Annals of the Entomological Society of America, 110(1), 2017, 19–36
doi: 10.1093/aesa/saw086
Review

Special Collection: Filth Fly–Microbe Interactions

A Review of Bacterial Interactions With Blow Flies (Diptera: Calliphoridae) of Medical, Veterinary, and Forensic Importance

Jeffery K. Tomberlin,[1,2] Tawni L. Crippen,[3] Aaron M. Tarone,[1] Muhammad F. B. Chaudhury,[4] Baneshwar Singh,[5] Jonathan A. Cammack,[1] and Richard P. Meisel[6]

[1]Department of Entomology, College Station, Texas A&M University, 2475 TAMU, Texas 77843-2475 (jktomberlin@TAMU.edu; tamlucilia@tamu.edu; jcammack_07@tamu.edu), [2]Corresponding author, e-mail: jktomberlin@TAMU.edu, [3]US Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, 2881 F&B Rd, College Station, Texas 77845 (tawni.crippen@ars.usda.gov), [4]USDA-ARS, SRU, University of Nebraska, Lincoln, NE (Muhammad.Chaudhury@ars.usda.gov), [5]Department of Forensic Science, Virginia Commonwealth University, Richmond, VA-23284 (bsingh@vcu.edu), and [6]Department of Biology and Biochemistry, University of Houston, 3455 Cullen Blvd, Houston, Texas 77204-5001 (rpmeisel@Central.UH.EDU)

Subject Editor: Dana Nayduch

Received 20 September 2016; Editorial decision 27 October 2016

Abstract

Blow flies are commonly associated with decomposing material. In most cases, the larvae are found feeding on decomposing vertebrate remains; however, some species have specialized to feed on living tissue or can survive on other alternate resources like feces. Because of their affiliation with such septic environments, these insects have close associations with microbes. Historically, a tremendous amount of research focused on these insects due to their veterinary importance. Within the past 40 yr, efforts have expanded this research to include areas such as systems ecology, forensics, and even wound debridement (maggot) therapy. Initial research efforts examining the relationship between microbes and these insects were hampered by the technology available. However, with the advent of high-throughput sequencing and modern molecular techniques, new avenues of research examining these interactions have opened up. The purpose of this article is to highlight the research exploring the interactions between microbes and blow flies with regards to blow fly biology, the application of such information to benefit humanity, and potential future pathways of research.

Key words: interkingdom communication, veterinary entomology, medical entomology, trophic interaction, succession

Blow Fly Biology

Blow flies (Diptera: Calliphoridae) (Figs. 1 and 2) are medium- to large-sized calyptrate flies, many of which are easily recognized for their metallic blue or green coloration. They occur throughout the year and have a global distribution except in areas of extreme cold. This particular family of fly is of great medical, veterinary, and forensic importance due to the resources they utilize and their association with people (Sanford et al. 2014), pets (Anderson and Huitson 2004), livestock (Axtell 1986), poultry (Axtell and Arends 1990), and aquaculture (Fig. 3; Esser 1991, Aak et al. 2011). Globally, well over 1,000 species from 150 genera have been described (Shewell 1987). Of these, 54 species in 17 genera occur in North America (Whitworth 2006).

The larvae of these flies are vermiform, and outside of the Hawaiian genus *Dyscritomyia*, which larviposits (Wells et al. 2002), adults deposit large numbers of eggs on a resource. In some cases, the resulting larvae of these flies inhabit decomposing vertebrate remains (Fig. 3; Hall 1948, Payne et al. 1968), while some are known to cause myiasis (Broce 1985). The larvae pass through three stadia prior to pupating and emerging as adults. Total development time can vary depending on species and environmental conditions encountered; in general, completion of development from egg to adult takes between 8–14 d. Adult blow flies frequent carrion (Hall 1948, Payne 1965, Pechal and Benbow 2016), wounds on vertebrates (Sanford et al. 2014), feces (Mann et al. 2015, Brodie et al. 2016), and even flowers (Fig. 4; Brodie et al. 2015). These resources are used as sites for locating and securing mates, obtaining nutrition to meet the requirements of oogenesis, or supporting the development of offspring. Because of the environment in which they inhabit, and their association with humans and other animals, a tremendous amount of research has been conducted to examine various aspects of their biology, including:

- **Development** (Byrd and Butler 1996, Byrd and Allen 2001, Boatright and Tomberlin 2010, Owings et al. 2014)

Fig. 1. Adult hairy maggot blow fly, *Chrysomya rufifacies*, resting on vegetation (photo credit: Heo, C.C.).

- **Genetics** (McKenzie and Whitten 1982, Warman et al. 2000, Concha et al. 2010, Concha et al. 2011, Sze et al. 2012, Anstead et al. 2015, Kotze et al. 2015)
- **Ecology** (Stensmyr et al. 2002, van der Niet et al. 2011, Benbow et al. 2015)
- **Role as vectors** (Greenberg 1973)
- **Control** (Klassen and Curtis 2005)
- **Behavior** (Spivak et al. 1991, Boulay et al. 2015, Boulay et al. 2016, Brodie et al. 2016, Liu et al. 2016)

These data have been applied in various ways including, but not limited to, forensics (Smith 1986, Haskell and Williams 2008, Byrd and Castner 2010, Tomberlin and Benbow 2015), and maggot therapy (Sherman et al. 2000).

