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Interkingdom responses of flies to bacteria mediated by fly physiology and bacterial quorum sensing

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Insect location and utilization of a resource is influenced by a host of variables including nutrients acquired prior to encountering a stimulus and age of the individual. For the carrion system, we hypothesized that volatiles to which primary colonizers, such as blow flies, respond are the same signalling molecules produced and utilized for quorum sensing by bacteria found on the resource. We provided freshly emerged blow flies, *Lucilia sericata*, different diets (blood or powdered milk) and assessed their behaviour in a dual-choice assay based on sex and ovarian status of 7-day-old or 14-day-old adults. We determined their preference between wild-type *Proteus mirabilis*, which is able to swarm (a quorum-sensing response), or mutated (by transposon mutagenesis) *P. mirabilis*, which is unable to swarm. In most instances, an individual's sex did not significantly influence its response. Age and diet appeared to regulate fly motivation and preference. Seven-day-old flies had a significantly greater probability of responding to the wild type than to the mutant, regardless of diet, but the percentage of milk-fed flies that responded was significantly smaller (85% less) than the percentage of blood-fed flies that responded. Blood-fed flies oviposited, whereas milk-fed flies did not. Seven-day-old flies oviposited predominately on the wild type, whereas 14-day-old flies oviposited predominately on the mutant. Our results demonstrate that the mechanism used by *L. sericata* for detecting a resource can be associated with bacterial quorum sensing, and that the physiological state of the insect influences its response. We also identified several differences in volatile compounds produced by the bacteria that could explain blow fly response.

The unpredictable occurrence of carrion within an ecosystem results in intense competition for these ephemeral resources. With regard to Diptera, arrival out of sequence in the decomposition process could be detrimental to the resulting offspring (Shorrocks & Bingley 1994; Lam et al. 2007) and their ability to locate a suitable mate (Norris 1965; Archer & Elgar 2003). Consequently, arthropods that utilize these resources have evolved highly sensitive sensory systems allowing for the quick discovery, colonization and utilization of these ephemeral resources (Dethier 1947; Spivak et al. 1991).

Bacteria associated with carrion release volatile organic compounds (VOCs) that mediate attraction and oviposition by blow flies (Diptera: Calliphoridae) (LeBlanc 2008). In many instances, specific bacteria are responsible for attracting myiasis-causing blow flies to wounds (Khoga et al. 2002). Bovine blood inoculated with one of eight bacteria species, or in combination, was examined for eliciting attraction and oviposition of *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae) (Chaudhury et al. 2010). All bacteria species elicited a response by the blow fly; however, *Proteus mirabilis* incubated in blood for 24 h was one of only two species to elicit an oviposition response.

Many of the VOCs associated with carrion decomposition have been surveyed (LeBlanc 2008). And, in some instances, the VOCs identified, such as indole, sodium sulfide (Urech et al. 2004) and

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putrescine (Dethier 1947), have been used to develop trapping methods for flies of medical and veterinary importance. Only more recently has it been determined that many of these compounds are also produced by bacteria, allowing them to make quorum sensing (QS)-based decisions. Putrescine was found to rescue the swarming ability (a QS-regulated behaviour) of an *rfaL* deficient *P. mirabilis* mutant. The inability of *P. mirabilis* to swarm resulted in less attraction to and oviposition by *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) than the wild type (Ma et al. 2012).

Arthropod responses to stimuli vary depending on their age and nutritional history. The response of the parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) to hosts is influenced by its age, experience and larval diet (Drost et al. 1988), with younger adults being more selective. Within the context of decomposition ecology, a poor adult diet influences mating behaviour of flies, as demonstrated in face flies, *Musca autumnalis* De Geer (Diptera: Muscidae), which reduce egg production and, consequently, hormones essential for inducing mating behaviour (Chaudhury & Ball 1973). In addition, responses of adult *Phormia regina* Meigen (Diptera: Calliphoridae) to yeast are influenced by the age and diet of the individual (Bowdan 1982). In both instances, older adults and those with poor nutrition are less selective when choosing mates or food. In the present study, we tested these factors together to determine their roles in regulating blow fly behaviour. Our objectives were (1) to determine the effect of age and nutritional history on the response of *L. sericata* to a *rfaL* deficient *P. mirabilis* mutant (QS inhibited) and the wild type, (2) to determine the role of sex and ovarian status, an indicator of physiological state, on the response of *L. sericata* to these bacteria and (3) to characterize VOCs produced by *rfaL*-deficient mutant and wild-type strains of *P. mirabilis* to identify candidate molecules involved in attraction and oviposition.

METHODS

Methods for this research were based on those described in Ma et al. (2012). *Proteus mirabilis* was isolated from *L. sericata* larvae and the mutant was created using transposon mutagenesis with the EX-Tn*5*™ <DHFR-1> Tnp transposome kit (Epicentre, Madison, WI, U.S.A.). The swarming mutation, *rfaL*, was selected because of the ability to restore swarming by use of the known fly attractant, putrescine (Ma et al. 2012). Using methods described below, wild type and *rfaL* mutant bacteria were screened (attraction and oviposition) and determined to not be repellant to flies (see Supplementary Table S1, Fig. S1). As this research was conducted with arthropods, no permit was required from the Office of Research Compliance and Biosafety, Texas A&M University.

