 2,3). However, response to 0.05 μg of DMDS did not differ based on the sex or ovarian status of individuals. 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 (nongravid: 0.25 μg; male: 0.005 μg) with females preferring to orient towards 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 ($F_{2,342} = 7.17$, $P = 0.0009$; Table 2). Percentage response ± SEM of 7–9-day-old *L. sericata* based on physiology (sex and ovarian status) to different doses of IND can be found in Table 4 and Fig. 2. Generally, for both sexes, the compound was attractive (64–66%) at the highest dose tested and repellent (53–65%) at the lowest dose, with only nongravid females preferring (57%) the intermediate dose.

Table 1
Type III tests of fixed effects of fly physiology on response to different volatile organic compounds (VOCs) at different concentrations in a Y-tube olfactometer

Tested VOCs	Effect	*df*	*F*	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

DMDS: dimethyldisulphide; IND: indole; PAA: phenylacetic acid; IBA: isobutylamine.

[1] Dose: response by flies to different doses.

[2] Physiology: sex and ovarian status of flies.

[3] Response to treatment: response to chemical dose versus control.

Table 2
Least squares means of the impact of fly physiology on responses to volatile organic compounds (VOCs) at different concentrations in a Y-tube olfactometer

VOCs	Effect	Dose (µg)	Physiology	Response to treatment	vs	Dose (µg)	Physiology	Response to treatment	Pr > $\lvert t \rvert$
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	ASC	Control	0.0091
	Dose * response to treatment	0.05	ASC	Compound	vs	5	ASC	Compound	0.0067
	Dose * response to treatment	5	ASC	Control	vs	5	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	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	M	Control	0.0027
	Dose * physiology * response to treatment	0.1	NG	Control	vs	0.1	NG	Compound	0.0059
	Dose * physiology * response to treatment	0.01	M	Control	vs	0.01	M	Compound	0.0025

DMDS: dimethyldisulphide; IND: indole; PAA: phenylacetic acid; IBA: isobutylamine; GF: gravid female; NG: nongravid female; M: male.

[1] Dose: response by flies to different doses.

[2] Physiology: sex and ovarian status of flies.

[3] Response to treatment: response to chemical dose versus control.

[4] ASC: data for all sex categories combined.

[5] ADC: data for all doses combined.

Table 3
Percentage response ± SE of *L. sericata* adults (7–9 days old) based on physiology (i.e. sex and ovarian status) to different doses of dimethyldisulphide (DMDS) when paired against a control in a Y-tube olfactometer

Doses (µg)	Physiology (n[1])	Percentage response ± SE	
		DMDS	Control
0.25	GF (20)	55.88±8.96	45.12±8.96
	NG (20)	66.18±16.40	33.82±16.40
	M (20)	45.42±10.10	54.58±10.10
0.05	GF (20)	46.41±9.69	53.59±9.69
	NG (20)	44.67±9.32	55.33±9.32
	M (20)	46.72±10.32	53.28±10.32
0.005	GF (20)	26.38±8.61	73.62±8.61
	NG (20)	47.67±8.32	62.33±8.32
	M (20)	64.57±9.42	35.43±9.42
0.0005	GF (20)	49.81±8.26	50.19±8.26
	NG (20)	43.41±8.10	56.59±8.10
	M (20)	49.34±10.04	50.66±10.04

GF: gravid female; NG: nongravid female; M: male.

[1] n = replicates.

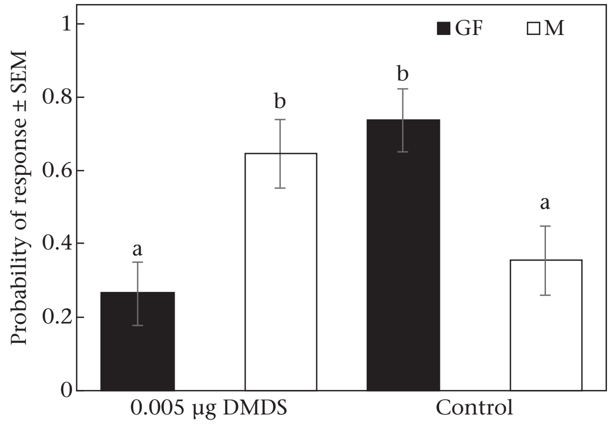

Figure 1. Mean probability of response ± SEM of *L. sericata* adults (7–9 days old) to dimethyldisulphide (DMDS) at 0.005 µg DMDS versus a control in a Y-tube olfactometer. Different letters above bars indicate a significant difference ($P < 0.05$). GF: gravid female ($N = 20$); M: male ($N = 20$).