Blow Fly Attraction to Bacteria

Research during the early 20th century that explored microbial interactions with blow flies was conducted from a veterinary perspective due to key species causing myiasis of livestock (Tillyard and Seddon 1933, Mackerras 1936). *Lucilia* sp. (Cragg 1956), such as, but not limited to, *L. sericata* (Meigen) (Diptera: Calliphoridae) (Hobson 1935) and *L. cuprina* (Wiedemann) (Diptera: Calliphoridae) (Barton Browne 1958), were notorious for causing sheep strike in the United Kingdom and Australasian regions, respectively, while *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) was decimating cattle in the United States and southward (Bishopp et al. 1917, Hall et al. 2016). Gravid flies of *C. hominivorax* are attracted to larval-infested wounds and the navels of newborn livestock, where the flies oviposit (Bushland 1960). Infested wounds appear to release odors resulting in attraction and subsequent oviposition. Volatile organic compounds (VOCs) from wounds appear to play a role in attracting gravid flies and stimulating oviposition (Bromel et al. 1983, Hammack and Holt 1983, Hammack et al. 1987, Hammack 1991). During the early 1920s,

Fig. 2. Adult secondary screwworm, *Cochliomyia macellaria*, resting on vegetation (photo credit: Heo, C.C.).

Fig. 3. Blow fly larvae decomposing fish carrion in Alaska, USA (photo credit: T.L. Crippen).

researchers began speculating that bacteria were regulating blow fly attraction and colonization of sheep (Hobson 1935), but until recently, technological barriers limited the ability of researchers to explore these interactions and their relevance to the ecology and biology of this diverse group of flies.

DeVaney et al (1973) showed that bacteria-produced factors in bovine blood were highly attractive to primary screwworm (*C. hominivorax*). They determined cultures of several Gram-negative bacteria isolated from screwworm larval rearing media, homogenized third instars of *C. hominivorax*, and infested wounds on sheep produced attractive odors. These results suggested bacterial contamination of oviposition substrates was a prerequisite for attraction and oviposition by gravid flies (DeVaney et al. 1973, Eddy et al. 1975). These workers showed that the source of odors in incubated bovine blood that was attractive to gravid flies was bacteria (and compounds produced by them). Olfactometer tests showed that the blood containing *Providencia* (previously *Proteus*) *rettgeri* was the most attractive. Comparatively, *Morganella* (previously *Proteus*) *morganii* was somewhat more attractive than *Proteus vulgaris* and significantly more so than *Proteus mirabilis*. They also compared the attraction of these bacteria species alone with the four species in combination, and found that the combination was considerably more attractive than *P. mirabilis*, *M. morganii*, or *P. vulgaris* alone, but there was no significant difference between this combination and *P. rettgeri* (Eddy et al 1975). They also tested a combination of 12 *Bacillus* species against the combination of four species mentioned above. Results from these studies showed that the *Bacillus* combination trapped only 3% while the *Morganella–Proteus–Providencia* combination trapped 34% of the flies. Eventually, the cultures of *P. rettgeri* were found to be the most attractive (Eddy et al. 1975).

Screwworm flies normally deposit more egg masses when they have a suitable substrate such as horse meat, liver, bovine blood, etc. Eddy et al. (1975) compared oviposition on substrates incubated with each of the *Morganella–Proteus–Providencia* species and the combination of these four species. Significantly more oviposition occurred when a combination of *Bacillus* species was used than *Proteus* or a combination of *Bacillus* sp. and *Proteus* sp. (Eddy et al. 1975). According to these authors, many of the results were not consistent in their studies. For instance, peak attraction of incubated blood was sometimes after 7 d of incubation and sometimes after 14 or 21 d. Similar inconsistent results were obtained with homogenized screwworm larvae (Eddy et al. 1975). Most likely, these variations were caused by un-even presence of bacteria in the test materials used.

The above work was followed by Hammack et al. (1987) using a steam distillate of culture medium inoculated with *P. rettgeri* to assess the attraction of *C. hominivorax* adults. Results showed that females with previtellogenic ovaries and males were not attracted. Gravid mated females of 10–12 d old were most strongly attracted. These results confirmed that *P. rettgeri* produces an attractant for screwworm flies, and that this attractant lures females rather than males, and older females than younger ones, and more mated than virgin females (Hammack et al. 1987). Later, Hammack (1991) examined factors affecting oviposition by *C. hominivorax* using host-originated fluids in laboratory bioassays. She reported that fresh blood (with no attractive odor) was as attractive for oviposition as the other attractive fluids tested including fluids from screwworm-infested wounds and cultures of *P. rettgeri*. However, she noted that oviposition varied depending on the substrates to which the blood was applied, suggesting that an interaction exists between olfactory cues and tactile stimuli to bring about oviposition (Hammack 1991). More recent work indicates color could also play an important role in blow fly foraging behavior (Brodie et al. 2014, Brodie et al. 2015).