Colony Maintenance

Lucilia sericata adults from Davis, CA, U.S.A. (Tarone et al. 2011) were maintained in 30 cm^3 BioQuip cages and provided a sugar water syrup ad libitum. The transcriptome for this strain has been sequenced (Sze et al. 2012). Flies were used in one of two treatments. Flies were either fed ad libitum blood collected from fresh beef liver (treatment 1), or fed a powdered milk and sugar mixture (treatment 2). Flies from each treatment were tested when they were 7 or 14 days old (posteclosion). Each replicate consisted of a single cage of flies (population) partitioned for use in both attraction and oviposition experiments. Therefore, each fly population was used for one experimental replicate.

Attraction Olfactometer

We used an olfactometer to measure responses of blow flies to treatments. The olfactometer consisted of two white PVC pipes, 15 cm long × 10 cm diameter (Charlotte Pipe, Charlotte, NC, U.S.A.), which extended from opposing walls of a 45 cm^3 Plexiglas cube (Fig. 1a). Proximal ends of each pipe were inserted into the cube and fitted with inverted funnels, thus allowing the flies to enter the pipe but reducing the likelihood of their retreating back into the cube. The inside of the pipes were lined with two odour-free sticky traps (Bell Laboratories Inc., Chicago, IL, U.S.A.). The distal end of the pipe

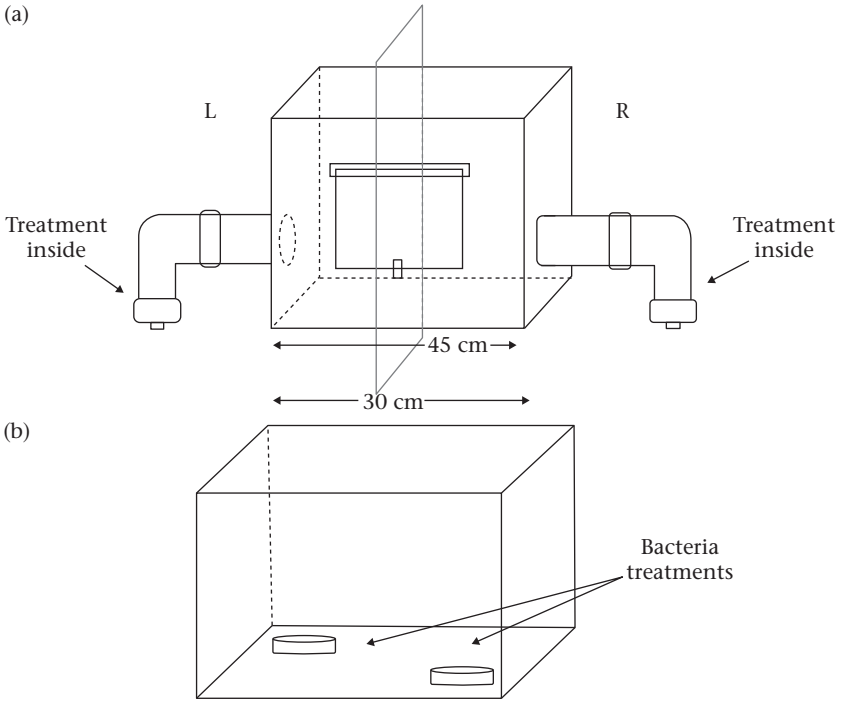

Figure 1. Olfaction device used to measure *L. sericata* (a) attraction to and (b) oviposition on wild-type and *rfaL* mutant *P. mirabilis*. Illustrations courtesy of C. Tyler (images not to scale).

was covered with nylon mesh and inserted into a white 90° angle PVC pipe, 15 cm long × 10 cm in diameter. The distal end of the 90° angle PVC pipe was capped with a 10 cm diameter cap on which the agar plate inoculated with bacteria was placed. Each pipe contained a separate treatment or control. The Plexiglas cage and all components were washed with odourless soap and dried prior to use during tests.

Attraction Assay

The fly attraction assay was performed at 21 °C under parallel fluorescent lights. Ma et al. (2012) showed that flies are attracted to 100 μl of 10^9 colony-forming units (cfu)/ml of wild-type *P. mirabilis* inoculated on a 17 × 100 mm Luria agar (LA) plate incubated at 37 °C for 24 h. We used this bacterial preparation procedure for both wild-type and mutant *P. mirabilis*, resulting in an average initial bacterial inoculum concentration of 3.83×10^9 cfu/ml for the wild type and 1.32×10^9 cfu/ml for the *rfaL* mutant. Approximately 250 *L. sericata* adults were placed into the olfactometer without food or water. Flies were allowed 24 h to make a decision to enter either pipe. A separate pipe apparatus was designated and used for each treatment (wild type and mutant) for all replicates. We alternated the side of the olfactometer used to deliver treatments between replicates to avoid side biases. We collected flies captured on the sticky traps within each pipe and those remaining in the central cage after 24 h, counted them and recorded their sex and ovarian status (i.e. gravid or nongravid).

Oviposition Assay

We performed the oviposition assay under the same conditions as the attraction assay (see Fig. 1b). Approximately 150 *L. sericata* adults were placed in a 30 cm^3 BioQuip cage with water and sugar provided ad libitum. LA plates (10 × 35 mm; USA Scientific Inc., Ocala, FL, U.S.A.) were inoculated with 13 μl of 10^9 cfu/ml *P. mirabilis* wild type or *rfaL* mutant and incubated at 37 °C for 24 h. The plate was covered with a mesh to prevent direct contact with bacteria and inserted into the centre of a 17 × 100 mm LA plate. We placed two treatments in opposing corners on the floor of the cage and rotated treatment placement between replicates to eliminate bias towards one side of the cage. Flies had contact with the LA agar ring surrounding the plate in the centre, but not with the bacteria growing on the plate, as previously described (Ma et al. 2012). We assessed the number of eggs deposited on the plate after 24 h.