Table 4
Percentage response ± SE of *L. sericata* adults (7–9 days old) based on physiology (i.e. sex and ovarian status) to different doses of indole (IND) when paired against a control in a Y-tube olfactometer

Dose (µg)	Physiology (n[1])	Percentage response ± SE	
		IND	Control
5	GF (20)	64.67±7.93	35.33±7.93
	NG (20)	64.11±9.36	35.99±9.36
	M (20)	66.98±6.39	33.02±6.39
0.5	GF (20)	42.83±8.05	57.17±8.05
	NG (20)	57.97±9.89	42.03±9.89
	M (20)	49.71±10.95	50.29±10.95
0.05	GF (20)	47.88±9.84	53.12±9.84
	NG (20)	38.19±9.36	61.81±9.36
	M (20)	34.55±9.75	65.46±9.75

GF: gravid female; NG: nongravid female; M: male.

[1] n = replicates.

PAA

Flies showed a sexually dimorphic and physiologically dependent response to PAA. Sex and ovarian status affected fly response to PAA ($F_{2,358} = 4.4$, $P = 0.0130$; Table 2). Percentage response ± SEM of 7–9-day-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. Females showed significant attraction to PAA within the range of doses tested (nongravid females: 62% to 0.1 µg; gravid females: 72% to 10 µg; Table 2, Fig. 3).

IBA

Flies showed a complex response to IBA. Responses differed significantly between control and IBA treatments ($F_{2,342} = 2.45$, $P = 0.0459$; Table 2). Relative to males, responses to the lower doses of IBA were greater for gravid females (25% greater) and nongravid

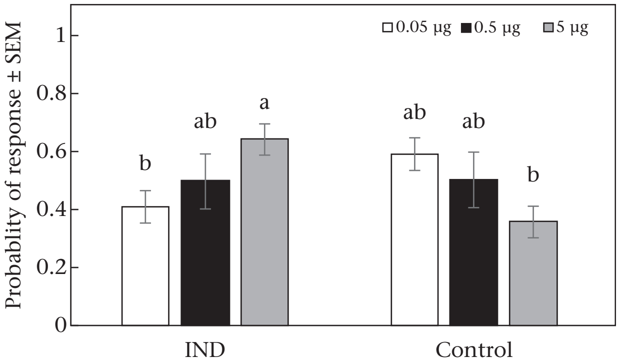

Figure 2. Mean probability of response ± SEM of *L. sericata* adults (7–9 days old) ($N = 60$ per dose) to different indole (IND) doses versus a control in a Y-tube olfactometer. Different letters above bars with the same colour (dose) indicate a significant difference ($P < 0.05$).

Table 5

Percentage response ± SE of *L. sericata* adults (7–9 days old) based on physiology (i.e. sex and ovarian status) to different doses of phenylacetic acid (PAA) when paired against a control in a Y-tube olfactometer

Doses (μg)	Physiology (n^1)	Percentage response ± SE	
		PAA	Control
10	GF (20)	72.08±9.41	28.92±9.41
	NG (20)	58.13±7.96	41.87±7.96
	M (20)	41.12±10.13	58.88±10.13
1	GF (20)	55.88±9.36	44.12±9.36
	NG (20)	55.35±8.43	44.65±8.43
	M (20)	49.82±9.36	50.18±9.36
0.1	GF (20)	59.93±6.53	40.07±6.53
	NG (20)	62.41±11.07	37.59±11.07
	M (20)	42.81±10.32	57.20±10.32

GF: gravid female; NG: nongravid female; M: male.