Chaudhury et al. (2002) continued the above research on screwworms using eight species of bacteria that were isolated from

Fig. 4. Adult blow flies on flowers in Alaska. Blow flies have been suggested as pollinators and by recent evidence they can digest pollen (Brodie et al. 2015) (photo credit: T.L. Crippen).

screwworm-infested animal wounds. The species of bacteria were: *Enterobacter cloacae*, *E. sakazakii*, *Klebsiella oxytoca*, *P. mirabilis*, *P. vulgaris*, *P. rettgeri*, *P. stuartii*, and *Serratia liquefaciens*. Both fertile and sterile (irradiated) male and female *C. hominivorax* were tested in a cage bioassay system for assessing attraction and oviposition using bovine blood inoculated with all eight species of bacteria and incubated for varying time periods. Substrates incubated for 48–72 h attracted more (>50% of flies were attracted to the resource) 7-d-old fertile females than did the substrates incubated for 24 and 96 h (<25% attraction). Significantly more fertile females were attracted to these substrates than sterile females (<20% attraction) of the same age group. Males of all tested age groups were unresponsive (<1% attraction). Oviposition tests lasting for 1 h resulted in significantly more oviposition in treated substrates compared to untreated control. Results indicate volatiles from five individual species of bacteria (*K. oxytoca*, *P. mirabilis*, *P. vulgaris*, *P. rettgeri*, and *P. stuartii*) were responsible for attracting more females resulting in more oviposition than volatiles from the remaining three species (*E. cloacae*, *E. sakazakii*, and *S. liquefaciens*) (Chaudhury et al. 2010). Volatiles from the same five species were also tested in a two-choice bioassay to study landing response and oviposition of the secondary screwworm, *C. macellaria* (Chaudhury et al. 2016). These tests showed that significantly more flies landed on substrates containing *P. mirabilis* than on substrates with other species of bacteria. Substrates treated with *K. oxytoca* attracted the least flies. Substrates containing bacteria incubated for 72 h attracted significantly more flies than those incubated for 24, 48, or 96h period. In 3-h duration oviposition tests, substrates with *P. rettgeri* attracted significantly more flies to oviposit than the other four species. The most oviposition events were recorded from substrates treated with all five species of bacteria. At least 72 h of incubation seems to be required to obtain the most active volatiles. These results suggest that *C. macellaria* uses similar chemical cues as *C. hominivorax* from bacteria volatiles as an oviposition attractant, supporting the generalizability of at least some blow fly interactions with microbes.

Interestingly, several of the key microbial players in the *Cochliomyia* studies also appear in the *Lucilia* system where myiasis manifests in association with animal feces in wool. The larvae of sheep blow flies *L. cuprina* and *L. sericata* are the primary cause of sheep myiasis in Australia, Europe, and New Zealand. The responses of these two species are similar and attraction to host and subsequent oviposition appear to involve volatile chemicals resulting from bacterial decomposition. Emmens and Murray (1982) selected

four species of bacteria isolated from the fleece of Merino sheep, namely, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E. cloacae*, and *P. mirabilis* for their study. Extracts from cultures of these bacteria were incorporated into nutrient agar and exposed to females of *L. cuprina* in cages. Odors from the cultures of bacteria attracted *L. cuprina* females to oviposit but the attraction was not consistent for all the cultures. The order of the bacterial species with respect to decreasing overall response was *P. mirabilis* with highest number of eggs laid, followed by *E. cloacae*, *B. subtilis* and least being *P. aeruginosa* (Emmens and Murray 1982). In another experiment, extracts from unsterile sheep fleeces treated with these four species of bacteria singly stimulated oviposition of *L. cuprina* equally during 24-h period; however, with increasing length of incubation, the cultures of *P. mirabilis*, *E. cloacae*, and *B. subtilis* became contaminated with increasing numbers of *P. aeruginosa* resulting in greater responses of the flies (Emmens and Murray 1983). The maximum number of eggs was deposited over 4-d-old cultures. This response was significantly associated with the presence of *P. mirabilis* and *E. cloacae* but not *B. subtilis*. The relatively lower oviposition responses to *P. aeruginosa* in pure culture as seen in these experiments and in previous studies (Emmens and Murray 1982) was thought to be due to different culturing methods used; however, the collective results obtained from these studies indicate that the effects of attractants are enhanced when bacteria are mixed. This was also evident in studies with the screwworm flies described in the previous section (Chaudhury et al. 2010). Others have treated baits with *P. mirabilis* cultured in a commercial medium or a gut mucus mixture and examined their level of attraction to blow flies in New Zealand sheep pastures (Morris et al. 1998). Females of *L. cuprina*, *Calliphora stygia* (F.) (Diptera: Calliphoridae), and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) were attracted to media culture. The gut mucus culture was significantly less effective. This is the first published record of sheep blow flies being attracted to bacterial odors in the field (Morris et al. 1998). In a study of interkingdom swarming signals, Ma et al. (2012) used *P. mirabilis* isolated from the salivary glands of *L. sericata* and identified several interkingdom signals between *P. mirabilis* and blow flies that influence blow fly attraction (Tomberlin et al. 2012, Liu et al. 2016).

Research during the early to mid-twentieth century, as well as more recently, determined blow flies respond to compounds associated with decomposition, such as indole (Hobson 1936, Hobson 1938, Dethier 1947). Grabbe and Turner (1973) extracted and fractionated bovine blood that had been inoculated and incubated; compounds isolated from the various fractions included phenol, p-cresol, indole, skatole, and ethanethiol. They found that a dilute aqueous mixture of indole, skatole, phenol, and p-cresol was attractive to screwworm flies in the laboratory. Similarly, Chaudhury et al. (2012) found that an aqueous slurry of media remaining after larval development to be attractive to gravid screwworm flies. The volatiles collected from these waste media using solid phase microextraction method yielded five electrophysiologically-active chemicals: dimethyl disulfide, dimethyl trisulfide, phenol, p-cresol, and indole (Chaudhury et al. 2014). A synthetic blend of these compounds was attractive to females of both primary and secondary screwworms (Chaudhury et al. 2014) as well as to female *L. sericata* (Chaudhury et al. 2015). Further research is necessary to identify and relate the many VOCs to the species of bacteria that produce them, and determine their specific roles as attractants, arrestants, and stimulants in host location, feeding, mating, oviposition, and other life processes of blow flies. However, it is again worth noting the similarities in attraction between *Cochliomyia* sp. and *Lucilia* sp. to similar classes of molecules. In some instances, these bacterial cues/signals are clearly important to numerous organisms (see Davis et al. 2013), indicating that the blow fly system is a useful model for dissecting insect and animal interactions with microbes.