Solid Phase Microextraction (SPME)

We conducted a preliminary survey to describe VOCs produced by wild-type and mutant *P. mirabilis*. We conducted two replicates for the wild type and three replicates for mutant *P. mirabilis*. Bacteria were prepared as described above on a 17 × 100 mm LA plate. The range of bacterial inoculum concentration was 1.00–5.60×10^9 cfu/ml for the wild type and 0.51–6.50×10^9 cfu/ml for the *rfaL* mutant. The plates were covered with a sterile 310 ml glass bell jar with a port on the top. Aluminium foil compressed by a top layer of Parafilm® was secured around the enclosure at the bottom to seal it from air exchange. The top port was fitted with a rubber stopper, covered with aluminium foil, with a centre hole through which the SPME fibre was inserted and again affixed with an aluminium foil-Parafilm® outer seal. SPME fibres (60 μm CW(PEG) SPME fibre; Supelco, Bellefonte, PA, U.S.A.) were exposed to the headspace of the bell jar at room temperature. VOC extraction was conducted for 30 min at 0, 7 and 24 h after inoculation. The jar covered 60.5% of the surface area of the 100 mm diameter plate (3.85 cm^2). Therefore, we converted measured quantities of volatiles to total volatiles emitted from the bacteriological plate by multiplying by 1.65.

Gas Chromatography-Mass Spectrometry

We performed gas chromatography-mass spectrometry (GC-MS) analysis of SPME fibre-extracted headspace volatile compounds on an Ultra GC/DSQ (ThermoElectron, Waltham, MA, U.S.A.). We used Rxi-5ms as a gas chromatographic column; the column was 60 m long, with an inner diameter of 0.25 mm and a film thickness of 0.25 μm (Restek, Bellefonte, PA, U.S.A.). Helium was used as a carrier gas at a constant flow of 1.5 ml/min and a split flow rate of 50 ml/min. Transfer line and ion source were held at 250 °C. The SPME fibre was manually placed into the GC injector, which was held at 225 °C in splitless mode, and the fibre was left in the injector for 3 min to desorb the VOCs. We used a narrow bore straight liner with an inner diameter of 0.8 mm (SGE; Austin, TX, U.S.A.) as an inlet. The column temperature was maintained at 40 °C for 3 min and raised to 250 °C at 20 °C/min. Mass spectra were acquired in full scan mode in the range of 15–300 m/z. Data acquisition and processing was carried out using Xcaliber (ThermoElectron). Volatile compound identification was carried out by comparison of mass spectrum and NIST 02 spectra library.

Experimental Design and Statistical Analysis

We performed four replicate experiments for attraction and oviposition preference to wild-type or mutant *P. mirabilis*. We based assessments on responses of males, gravid females, nongravid females and all flies (summary of flies to respond). We analysed behaviour data with PROC GLIMMIX (SAS 2011), which is a generalized linear mixed model (GLMM). The probability (P) of attraction and oviposition responses by *L. sericata* to the wild-type or mutant *P. mirabilis* was examined with sex, age and nutrition as fixed factors and replicate as a random factor. We used the same procedure to determine differences in levels of response across age and nutrition treatments. Only variables that were statistically significant ($P < 0.05$) are presented in tables. VOCs profiles could not be statistically tested between the wild-type and mutant *P. mirabilis* because there were only two replicates for the wild-type treatments. However, there were two compounds, isobutyl amine and phenylethyl alcohol, with much larger and obvious peak areas (>75% higher than the average of all other molecules) that represented the wild-type and mutant profiles. Therefore, we compared these peak areas to the average area of the remaining compounds to provide a relative percentage difference of isobutyl amine and phenylethyl alcohol in the profiles and between the two treatments.

RESULTS

Preliminary experiments confirmed that the wild-type and mutant *P. mirabilis* attracted *L. sericata* (Table S1, Fig. S1). In subsequent experiments comparing fly response to wild-type and mutant *P. mirabilis*, nutrition affected the level of fly attraction. Individuals fed powdered milk as a protein source responded 85% less overall than those fed blood across all trials (Fig. 2). Unlike blood-fed flies, those provided with powdered milk failed to respond in 50% of the replicates as well. Flies were examined for the influence of age, sex and nutrition on the probability of making a choice versus not making a choice (staying in the centre of the olfactometer). Estimated probability values for making a choice (adjusted for replicate) are presented in Table 1. The replicate variance was not significantly different from 0 but was included in the final model to adjust for replicate differences. Age ($P < 0.0001$),

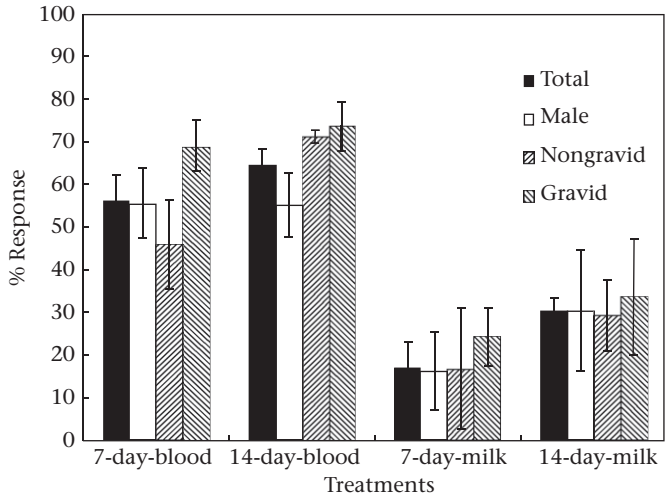

Figure 2. Mean ± SE percentage response of adult *L. sericata* to *P. mirabilis*, with responses to wild-type and *rfaL* mutant strains combined (see Table 2). Males and females were either fed powdered milk or blood, and tested at 7 and 14 days of age.