[1] n = replicates.

females (35% greater); nongravid females were attracted (~70%) to IBA at 0.1 μg (Fig. 4), whereas males showed significant repellence (~70%) to IBA at 0.01 μg (Fig. 4). Percentage response ± SEM of 7–9-day-old *L. sericata* of different sexes to different IBA doses are presented in Table 6. Generally, gravid and nongravid females appeared to orient towards 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 findings suggest that *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 and physiological state (i.e. gravid versus nongravid) 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 results indicate that 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 results suggest that different individuals of the same species (male, gravid female, nongravid female) would be attracted to different concentrations of fly trap components. These results could also be useful when assessing VOC 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 active decay (Dekeirsschieter et al., 2009) and has previously been demonstrated to attract *L. sericata* (Frederickx, Dekeirsschieter, Verheggen, et al., 2012). However, dose responses and associated preferences of *L. sericata* to the tested compounds as related to physiological status were not reported. We determined here that responses of flies to DMDS differed significantly 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 has probably already been colonized and is 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 is important 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 that 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 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 as previously discussed, is commensal with *L. sericata* (Erdmann, 1987). This compound produced by *P. mirabilis* functions as an antibiotic (Erdmann, 1987) and potentially aids in *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 differentially produced by mutant and wild-type *P. mirabilis* (Tomberlin et al., 2012) that affects *L. sericata* behaviour. Similar compounds are known to be present as VOCs produced by blow fly larvae during 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 whether 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 towards 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 produced during decomposition of vertebrate remains and is a known fly attractant (Dekeirsschieter et al., 2009). This molecule, which is highly differentially produced between mutant and wild-type bacteria, also affects *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 as 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 quorum-sensing 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, nongravid 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 except at the highest

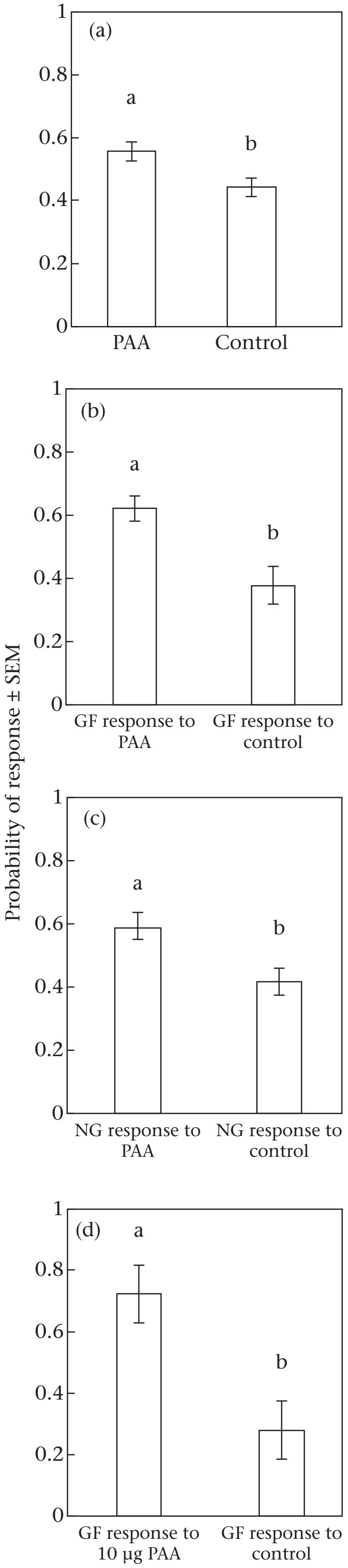

Figure 3. Mean probability of response ± SEM of all *L. sericata* adults (7–9 days old) to different phenylacetic acid (PAA) doses versus a control in a Y-tube olfactometer: (a) overall response for all fly categories ($N = 180$) and doses combined; (b) gravid female ($N = 60$) response to all PAA doses combined; (c) nongravid female ($N = 60$) response to all PAA doses combined; (d) gravid female ($N = 20$) response to 10 μg PAA versus control. Different letters above bars indicate a significant difference ($P < 0.05$). GF: gravid female; NG: nongravid female.

Table 6
Percentage response ± SE of *L. sericata* adults (7–9 days old) based on physiology (i.e. sex and ovarian status) to different doses of isobutylamine (IBA) when paired against a control in a Y-tube olfactometer

Doses (μg)	Physiology (n^1)	Percentage ± SE response	
		IBA	Control
1	GF (20)	54.98±8.84	44.02±8.84
	NG (20)	68.77±8.08	31.23±8.08
	M (20)	68.30±9.63	31.70±9.63
0.1	GF (20)	50.02±9.73	49.98±9.73
	NG (20)	70.91±8.03	29.09±8.03
	M (20)	35.46±10.16	64.54±10.16
0.01	GF (20)	62.68±6.95	47.32±6.95
	NG (20)	65.05±7.29	34.95±7.29
	M (20)	30.05±9.64	69.95±9.64

GF: gravid female; NG: nongravid female; M: male.