At the time the aforementioned studies were conducted, the ecological relevance of the VOCs was not known. However, within the past decade it was determined that these volatiles are associated with bacterial activity, specifically bacterial communication and decision-making (i.e., quorum sensing (Lee et al. 2007)). More recent research is beginning to work toward bridging the nutritional ecology of bacteria with blow fly behavioral ecology, as many of these volatiles are by-products of the break-down of essential amino acids (Liu et al. 2016). Specific examples of VOCs include dimethyl disulfide, which is produced by the breakdown of methionine (Hayward et al. 1977), is a recognized by-product of vertebrate carrion decomposition (Vass et al. 2002, Paczkowski et al. 2015), and a regulator of blow fly attraction to such resources (Urech et al. 2004). Other by-products include isobutyl amine and phenylacetic acid, which are produced by the degradation of valine (Richardson 1966) and phenylalanine (Erdmann and Khalil 1986), respectively. Indole is produced when tryptophan is broken down (Sasaki-Imamura et al. 2010). In many cases, the response of blow flies to these volatiles is sex and ovarian-status specific. Males responded to dimethyl disulfide, which is produced late in the decomposition process (Paczkowski et al. 2015), a time when virgin females are attracted to the resource (Mohr and Tomberlin 2014, 2015), potentially for a protein meal necessary for oogenesis. In contrast, gravid flies respond to phenylacetic acid, which could indicate the presence of beneficial bacteria for resulting offspring (Liu et al. 2016). Interestingly, males do not respond to phenylacetic acid. However, this could be biologically relevant, as the ability to secure a mate would be highly unlikely as virgin females would be absent. Additionally, female blow flies typically mate only once. Females were highly attracted by phenylacetic acid and isobutyl amine, while males offered no response or were repelled (Liu et al. 2016). These results are significant, as they indicate the presence of beneficial bacteria (see discussion of *Proteus mirabilis* below), and that blow flies are potentially responding to microbial activity in relation to nutritional value of the resource they are attempting to access.

Role of Bacteria in Growth and Development

Many bacteria have a mutually-beneficial relationship with their hosts. Flies have been shown to be dependent on bacteria and their metabolic products for growth and development, as the immature stages of many fly species fail to develop in the absence of bacteria (Schmidtmann and Martin 1992, Zurek et al. 2000). In most studies, flies show the best survival rates in unsterilized or mixed bacterial environments, but some bacteria species enhance the development and survival rates of different fly species: *Escherichia coli* and *Lactobacillus plantarum* in the face fly, *Musca autumnalis* DeGeer (Diptera: Muscidae) (Hollis et al. 1985); *Streptococcus sanguis*, *Staphylococcus* sp., and *E. coli* in the house fly, *Musca domestica* L. (Diptera: Muscidae) (Schmidtmann and Martin 1992, Watson et al. 1993, Zurek et al. 2000); *Acinetobacter* sp., *Flavobacterium odoratum*, *Citrobacter freundii* and *Serratia fanticola* in the stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae) (Lysyk et al. 1999, Romero et al. 2006); and bacteria from families of Pseudomonadaceae, Corynebacteriaceae, Micrococcaceae, and Bacillaceae in the horn fly, *Haematobia irritans* L. (Diptera: Muscidae) (Perotti et al. 2001).

Perhaps the increased survival rates seen with mixed bacterial environments are because different bacteria are more advantageous at different stages of growth dependent on the metabolites they produce and the nutritional needs of the arthropod at that stage. Zurek et al. (2000) collected third-instars of house fly larvae from two common sources found at animal production facilities. They found that *Bacillus coagulans*, *Bacillus* sp., *Clavibacter michiganese*, *Corynebacterium aquaticum*, *Lactococcus garviae*, *Microbacterium esteraromaticum*, *Microbacterium lacticum*, *Microbacterium liquefaciens*, *Ochrobacter anthropic*, *Sphingobacterium spiritivorum*, *Sphinomonas capsulataa*, *Staphylococcus epidermidis*, *Staphylococcus lentus*, *Streptococcus sanguis*, *Xanthobacter flavus*, and *Yersinia pseudotuberculosis*, all supported larval growth to some extent to the pupal stage, whereas *Corynebacterium seminale*, *Gordona amarae*, *Microbacterium barkei*, *Morganella morganii*, *Providencia rettgeri*, *Providencia stuartii*, and *Serratia marcescens* did not. *Streptococcus sanguis* and *Sphingobacterium spiritivorum* supported larval development through eclosion as adult flies, whereas *Bacillus* sp. and *Staphylococcus epidermidis* did not.