Table 2
Mean ± SE percentage response of *L. sericata* adults of different ages, nutritional background, sex and ovarian status to wild-type and *rfaL* mutant *P. mirabilis* in attraction assays (replicates, $N = 4$)

Age and nutrition	Sex and ovarian status	Mean percentage response ± SE (N)	
		Wild type	*rfaL* mutant
Blood-fed			
7-day-old	All	54.3±7.8 (282)	45.7±7.8 (158)
	Male	58.1±9.0 (136)	41.9±9.0 (78)
	Nongravid	62.0±10.4 (68)	38.0±10.4 (37)
	Gravid	58.4±7.9 (78)	41.6±7.9 (43)
14-day-old	All	61.5±5.8 (372)	38.5±5.8 (203)
	Male	72.4±8.2 (144)	27.6±8.2 (56)
	Nongravid	55.2±8.3 (172)	44.8±8.3 (103)
	Gravid	57.7±9.1 (56)	42.3±9.1 (44)
Milk-fed			
7-day-old	All	39.2±17.3 (130)	60.8±17.3 (157)
	Male	39.7±21.3 (63)	60.3±21.3 (67)
	Nongravid	37.7±10.1 (46)	62.3±10.1 (66)
	Gravid	40.4±37.6 (21)	59.6±37.6 (24)
14-day-old	All	55.7±21.1 (107)	44.3±21.1 (78)
	Male	57.9±26.9 (47)	42.1±26.9 (36)
	Nongravid	55.7±22.1 (41)	44.3±22.1 (32)
	Gravid	30.5±36.3 (19)	69.5±42.2 (10)

nutrition ($P < 0.0001$), sex ($P < 0.0001$), age*nutrition ($P < 0.0001$), nutrition*sex ($P = 0.0037$) and the three-way interaction of age*nutrition*sex ($P < 0.0001$) were significant in explaining whether flies made a choice in the olfactometer. The two-way interaction of age*sex was not significant ($P = 0.4816$) in explaining choice or no choice in the olfactometer.

Attraction data to wild-type and *rfaL* mutant *P. mirabilis* are presented in Table 2. Estimated values for the probability of attraction, adjusted for replicate, are presented in Table 3. The replicate variance was not significantly different from 0 but was included in the final model to adjust for replicate differences. Age ($P = 0.0147$) and nutrition ($P < 0.0001$) were significant, whereas sex ($P = 0.1656$), age*nutrition ($P = 0.0732$) and all other two- and three-way interactions ($P > 0.20$) were not significant in explaining the response of the flies to the wild-type or mutant *P. mirabilis*. The level of blow fly attraction to the different treatments was dependent on nutritional history and age (Table 2).

Blow fly oviposition was influenced by nutritional history. At 7 days of age, blood-fed flies significantly preferred ovipositing on the wild-type *P. mirabilis* rather than on the *rfaL* mutant (Table 4), but at 14 days of age, they laid more eggs on the mutant, but not at a significant level, possibly indicating an influence of age on preference. In addition, poor nutrition was detrimental to oviposition, as no milk-fed flies laid eggs during the experiments. Dissections of flies indicated that a smaller percentage of milk-fed females were gravid compared with those fed blood from beef liver.

Estimated values for the probability of oviposition, adjusted for replicate, are presented in Table 5. Replicate variance did not differ significantly from 0 but was included in the final model to adjust for replicate differences. Age ($P = 0.0014$) significantly predicted whether flies would oviposit on the wild-type or the mutant *P. mirabilis*.

Figure 3 shows VOCs emitted from wild-type and mutant strains 24 h after inoculation. The identified compounds from both *P. mirabilis* strains were of various functional groups, including alcohol, aldehyde, amine and ester, which are known blow fly attractants (Table 6).

All VOCs detected in the wild type were also detected in the *rfaL* mutant except 3-methyl butanal, indole and 5-methyl-2-phenyl-2-hexanal. In addition, all the compounds listed in Table 6 were not detected at 0 or 7 h after collection, with the exception of dimethyl disulfide, which was detected at 7 h for the wild type only. Peak area of each identified compound was calculated to show their relative concentration as used in the olfactometer (Table 6). Most of the compounds detected in the wild type had similar peak areas in

Table 1
Estimated probability, adjusted for replicate, for the attraction assay with fixed variables related to *L. sericata* choice or no choice to wild-type or *rfaL* mutant *P. mirabilis* (replicates, $N = 4$)

Factor	Estimated P (SE)	Estimated odds $(P/1 - P)$*
7-day-old	0.3739 (0.0486)	0.5872
14-day-old	0.4649 (0.0525)	0.8688
Blood-fed	0.5768 (0.0510)	1.3629
Milk-fed	0.2757 (0.0420)	0.3806
Sex: gravid	0.4941 (0.0557)	0.9767
Sex: male	0.3496 (0.0473)	0.5375
Sex: nongravid	0.4158 (0.0510)	0.7117