[1] n = replicates.

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 be important in guiding the decisions of blow flies of all classes.

Interestingly, IBA may also play a role in a less appreciated aspect of blow fly biology, pollination (Heath, 1982). Several studies have demonstrated the role of blow flies in pollination of 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 they 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 we used to analyse the data 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 use 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 influencing fly

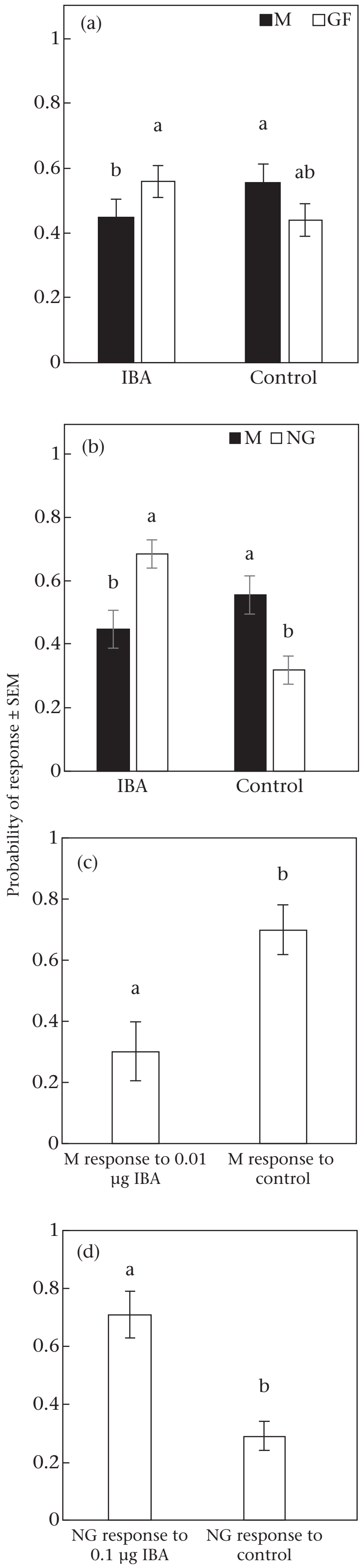

Figure 4. Mean probability of response ± SEM for *L. sericata* adults (7–9 days old) to different isobutylamine (IBA) doses versus a control in a Y-tube olfactometer: (a) male ($N = 60$) versus gravid female ($N = 60$) response across IBA doses versus control; (b) male ($N = 60$) versus nongravid female ($N = 60$) response across IBA doses versus control; (c) male ($N = 20$) response to IBA at 0.01 μg versus control; (d) nongravid female ($N = 20$) response to IBA at dose 0.1 μg versus control. Different letters above bars indicate a significant difference ($P < 0.05$). M: male; GF: gravid female; NG: nongravid female.

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), interpretation of evidence in forensic entomology (Tomberlin, Benbow, et al., 2011; Tomberlin, Mohr, et al., 2011) and the role of filth-feeding flies in pollination biology (Gardener & Gillman, 2002), and it can advance our understanding of how flies influence the proliferation and distribution of pathogens into the environment.

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References

Ashworth, J. R., & Wall, R. (1994). Responses of the sheep blowflies *Lucilia sericata* and *L. cuprina* to odour and the development of semiochemical baits. *Medical and Veterinary Entomology, 8*(4), 303–309. http://dx.doi.org/10.1111/j.1365-2915.1994.tb00093.x.

Barnes, K. M., Gennard, D. E., & Dixon, R. A. (2010). An assessment of the antibacterial activity in larval excretion/secretion of four species of insects recorded in association with corpses, using *Lucilia sericata* Meigen as the marker species. *Bulletin of Entomological Research, 100*, 635–640. http://dx.doi.org/10.1017/S0007485309990071X.

Barton, P. S., Cunningham, S. A., Macdonald, B. C. T., McIntyre, S., Lindenmayer, D. B., & Manning, A. D. (2013). Species traits predict assemblage dynamics at ephemeral resource patches created by carrion. *PLoS One, 8*(1), e53961. http://dx.doi.org/10.1371/journal.pone.0053961.