As suggested by optimal foraging theory, an inferior competitor (potentially blow fly larvae in the case of carrion) could avoid such challenges simply by eliminating the competitor (potentially bacteria). Greenberg (1968) previously determined a five log reduction of Gram-positive and Gram-negative bacteria exposed to conditions experienced in the midgut of larval *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). Mumcuoglu et al. (2001) observed similar interactions between *L. sericata* and *E. coli*. They determined only 18% of *E. coli* ingested by *L. sericata* larvae was present in the hindgut. Reducing bacterial counts could free up nutrients for larval use as well as decrease the likelihood of becoming infected and killed. In fact, larvae of *C. macellaria* developed significantly faster (7.8 and 7.7 d) and had greater survivorship (88% and 94%) on sterile blood agar or a modified Harris rearing media, respectively, than those provided the same diets inoculated with bacteria, such as *E. coli* O157:H7 (9.1 d and 35.7%), *Providencia* sp. (9.7 d and 17.3%), *E. faecalis* (8.2 d and 75.5%), *Ochrobactrum* sp. (8.4 d and 81%), or a mixture (9.3 d and 27.5%; Ahmad et al. 2006).

While some bacteria are harmful to fly larvae, one in particular, *Proteus mirabilis*, has been considered beneficial. Blow fly colonization of vertebrate carrion results in increased pH, which is hypothesized to reduce competing or pathogenic bacteria, due to *P. mirabilis* activity (Barnes et al. 2010). This bacterium is able to persist in the digestive tract of *L. sericata* through immature development to the adult stage (Wei et al. 2014a). Additionally, the bacterial mortality recorded by Greenberg (1968), which was previously discussed, was suspected to be due to pH-specific activities of by-products, or "mirabilicides", produced by *P. mirabilis* (Barnes et al. 2010). Furthermore, what is most interesting is that beetle species that colonize carrion after blow flies, decrease the pH and their excretion/secretions (ES) kill *P. mirabilis* (Barnes et al. 2010). One could speculate that by doing so, such modifications would reduce beetle competition with fly larvae utilizing the same resource. These observations paint a picture of microbial warfare among insects competing for a resource, with different microbial tolerances as a mechanism by which different organisms can come to dominate or be excluded from ephemeral carrion resources (Janzen 1977, Burkepile et al. 2006). In this instance, *P. mirabilis* and *L. sericata* seem to be in alliance against other insects (like carrion beetles) and bacteria (those killed by "mirabilicides").

Since this initial work, a tremendous amount of research exploring the antimicrobial properties of the ES produced by bacteria associated with blow fly larvae and the larvae themselves has been conducted (Cazander et al. 2009a, Cazander et al. 2009b, Cazander et al. 2010, Barnes and Gennard 2011, Cazander et al. 2012, Barnes and Gennard 2013, Cazander et al. 2013). Two of these antimicrobials, phenyl acid acid (noted above) and phenylacetaldehyde, were identified from *P. mirabilis* isolated from larvae of *C. hominivorax* (Erdmann and Khalil 1986). Genetic and microscopic evidence indicating that *P. mirabilis* can reside in *L. sericata* salivary glands (Singh et al. 2015, Blenkiron et al. 2015), which are relatively microbe free, supports the concept that these two species coevolved to kill other microbes. However, it should be noted that while many bacteria are suppressed by ES, some were able to survive pupation of *C. macellaria*, indicating some level of "resistance" to being digested by the larvae (Ahmad et al. 2006). This ability to persist in the alimentary canal of the insect through development is cause for concern as these flies could serve as mechanical vectors for these pathogens, as well as creating an environment in which antibiotic resistance is developed and amplified in some circumstances (Wei et al. 2014a,b), but also provides further support for the concept that certain flies and microbes act as mutualists to compete against others.

Molecular Techniques for Evaluating Bacterial–Blow Fly Interactions

Like all other organisms, development and survival of blow flies also depends on their associated microbiome. Hence, to better understand how bacteria associated with blow flies regulate physiology and behavior of blow flies, the first step is to understand the microbiome associated with the fly species that colonize carrions. However, our current understanding of bacteria associated with blow fly species is limited (Thompson et al. 2013, Wei et al. 2014a, Singh et al. 2015, Pechal and Benbow 2016). This is mainly because culture-based methods used in the past were not able to discover the majority of bacteria associated with insects (Thompson et al. 2013). New culture-independent sequencing technologies (i.e. next-generation sequencing technologies) are capable of performing comprehensive surveys of bacteria (both rare and abundant) in a cost effective and timely manner. To do this, the first step is the selection of DNA extraction methods that give relatively unbiased estimates of the presence of both Gram-positive and Gram-negative bacteria. Bacterial DNA from different life stages of blow flies can be extracted either by using organic extraction methods (Zheng et al. 2013, Singh et al. 2015) or by using commercial kits (Iancu et al. 2016).

Extracted DNA can be used either for amplification and sequencing of targeted marker loci (e.g., 16S ribosomal DNA (16s rDNA) for prokaryotes, 18S ribosomal DNA (18S rDNA) for eukaryotes) or for direct whole genome shotgun sequencing (i.e. metagenomic sequencing). In marker gene sequencing approaches, different variable regions of targeted loci are amplified using barcoded universal primers and then barcoded-amplified products are pooled and sequenced using next-generation sequencing platform of choice (e.g., Hiseq/Miseq (Illumina Inc. USA), Ion PGM System (Thermo Fisher Scientific, USA)). On the other hand, in the metagenomic sequencing approach, DNA from the whole community is sheared and then directly sequenced using next-generation sequencing platform of choice (e.g., Hiseq/Miseq (Illumina Inc. USA)). Both approaches have some advantages. Marker gene-based sequencing approaches are comparatively cheap, provide information on both rare and abundant microbial taxa, and are easy to analyze (as user friendly bioinformatics pipelines are freely available (e.g., QIIME