* Estimated probability values for all 12 combinations of age, sex and nutrition can be obtained from the following model: (log odds (LO) = −0.3625 − 0.8554 × age − 0.2599 × nutrition − 0.6033 × sex1 − 0.8749 × sex2 + 2.3207 × age × nutrition + 1.0019 × age × sex1 + 0.9004 × age × sex2 + 1.5998 × nutrition × sex1 + 1.2186 × nutrition × sex2 − 1.5241 × age × nutrition × sex1 − 1.8623 × age × nutrition × sex2), where age = 1 for 14-day-old; age = 0 for 7-day-old; nutrition = 1 for blood-fed; nutrition = 0 for milk-fed; sex1 = 1 and sex2 = 0 for gravid females; sex1 = 0 and sex2 = 1 for males; sex1 = 0 and sex2 = 0 for nongravid females. Values calculated can then be computed with $P = \exp(LO)/(1 + \exp(LO))$.

Table 3
Estimated probability, adjusted for replicate, with fixed variables related to *L. sericata* attraction to wild-type or *rfaL* mutant *P. mirabilis* (replicates, $N = 4$)

Factor	Estimated P (SE)	Estimated odds $(P/1 - P)$*
7-day-old	0.5338 (0.0362)	1.1450
14-day-old	0.6078 (0.0363)	1.5497
Blood-fed	0.6349 (0.0318)	1.7390
Milk-fed	0.5051 (0.0401)	1.0206
Sex: gravid	0.5628 (0.0456)	1.2873
Sex: male	0.5954 (0.0354)	1.4716
Sex: nongravid	0.5552 (0.0376)	1.2482

* Estimated probabilities for all 12 combinations of age, sex and nutrition can be obtained from the following model: (log odds (LO) = −0.3519 + 0.5197 × age + 0.8049 × nutrition − 0.04258 × sex1 + 0.1965 × sex2 − 0.4312 × age × nutrition), where age = 1 for 14-day-old; age = 0 for 7-day-old; nutrition = 1 for blood-fed; nutrition = 0 for milk-fed; sex1 = 1 and sex2 = 0 for gravid females; sex1 = 0 and sex2 = 1 for males; sex1 = 0 and sex2 = 0 for nongravid females. Values calculated can then be computed with $P = \exp(LO)/(1 + \exp(LO))$.

Table 4
Mean ± SE percentage oviposition on wild-type and *rfaL* mutant *P. mirabilis* by blood-fed *L. sericata* of different ages, sex and ovarian status in dual-choice behavioural assays (replicates, $N = 4$)

Age	Oviposition	
	Wild type (*N*)	*rfaL* mutant (*N*)
7-day-old	62.0±13.0 (1480)	38.0±13.0 (1000)
14-day-old	48.8±6.2 (1080)	51.2±6.2 (1134)

Table 5
Estimated probability, adjusted for replicate, with fixed variables related to blood-fed *L. sericata* oviposition on wild-type or *rfaL* mutant *P. mirabilis* (replicates, $N = 4$)

Factor	Estimated *P*	Estimated odds ($P/1 - P$)*
7-day-old	0.5832	1.3992
14-day-old	0.4706	0.8889

* Estimated probabilities for ages can be obtained from the following model: (log odds (LO) = 0.3360 − 0.4538 × age), where age = 1 for 14-day-old; age = 0 for 7-day-old. Values calculated can then be computed with $P = \exp(LO)/(1 + \exp(LO))$.

Table 6
Compounds identified using SPME GC-MS emitted from mutant and wild-type *P. mirabilis* 24 h after inoculation

Identified compound	Retention time (min)	Peak area×10^7		Peak area ratio (mutant: wild type)
		rfaL mutant	Wild type	
1-Propanol, 2-methyl	4.42	1.32	0.7	1.9
Butanal, 3-methyl	4.71	0.0	0.3	0.0
1-Butanol, 3-methyl	5.66	65.0	85.0	0.8
Disulfide, dimethyl*	5.84	0.9	6.9	0.1
Benzaldehyde	8.58	0.3	0.3	1.0
Dimethyltrisulfide	8.64	0.2	0.3	0.7
1-Propanol, 3-(methylthio)	8.73	0.5	0.8	0.6
Benzyl alcohol	9.29	0.2	0.5	0.4
Acetaldehyde, phenyl	9.32	0.2	4.3	0.0
Phenylethyl alcohol	9.92	64.0	76.4	0.8
Acetic acid, 2-phenylethyl ester	11.04	2.5	2.6	0.9
Indole*	11.42	0.0	4.6	0.0
5-Methyl-2-phenyl-2-hexenal	12.67	0.0	0.3	0.0

* Known fly attractants (Urech et al. 2004).

the mutant. However, two compounds (phenylethyl alcohol and isobutyl amine) had much larger mean peak areas compared with the other compounds (Fig. 4). The phenylethyl alcohol peak area was about 96% greater than the average of all other compounds (excluding phenylethyl alcohol and isobutyl amine) for the mutant, but only about 78% greater in the wild type. A reverse relationship was found for isobutyl amine: the peak area for isobutyl amine was about 90% and 96% greater than the average peak area for mutant and wild-type strains, respectively (Fig. 4). When these two compounds were compared between wild-type and mutant strains, isobutyl amine was 46.6% greater in the wild type, while phenylethyl alcohol was 87.7% greater in the mutant profiles (Fig. 5). The slight variation in peak area evident in some of the compounds could be due to various experimental factors, such as bacterial concentration variability in inoculums and after 24 h growth, differences in time before the fibre was subjected to GC/MS and air tightness of sample collection.