Barton, P., Weaver, H., & Manning, A. (2014). Contrasting diversity dynamics of phoretic mites and beetles associated with vertebrate carrion. *Experimental and Applied Acarology, 63*(1), 1–13. http://dx.doi.org/10.1007/s10493-013-9758-7.

Benbow, M. E., Tomberlin, J. K., & Tarone, A. M. (2015). *Carrion ecology, evolution, and their applications*. Boca Raton, FL: CRC Press.

Bexfield, A., Nigam, Y., Thomas, S., & Ratcliffe, N. A. (2004). Detection and partial characterisation of two antibacterial factors from the excretions/secretions of the medicinal maggot *Lucilia sericata* and their activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Microbes and Infection, 6*(14), 1297–1304. http://dx.doi.org/10.1016/j.micinf.2004.08.011.

Brodie, B. S., Smith, M. A., Lawrence, J., & Gries, G. (2015). Effects of floral scent, color and pollen on foraging decisions and oocyte development of common green bottle flies. *PLoS One, 10*(12), e0145055. http://dx.doi.org/10.1371/journal.pone.0145055.

Brodie, B. S., Wong, W. H. L., VanLaerhoven, S., & Gries, G. (2015). Is aggregated oviposition by the blow flies *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae) really pheromone-mediated? *Insect Science, 22*, 651–660. http://dx.doi.org/10.1111/1744-7917.12160.

Bromel, M., Duh, F. M., & Erdmann, G. R. (1983). Bacteria associated with the screwworm fly *Cochliomyia hominivorax* (Coquerel) and their metabolites. *Endocytobiology, 2*, 791–800.

Brundage, A. L. (2012). *Fitness effects of colonization time of* Chrysomya rufifacies *and* Cochliomyia macellaria*, and their response to intra- and inter-specific eggs and egg-associated microbes* (Ph.D. Dissertation). College Station, TX: Texas A&M University.

Brundage, A. L., Benbow, M. E., & Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. *Ecological Entomology, 39*, 539–547. http://dx.doi.org/10.1111/een.12128.

Carter, D. O., Metcalf, J. A., Bibat, A., & Knight, R. (2015). Seasonal variation of postmortem microbial communities. *Forensic Science Medicine, and Pathology, 11*, 202–207. http://dx.doi.org/10.1007/s12024-015-9667-7.

Cazander, G., van Veen, K. E., Bernards, A. T., & Jukema, G. N. (2009). Do maggots have an influence on bacterial growth? A study on the susceptibility of strains of six different bacterial species to maggots of *Lucilia sericata* and their excretions/secretions. *Journal of Tissue Viability, 18*(3), 80–87. http://dx.doi.org/10.1016/j.jtv.2009.02.005.

Cerovsky, V., Zdarek, J., Fucik, V., Monincova, L., Voburka, Z., & Bem, R. (2010). Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. *Cellular and Molecular Life Sciences, 67*(3), 455–466. http://dx.doi.org/10.1007/s00018-009-0194-0.

Chaudhury, M. F., Zhu, J. J., & Skoda, S. R. (2016). Bacterial volatiles attract gravid secondary screwworms (Diptera: Calliphoridae). *Journal of Economic Entomology*. http://dx.doi.org/10.1093/jee/tov390. Advance online publication.

Currah, L., & Ockendon, D. J. (1983). Onion pollination by blowflies and honeybees in large cages. *Annals of Applied Biology, 103*(3), 497–506. http://dx.doi.org/10.1111/j.1744-7348.1983.tb02789.x.

Daeschlein, G., Mumcuoglu, K. Y., Assadian, O., Hoffmeister, B., & Kramer, A. (2007). In vitro antibacterial activity of *Lucilia sericata* maggot secretions. *Skin Pharmacology and Physiology, 20*(2), 112–115. http://dx.doi.org/10.1159/000097983, 000097983 [pii].

Dekeirsschieter, J., Frederickx, C., Lognay, G., Brostaux, Y., Verheggen, F. J., & Haubruge, E. (2013). Electrophysiological and behavioral responses of *Thanatophilus sinuatus* Fabricius (Coleoptera: Silphidae) to selected cadaveric volatile organic compounds. *Journal of Forensic Sciences, 58*, 917–923.

Dekeirsschieter, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., et al. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic Science International, 18*, 46–53. http://dx.doi.org/10.1016/j.forsciint.2009.03.034.

Dethier, V. G. (1947). *Chemical insect attractants and repellents*. Philadelphia, PA: Blakiston.