(Caporaso et al. 2010), mothur (Schloss et al. 2009), RDPipeline (Cole et al. 2014)). Metagenomic sequencing approaches provide information not only on microbial community structure but also on microbial function, and avoid PCR-based biases associated with marker gene approaches (Lee et al. 2012). Recovery of rare taxa requires high sequencing depth in metagenomic sequencing approaches, which ultimately increases sequencing cost, and complicates already complicated metagenomic data analysis pipelines (Sharpton 2014). One method for balancing the advantages of both methods is to use single-molecule information to extrapolate the metagenomic gene content by using prior knowledge of sequenced bacterial genomes (Langille et al. 2013). Given the importance of blow fly interactions with microbes, we predict many more studies of microbial communities in blow flies in the coming years.

Bacteria Associated With Blow Flies

Blow flies and other filth flies (e.g., Sarcophagidae and Muscidae) use decaying organic matter for nutrition and larval habitat and thus often interact with and transmit pathogens of human disease. The US Federal Drug Administration identified 21 filth fly species as significant threats to human health (Olsen 1998). These flies are synanthropic and thus have preferential associations with human populations, increasing the risk for pathogenic bacterial transfer (Polvony 1971, Greenberg 1973). Many bacteria, pathogens included, can exist in biofilms (i.e., complex structures composed of components that include proteins, glycoproteins, DNA, and carbohydrates that are constructed by microbes as a habitat (Costerton 2007)) in facilities such as hospitals that flies can easily interact with just by contact, and can lead to large numbers of human bacterial infections (Barraud et al. 2009). Additionally, these flies can harbor and transfer antibiotic resistant bacteria, some with multidrug resistance (Graham et al. 2009). Despite the importance of the blow fly to carrion decomposition and to pathogen dispersal, very little has been done to investigate their microbiomes. In addition to studies noted above, Caballero et al. (1996) documented by culture technique the bacterial content of *C. hominivorax* during sheep myiasis. The most prominent genera were *Escherichia*, *Proteus*, *Providencia*, *Staphylococcus*, and *Streptococcus*. Singh et al. (2015) compared the bacterial community structure associated with different stages of *L. sericata* and *L. cuprina* from colony raised flies reared on beef liver, using metgenomic analyses. They determined that the majority of bacteria came from the phyla Proteobacteria, Firmicutes and Bacteriodetes. Proteobacteria is a Gram-negative phylum that contains many pathogens, such as *Escherichia coli*, *Wolbachia*, *Bordetella*, and *Salmonella*. Bacteria from this phylum encompass a wide variety of metabolic capabilities, including chemoautotrophs that utilize hydrogen gas, ammonia, and methane during the decomposition of organic matter. The Firmicutes are a group of Gram-positive bacteria with low –G + C content. Many are very resistant to environmental desiccation and often produce endospores to survive extreme conditions. This phylum contains pathogens such as *Clostridia* and *Bacilli*. Bacteriodetes are Gram-negative rod shaped bacteria that are also widely distributed in the environment. While they can be opportunistic pathogens but most are not pathogenic.

Most bacterial genera identified in the Singh study were shared amongst the two fly species (*L. sericata* and *L. cuprina*) investigated (Singh et al. 2015). *Providencia*, *Ignatzchineria*, and *Lactobacillus* constituted several of the most dominant populations shared between the two species. *Providencia*, already noted above as a key component of the blow fly microbiome, is a member of the family Enterobacteriaceae. It can be an opportunistic pathogen living in soil, water, and sewage, causing diarrhea with fever and tachycardia, and leading to low blood pressure. The other Proteobacterium, *Ignatzchineria* was first isolated as the dominant species in the anterior portion of the digestive tract in larval *Wohlfahrtia magnifica* Schiner (Diptera: Sarcophagidae) flesh flies (Toth et al. 2006, Gupta et al. 2011). This species has been associated with myiasis, but was not generally associated with severe human disease until reports in which it was isolated in cases of bacteremia and urinary tract infection (Maurin et al. 2007, Roudiere et al. 2007, Barker et al. 2014, Le Brun et al. 2015). Neither the mechanism nor the epidemiology of an *Ignatzchineria* infection has been defined and the cases were all associated with a corresponding fly larvae infestation where *Ignatzchineria* was isolated concurrent with other bacteria, such as *Enterococcus* and *Providencia* (Le Brun et al. 2015). *Lactobacillus* is a Firmicute that is a member of the lactic acid bacterial group. These bacteria constitute a major portion of the microbiota of the gut and other body sites. They produce alcohol and lactic acid from sugars which lowers pH and that controlled fermentation is exploited by industry in the production of items such as yogurts, beer, sourdough bread, cider, other fermented foods and animal feeds. It is worth noting that this genus was also found in high relative abundance in the remarkably microbe-depauperate *L. sericata* salivary gland, along with *Proteus* (Singh et al. 2015). In combination, the metabolic by-products of these two groups would be expected to neutralize one another, which may be important to salivary gland function. *Lactobacillus* can also inhibit the activity of other microorganisms by producing bacteriocins with antimicrobial and antifungal properties (Inglin et al. 2015).