DISCUSSION

Arthropods such as blow flies that utilize carrion as a resource respond to VOCs associated with the resource. These VOCs were predominately thought to be associated with the decomposition process (Vass et al. 2002), as well as with bacteria associated with the resource (Chaudhury et al. 2010), and while in some instances these compounds can be attractive, in others, they can be repellant. Burkepile et al. (2006) found that fish carcasses treated with antibiotics, which suppress associated bacterial communities, were utilized longer than untreated fish carcasses. They concluded that the repellant compounds, which reduce competition between scavengers for these resources, are released by bacteria.

We hypothesized that many bacterially produced VOCs are associated with QS signalling (Ma et al. 2012). *Proteus* is a common bacterium associated with blow flies and decomposing resources (Chaudhury et al. 2002, 2010; Barnes et al. 2010), and we used it as a model to examine this hypothesis. *Proteus mirabilis*, genetically

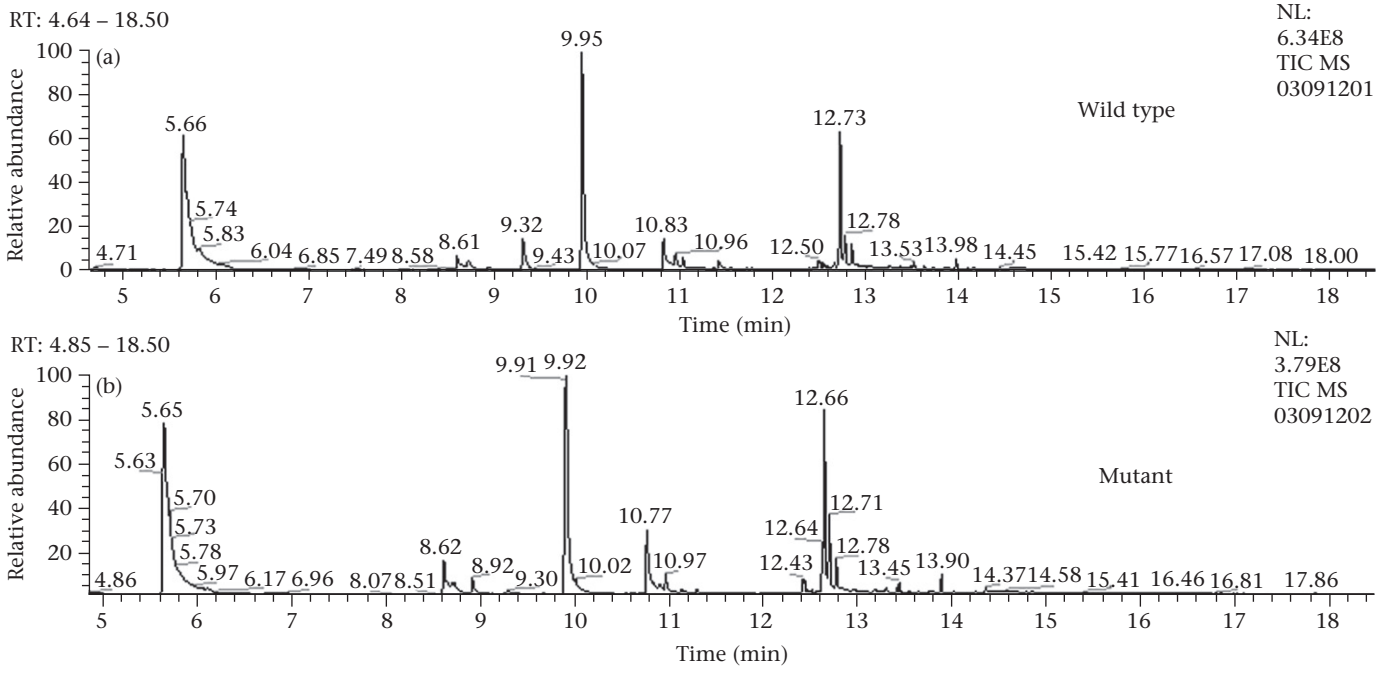

Figure 3. Total ion chromatogram (TIC) obtained for (a) wild-type and (b) mutant *P. mirabilis* by SPME GC-MS extracted from the headspace 24 h after inoculation.

modified to inhibit the QS-regulated swarming response, was less attractive to *L. sericata*, as measured by oviposition, than the wild type (Ma et al. 2012). As pointed out by Ma et al. (2012), these results have broad ramifications for behavioural ecology since they demonstrate that arthropods at higher trophic levels are responding to the decision-making process occurring at the microbial level.