Dethier, V. G. (1956). Repellents. *Annual Review of Entomology, 1*, 181–202. http://dx.doi.org/10.1146/annurev.en.01.010156.001145.

Erdmann, G. R. (1987). Antibacterial action of myiasis-causing flies. *Parasitology Today, 3*(7), 214–216. http://dx.doi.org/10.1016/0169-4758(87)90062-7.

Erdmann, G. R., & Khalil, S. K. W. (1986). Isolation and identification of two antibacterial agents produced by a strain of *Proteus mirabilis* isolated from larvae of the screwworm (*Cochliomyia hominivorax*) (Diptera: Calliphoridae). *Journal of Medical Entomology, 23*, 208–211. http://dx.doi.org/10.1093/jmedent/23.2.208.

Flores, M., Longnecker, M., & Tomberlin, J. K. (2014). Effects of temperature and tissue type on *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart) development. *Forensic Science International, 245*, 24–29. http://dx.doi.org/10.1016/j.forsciint.2014.09.023.

Forbes, S. L., & Perrault, K. A. (2014). Decomposition odour profiling in the air and soil surrounding vertebrate carrion. *PLoS One, 9*(4), e95107. http://dx.doi.org/10.1371/journal.pone.0095107.

Frederickx, C., Dekeirsschieter, J., Brostaux, Y., Wathelet, J.-P., Verheggen, F. J., & Haubruge, E. (2012). Volatile organic compounds released by blowfly larvae and pupae: new perspectives in forensic entomology. *Forensic Science International, 219*, 215–220. http://dx.doi.org/10.1016/j.forsciint.2012.01.007.

Frederickx, C., Dekeirsschieter, J., Verheggen, F. J., & Haubruge, E. (2012). Responses of *Lucilia sericata* Meigen (Diptera: Calliphoridae) to cadaveric volatile organic compounds. *Journal of Forensic Sciences, 57*, 386–390. http://dx.doi.org/10.1111/j.1556-4029.2011.02010.x.

Gardener, M. C., & Gillman, M. P. (2002). The taste of nectar: a neglected area of pollination ecology. *Oikos, 98*(3), 552–557.

Hayward, N. J., Jeavons, T. H., & Nicholson, A. J. (1977). Methyl mercaptan and dimethyl disulfide production from methionine by *Proteus* species detected by head-space gas-liquid chromatography. *Journal of Clinical Microbiology, 6*, 187–194.

Heath, A. (1982). Beneficial aspects of blowflies (Diptera: Calliphoridae). *New Zealand Entomologist, 7*(3), 343–348.

Hobson, R. P. (1936). Sheep blow fly investigations. III. Observations on the chemotropism of *Lucilia sericata*. *Annals of Applied Biology, 23*, 845–851. http://dx.doi.org/10.1111/j.1744-7348.1936.tb06130.x.

Howlett, B. G. (2012). Hybrid carrot seed crop pollination by the fly *Calliphora vicina* (Diptera: Calliphoridae). *Journal of Applied Entomology, 136*(6), 421–430. http://dx.doi.org/10.1111/j.1439-0418.2011.01665.x.

Huntington, T. E., & Higley, L. G. (2010). Decomposed flesh as a vitellogenic protein source for the forensically important *Lucilia sericata* (Diptera: Calliphoridae). *Journal of Medical Entomology, 47*(3), 482–486. http://dx.doi.org/10.1603/Me09052.

Hyde, E. R., Haarmann, D. P., Lynne, A. M., Bucheli, S. R., & Petrosino, J. F. (2013). The living dead: bacterial community structure of a cadaver at the onset and end of the bloat stage of decomposition. *PLoS One, 8*(10), e77733. http://dx.doi.org/10.1371/journal.pone.0077733.

Hyde, E. R., Haarmann, D. P., Petrosino, J. F., Lynne, A. M., & Bucheli, S. R. (2014). Initial insights into bacterial succession during human decomposition. *International Journal of Legal Medicine, 123*(3), 661–671. http://dx.doi.org/10.1007/s00414-014-1128-4.

Jaklic, D., Lapanje, A., Zupancic, K., Smrke, D., & Gunde-Cimerman, N. (2008). Selective antimicrobial activity of maggots against pathogenic bacteria. *Journal of Medical Microbiology, 57*(5), 617–625. http://dx.doi.org/10.1099/jmm.0.47515-0.