In contrast to their role in transmission of bacteria, blow flies may also actively participate in the eradication of pathogens. Early on, the ES of blow flies were determined to contain bactericidal substances (Greenberg et al. 1970). *Lucilia sericata* larvae are used to debride wounds and contain substances with antimicrobial properties (Blueman and Bousfield 2012), such as a DNAse capable of degrading genomic bacterial DNA (Brown et al. 2012). Flies also produce insect defensins (i.e. lucifensin; Cerovsky et al. 2010) with antimicrobial properties, and some bacteria that flies carry are capable of killing other bacteria, such as *Proteus* sp., which produce mirabilicides (i.e. phenylacetic acid and phenylacetaldehyde; Erdmann and Khalil 1986). Additionally, larvae express lysozymes in their midgut, which kill bacteria during their passage through the midgut (Valachova et al. 2014). Some of these compounds appear to have selective capabilities; the ES of *L. sericata* was more effective against Gram-positive bacteria, like *Staphylococcus aureus*, than against Gram-negative bacteria, like *Proteus* sp. and *Pseudomonas* sp. (Jaklic et al. 2008). Many questions remain about how these compounds are deployed. For example, do flies use ES compounds to selectively control the microbial community structure in their environment to their own advantage, such as to deter predators and competitors or attract mates?

Horizontal and Vertical Transmission

Microbes have limited ability for dispersal by self-propelled motility (e.g. flagella, axial filament, or gliding); and instead rely on other means for spatial and temporal transmission. Horizontal transmission of microbes is the passage of the symbiont from one host to another unrelated host, and vertical transmission of microbes is the passage of the symbiont from parents to offspring (Fine 1975). A well-studied alpha-proteobacteria that is vertically transmitted

among a variety of arthropods, including some blow flies, is *Wolbachia* (Stouthamer et al. 1999, Baudry et al. 2003, Mingchay et al. 2014). Studies suggest that a benefit of vertically-transmitted infection is the prevention of more virulent infection, by horizontally transmitted organisms (Lively et al. 2005). *Wolbachia* also has the capability to cause reproductive isolation between infected and uninfected flies, although no such evidence for *Wolbachia*-induced reproductive isolation has been documented in blow flies (Baudry et al. 2003, Mingchay et al. 2014).

Horizontal transmission of pathogenic bacteria by the exterior surfaces and mouthparts, of flies, along with internal transfer via feces and vomit has long been known to occur in many fly species (Förster et al. 2007, 2009; Pava-Ripoll et al. 2012). Blaak et al. (2014) demonstrated the horizontal acquisition of *Escherichia coli* with extended-spectrum antibiotic resistance to most beta-lactam antibiotics by blow flies at a poultry facility. Horizontal transmission of various bacteria in blow flies was recently assessed in a metagenomic study of *L. sericata* and *L. cuprina*, that characterized and compared the bacteria on adult flies and the fresh liver before they oviposited on it, followed by the resulting larvae and the aged liver after larval development (Singh et al. 2015). Of the 138 genera found, adult flies had 23 unique genera, larvae had 6, fresh liver had 14, and aged liver had 40. There were 15 genera shared by all, of which *Proteus*, *Enterococcus*, and *Lactobacillus* (all described above) were the most dominant. Thirteen bacterial genera were found on the aged liver that were not present on the fresh liver, but were present on the adult flies; thus representing horizontal transfer from the adult flies to the liver; the dominant bacteria of these was *Staphylococcus*. Additionally, four bacteria were found associated with the larva that were present on the liver, but not on adult flies, representing horizontal transfer from the liver to the larvae; the dominant bacteria of these were *Vagococcus* and *Lactococcus*. No bacteria were found shared between only adults and larvae and not liver, so while vertical transmission could have occurred it is not possible to isolate that from horizontal transmission in this experimental design. Interestingly there were many bacteria (88 genera) present on the liver that were not transferred to the larvae. Perhaps larval ES or environmental conditions (i.e. temperatures within the larval mass) prevented the transfer of more genera to the larvae.

Dispersal strategies for bacteria include many routes, such as the avoidance of elimination during insect development discussed above, the most prominent being physical interaction with a contaminated host; the exchange through contact with airborne droplets or light weight particles containing bacteria that can remain airborne for long periods, such as fungal spores; and fecal to oral, wound or mucosal surface transmission. There is also indirect contact with an environmental substance or surface, or a host, such as a fly that retains the microbe after it was acquired by contact with a contaminated host or other surface (Barro et al. 2006). Ebert (2013) reviews the many symbionts, microbes among them, that utilize multiple methods of transmission (mixed-mode transmission), not just exclusively one (single-mode transmission). Combinations of these strategies can enhance the likelihood of persistence depending on the ecological conditions and plays a role in increased genetic drift and the evolution of virulence and genome architecture (Moran and Baumann 2000, Ebert 2013). Symbionts can persist within a population under a wider range of ecological conditions if they have mixed versus single modes of transmission (Lipsitch et al. 1995a, Lipsitch et al. 1995b).