Most of the VOCs we detected in our study are produced by many microorganisms (Joffraud et al. 2001; Schultz & Dickschat 2007; Chaudhury et al. 2010); however, we determined that emissions from mutant and wild-type *P. mirabilis* differed. These differences could be factors regulating bacterial functions being monitored by blow flies to ascertain resource appeal. Putrescine, sodium hydroxide, potassium hydroxide and ammonia, to name a few, restore swarming ability in a *P. mirabilis* mutant (Ma et al. 2012). These compounds are also known fly attractants (Dethier 1947). Indole is also a QS molecule (Bansal et al. 2009) and a known fly attractant (Urech et al. 2004). Dimethyl disulfide is also differentially regulated in the mutant and is a known fly attract (Urech et al. 2004). Future studies should examine whether the amines that we detected serve complementary roles as QS molecules and fly attractants. This is especially evident since putrescine, which is a known QS molecule and fly attractant, did not appear to explain differences in wild-type and mutant VOCs even though it can rescue the swarming behaviour of the *rfaL* mutant. It could be

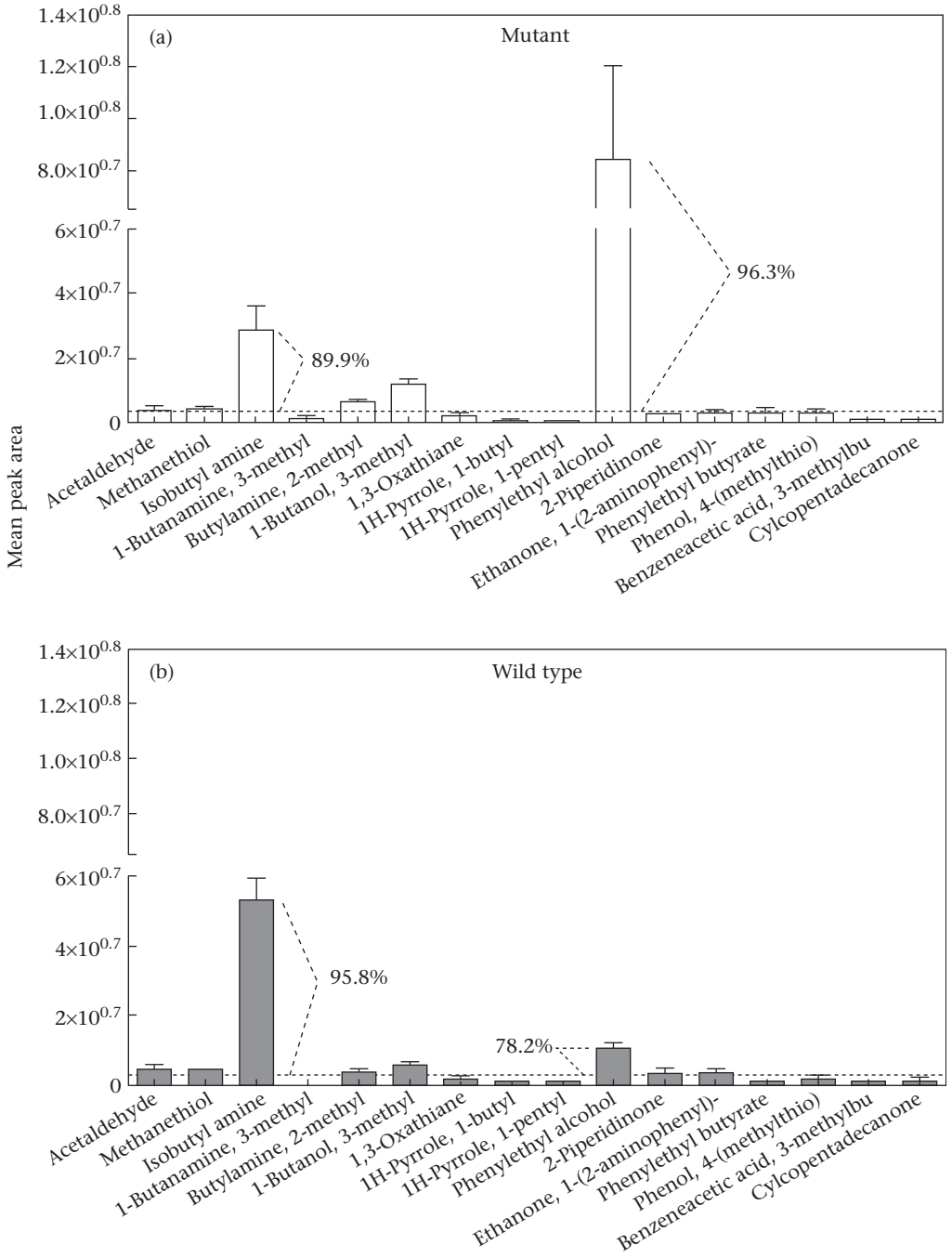

Figure 4. Mean ± SE compound peak area of (a) mutant and (b) wild-type *P. mirabilis* analysed by SPME GC-MS extracted from the headspace 24 h after inoculation.