Jürgens, A., & Shuttleworth, A. (2015). Carrion and dung mimicry in plants. In M. E. Benbow, J. K. Tomberlin, & A. M. Tarone (Eds.), *Carrion ecology, evolution, and their applications* (pp. 361–386). Boca Raton, FL: CRC Press.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell Biology, 78*(1), 80–88. http://dx.doi.org/10.1046/j.1440-1711.2000.00886.x.

Kasper, J., Mumm, R., & Ruther, J. (2012). The composition of carcass volatile profiles in relation to storage time and climate conditions. *Forensic Science International, 223*(1), 64–71. http://dx.doi.org/10.1016/j.forsciint.2012.08.001.

Lee, J., Jayaraman, A., & Wood, T. K. (2007). Indole is an inter-species biofilm signal mediated by SdiA. *BMC Microbiology, 7*, 42. http://dx.doi.org/10.1186/1471-2180-7-42.

Lee, J., Zhang, X.-S., Hegde, M., Bentley, W. E., Jayaraman, A., & Wood, T. K. (2008). Indole cell signaling occurs primarily at low temperatures in *Escherichia coli*. *International Society for Microbial Ecology Journal, 2*, 1007–1023. http://dx.doi.org/10.1038/ismej.2008.54.

Libert, S., Zwiener, J., Chu, X., VanVoorhies, W., Roman, G., & Pletcher, S. D. (2007). Regulation of *Drosophila* life span by olfaction and food-derived odors. *Science, 315*(5815), 1133. http://dx.doi.org/10.1126/science.1136610.

Ma, Q., Fonseca, A., Liu, W. Q., Fields, A. T., Pimsler, M. L., Spindola, A. F., et al. (2012). *Proteus mirabilis* interkingdom swarming signals attract blow flies. *International Society for Microbial Ecology Journal, 6*(7), 1356–1366. http://dx.doi.org/10.1038/ismej.2011.210.

Metcalf, J. L., Wegener Parfrey, L., Gonzalez, A., Lauber, C. L., Knights, D., Ackermann, G., et al. (2013). A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. *Elife, 2*, e01104. http://dx.doi.org/10.7554/eLife.01104.

Mikkelsen, L. L., Naughton, P. J., Hedemann, M. S., & Jensen, B. B. (2004). Effects of physical properties of feed on microbial ecology and survival of *Salmonella enterica* serovar Typhimurium in the pig gastrointestinal tract. *Applied and Environmental Microbiology, 70*, 3485–3492. http://dx.doi.org/10.1128/aem.70.6.3485-3492.2004.

Mohr, R. M., & Tomberlin, J. K. (2014). Environmental factors affecting early carcass attendance by four species of blow flies (Diptera: Calliphoridae) in Texas. *Journal of Medical Entomology, 51*, 702–708. http://dx.doi.org/10.1603/ME13149.

Paczkowski, S., Nicke, S., Ziegenhagen, H., & Schütz, S. (2015). Volatile emission of decomposing pig carcasses (*Sus scrofa domesticus* L.) as an indicator for the postmortem interval. *Journal of Forensic Sciences, 60*(Suppl.), S130–S137. http://dx.doi.org/10.1111/1556-4029.12638.

Paczkowski, S., & Schütz, S. (2011). Post-mortem volatiles of vertebrate tissue. *Applied Microbiology and Biotechnology, 91*(4), 917–935. http://dx.doi.org/10.1007/s00253-011-3417-x.

Pechal, J. L., Crippen, T. L., Benbow, M. E., Tarone, A. M., Dowd, S., & Tomberlin, J. K. (2013). The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. *International Journal of Legal Medicine, 128*, 193–205. http://dx.doi.org/10.1007/s00414-013-0872-1.

Richardson, M. (1966). Studies on the biogenesis of some simple amines and quaternary ammonium compounds in higher plants: isoamylamine and isobutylamine. *Phytochemistry, 5*(1), 23–30. http://dx.doi.org/10.1016/S0031-9422(00)85078-5.

Sasaki-Imamura, T., Yano, A., & Yoshida, Y. (2010). Production of indole from l-Tryptophan and effects of these compounds on biofilm formation by *Fusobacterium nucleatum* ATCC 25586. *Applied and Environmental Microbiology, 76*(13), 4260–4268. http://dx.doi.org/10.1128/aem.00166-10.