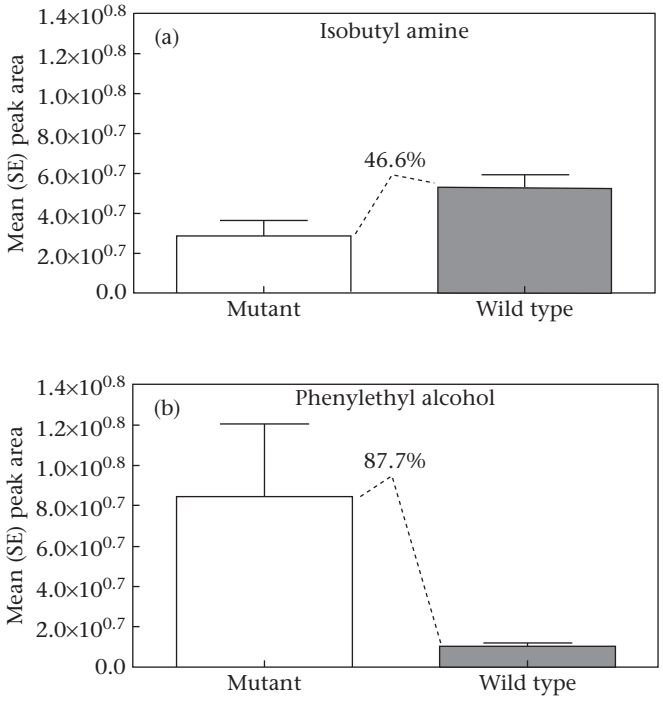

Figure 5. Mean ± SE compound peak area of (a) isobutyl amine and (b) phenylethyl alcohol for mutant and wild-type *P. mirabilis*.

that putrescine and isobutyl amine, which is a dominant compound of the wild type, are sufficiently similar in structure to elicit the swarming response.

We also found that decision making by *L. sericata* is affected by nutrition. Flies provided blood rather than powdered milk as a protein source showed a significantly greater level of response to wild-type *P. mirabilis*. This divergence in response could be due to the nutritional quality of the food resource, although colonies of flies are often maintained on powdered milk as a protein source (Boatright & Tomberlin 2010). Nevertheless, nutrient quality and quantity could influence fly physiology and egg production and dampen engagement of associated behavioural cascades (Stoffolano 1975). In the blow fly *P. regina*, poor nutrition reduces mating by 80% (Stoffolano 1975) and reduces subsequent motivation for locating and selecting a resource (Yin et al. 1999). Older flies showed different resource preferences than younger flies. This could be an indication of reduced selectivity of adults for a quality resource in an attempt to locate and oviposit on any resource available, potentially increasing fecundity. This response could be due to age of the individuals (Drost et al. 1988). Lack of experience in locating and evaluating resources could also be a factor regulating selectivity; however, the age cohorts and associated treatments in our experiments had no previous oviposition experience.

Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae) is receptive to mating only after feeding on a quality protein source (Browne et al. 1976), and subsequent oviposition typically occurs as a group (Barton Browne et al. 1969). In our experiments, the most likely explanation for the differences in response between milk-fed and blood-fed flies was the failure of milk-fed females to obtain appropriate nutrients for mating and subsequent oviposition. Even though the flies received unlimited powdered milk, the quantity of the resource did not compensate for its low quality. Thus, *L. sericata* may not vary its intake based on the quality of the resource, as demonstrated for *L. cuprina* (Browne & Gerwen 1992).

Resource quality can be decisive in determining the behavioural paths of arthropods. If arthropods have access to a high-quality resource (i.e. cow liver in this study) that contains the necessary cues, they can perform behaviours associated with a 'success-motivated search', which allows them to gravitate towards other resources, for consumption or potential oviposition (Bell 1990). In contrast, if insects consume low-quality resources (i.e. powdered milk in this study) or resources lacking essential stimuli (e.g. key bacterial species), then they will show other behaviours not associated with a success-motivated search, such as quiescence, limited searching and no oviposition. In the case of *L. sericata*, we think that bacteria associated with beef liver, *P. mirabilis* (Barnes et al. 2010), stimulated behaviours associated with a success-motivated search, whereas those associated with powdered milk did not. While not measured in this study, it is probable that the bacterial community associated with the powdered milk differs markedly from what the flies would encounter in nature.

Our results demonstrate that the physiological state of *L. sericata* affects its response to *P. mirabilis* with a mutated swarming ability mediated by putrescine and QS. Specifically, nutrition significantly affected the flies' motivation and preference for the mutant or wild-type *P. mirabilis*. Our methods will enable future research on questions related to interkingdom communication between bacteria and flies. While we used a response time of 24 h, we suggest future research examine the time course and dose–response curves needed for flies to respond. It is possible that less time might be required to measure the response of the flies to a target (i.e. bacteria or other resource) without concerns of aggregation or oviposition pheromones (Barton Browne et al. 1969) confounding the results.

For many systems, multitrophic-level interactions are initiated from the primary resource up through the ecosystem. In the case of our carrion system, resource (carrion) quality dictates the response of associated bacteria, which in turn influences the response (i.e. detection, attraction and colonization) of saprophytic arthropods (Tomberlin et al. 2011a, b). We have shown that (1) *Proteus* attracts *L. sericata* blow flies and encourages oviposition, (2) *L. sericata* shows differential attraction and oviposition responses to mutant *Proteus* strains that lack swarming capabilities (and in some cases, that lack QS signalling) and (3) the behavioural response of *L. sericata* to mutant and wild-type *Proteus* is dependent on its physiological state (nutrition and age). This paints a specific picture of carrion ecology, wherein decomposing remains potentially affect the physiology of associated microbial communities, and consequently, the QS signals they produce, which can be exploited by eavesdropping arthropods to evaluate the quality of the resource. A lack of attraction and colonization could prevent subsequent development of offspring, resulting in adults that are less fit and unable to distinguish quality resources from those of less quality, generating landscape-level variation (Tack et al. 2011) where some flies target and colonize specific resources. Our results help explain how flies detect and select oviposition resources (i.e. QS-mediated interkingdom eavesdropping) and the potential for this behaviour to influence variation in arthropod community structure.

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Supplementary Material

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