Sherman, R. A., Hall, M. J. R., & Thomas, S. (2000). Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annual Review of Entomology, 45*, 55–81.

Shorrocks, B., & Bingley, B. (1994). Priority effects and species coexistence: experiments with fungal-breeding *Drosophila*. *Journal of Animal Ecology, 63*, 799–806. http://dx.doi.org/10.2307/5257.

Singh, B., Crippen, T., Zheng, L. Y., Fields, A. T., Yu, Z. N., Ma, Q., et al. (2015). A metagenomic assessment of the bacteria associated with *Lucilia sericata* and *Lucilia cuprina* (Diptera: Calliphoridae). *Applied Microbiology and Biotechnology, 99*(2), 869–883. http://dx.doi.org/10.1007/s00253-014-6115-7.

Smith, B. N., & Meeuse, B. J. D. (1966). Production of volatile amines and skatole at anthesis in some *Arum* lily species. *Plant Physiology, 41*(2), 343–347. http://dx.doi.org/10.1104/pp.41.2.343.

Stadler, S., Stefanuto, P.-H., Brokl, M., Forbes, S. L., & Focant, J.-F. (2013). Characterization of volatile organic compounds from human analogue decomposition using thermal desorption coupled to comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *Analytical Chemistry, 85*(2), 998–1005. http://dx.doi.org/10.1021/ac302614y.

Statheropoulos, M., Agapiou, A., Spiliopoulou, C., Pallis, G. C., & Sianos, E. (2007). Environmental aspects of VOCs evolved in the early stages of human decomposition. *Science of the Total Environment, 385*, 221–227. http://dx.doi.org/10.1016/j.scitotenv.2007.07.003.

Statheropoulos, M., Spiliopoulou, C., & Agapiou, A. (2005). A study of volatile organic compounds evolved from the decaying human body. *Forensic Science International, 153*, 147–155. http://dx.doi.org/10.1016/j.forsciint.2004.08.015.

Sturgill, G., & Rather, P. N. (2004). Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Molecular Microbiology, 51*(2), 437–446. http://dx.doi.org/10.1046/j.1365-2958.2003.03835.x.

Sze, S.-H., Dunham, J. P., Carey, B., Chang, P. L., Li, F., Edman, R. M., et al. (2012). A de novo transcriptome assembly of *Lucilia sericata* (Diptera: Calliphoridae) with predicted alternative splices, single nucleotide polymorphisms, and transcript expression estimates. *Insect Molecular Biology, 21*, 205–221. http://dx.doi.org/10.1111/j.1365-2583.2011.01127.x.

Tomberlin, J. K., Benbow, M. E., Tarone, A. M., & Mohr, R. M. (2011). Basic research in evolution and ecology enhances forensics. *Trends in Ecology & Evolution, 26*, 53–55. http://dx.doi.org/10.1016/j.tree.2010.12.001.

Tomberlin, J. K., Crippen, T. L., Tarone, A. M., Singh, B., Adams, K., Rezenom, Y. H., et al. (2012). Interkingdom responses of flies to bacteria mediated by fly physiology and bacterial quorum sensing. *Animal Behaviour, 84*, 1449–1456. http://dx.doi.org/10.1016/j.anbehav.2012.09.013.

Tomberlin, J. K., Mohr, R., Benbow, M. E., Tarone, A. M., & VanLaerhoven, S. L. (2011). A roadmap for bridging basic and applied research in forensic entomology. *Annual Review of Entomology, 56*, 401–421. http://dx.doi.org/10.1146/annurev-ento-051710-103143.

Tomberlin, J. K., Rains, G. C., Allan, S. A., Sanford, M. R., & Lewis, W. J. (2006). Associative learning of odor with food or blood-meal by *Culex quinquefasciatus* Say (Diptera: Culicidae). *Naturwissenschaften, 93*, 551–556.

Urech, R., Green, P. E., Rice, M. J., Brown, G. W., Duncalfe, F., & Webb, P. (2004). Composition of chemical attractants affects trap catches of the Australian sheep blowfly, *Lucilia cuprina*, and other blowflies. *Journal of Chemical Ecology, 30*, 851–866.

Valone, T. J. (1989). Group foraging, public information, and patch estimation. *Oikos, 56*(3), 357–363. http://dx.doi.org/10.2307/3565621.