The interdependence between insects and bacteria is more amalgamated than simply for growth augmentation. Blow fly oviposition is also induced by metabolic products of bacteria, some of which were noted above (Hammack et al. 1987, Chaudhury et al. 2010). In an interesting study by Pechal (2012), the microbiome associated with decomposing swine carcasses that were accessible to flies or in which the flies were excluded was characterized over five days. They demonstrated significant changes in the bacterial community during decomposition between insect access and exclusion carcasses. When flies were not present, Proteobacteria was a dominant taxon throughout the 5-d sampling period, whereas relative abundance of Firmicutes decreased as decomposition progressed. However, when flies were present, the opposite occurred, as Proteobacteria decreased over time and Firmicutes became the dominant taxon by the fifth day of decomposition. At the genus level, *Psychrobacter* and *Moraxella* were dominant for both exclusion and access carcasses. But other bacterial succession patterns differed as decomposition progressed. Without insect access, *Aeromonas* and *Shewanellaceae* were detected only on the first day, *Peptostretpococcus* was detected only on the fifth day and *Proteus* transitioned to the dominant taxon by the third and fifth day. When insects were present, *Providencia* was dominant on the first and third day, but Bacillales was dominant by the fifth day. *Proteus* and *Corynebacterium* were present on the third day of decomposition, but by the fifth day, *Psychrobacillus* and *Ignatzschineria* were the dominant taxa and *Clostridium sensu stricto* (10%) was also detected. Additionally, Pechal and Benbow (2016) investigated the internal microbiome between *Calliphora terraenovae* Macquart (Diptera: Calliphoridae) utilizing streams where decomposing salmon carrion was a readily available resource or streams without carcasses. Bacteroidetes, Firmicutes, and Proteobacteria were the dominant phyla found increased on the *C. terraenovae* larval samples. They shared microbial communities with their food resource (salmon carrion); for example, Firmicutes, dominated by *Vagococcus*, *Clostridium*, and the candidate family Tissierellaceae increased due to this interaction.

We know from previous studies that both *Proteus* and *Providencia* are important players in the attraction of blow flies (Hammack et al. 1987, Chaudhury et al. 2010, Ma et al. 2012, Tomberlin et al. 2012), but what we do not know is the extent to which these bacteria are transported by the flies and seed the carcass as a result of the fly interaction or the interkingdom communication with the bacteria already associated with carcasses. While the microbiome of the fly was not analyzed in the Pechal (2012) study, it is interesting to note that an insect-associated genus, *Ignatzschineria*, was not a dominant member of the bacterial community on exclusion carcasses, but was in the later stages of the accessible carcasses, eluding to the likelihood that the insects played a role in the presence of this genus on the carcass. On the other hand, *Proteus* was dominant in the later stages of the exclusion carcass, indicating the bacteria were already present on the carcass or came from the environment and that the blow fly species that secondarily colonize the remains may be responding to its olfactory cues to select oviposition sites on the resource. This observation is interesting, given the non-intuitive response of *Proteus* to the presence of insect colonization, considering the empirical evidence to suggest a mutualism between *Proteus* and some blow flies. This result would suggest that such mutualism may be more complex than the simple laboratory experiments would suggest. Alternatively, it may be the case that other members of the carrion insect community are capable of eliminating *Proteus*, even in the presence of their hypothesized mutualists.

Microbial Genomes

While bacterial community studies are invaluable for providing information regarding common and important members of blow fly

associated communities of bacteria, there can be additional valuable information gleaned from the whole genomes of specific species and strains isolated from blow flies and blow fly resources. One area where this endeavor is important relates to bacterial community studies. For example, algorithms can be used to predict community function from the community structure information provided by the 16S rDNA gene (Langille et al. 2013). This is done by extrapolating from the presence of specific taxa (via 16S sequences) to the gene compositions of entire genomes and communities using microbial genome databases to provide a prediction of metabolic potential estimated from the types and predicted functions of genes in the sequenced genomes associated with 16S sequences. This capability enables researchers to extend their interpretations of 16S data to the expected functionality of the community, facilitating the ability to ask questions like, does the community have the ability to catabolize a specific amino acid or produce a specific antibiotic? Such capability with blow fly bacteria communities may allow us to expand our understanding of the specific biological processes involved in fly–microbe interactions. However, the quality of a result from any application that relies on a database will depend greatly on the content of the database. Thus, it is imperative that genomes of additional blow fly-derived bacteria be sequenced and deposited into relevant databases to enable the best results from functional predictions. This task is most important when there are blow fly associated bacteria for which there is no known genome sequence, such as the *Ignatzshineria* commonly found with blow flies. Genome sequences for strains from this genus would enhance our ability to understand mechanisms by which these bacteria interact with flies.

Even in instances where a bacterial genome has been sequenced for a particular taxon, it may still be important to produce additional genome sequences for blow fly-derived strains. For example, the genus *Proteus*, and specifically *P. mirabilis*, has been noted numerous times in this review as important bacteria that interact with blow flies. *Proteus* sp. are also important to medicine and ecology (Drzewiecka 2016), and numerous strains of the genus, including *P. mirabilis*, have been sequenced (Pearson et al. 2008, Sullivan et al. 2013, Mac Aogáin et al. 2016). Recently, an *L. sericata*-derived strain of *P. mirabilis* was sequenced and assembled (Yuan et al. 2016), revealing some interesting features of that strain. First, there are two high quality reference genomes for the species and the blow fly derived strain is much more similar to the BB2000 strain used to study self-recognition during swarming of *P. mirabilis* (Gibbs et al. 2008, Cardarelli et al. 2015) than is was to the original reference genome obtained from a clinical strain of the species. Second, it is clear that the fly-derived strain contains lineage specific insertions and deletions. It remains to be seen if there are genes relevant to fly interactions in those regions of the genome, but such a hypothesis will be important to test. It is clear that *P. mirabilis* and other bacteria often have a core genome (composed of sequences shared by all members) as well as numerous auxiliary components (composed of sequences not shared by all members of the species and sometimes unique to one strain; Collins and Higgs 2012). Evolutionary comparisons, such as those described below, of fly-derived and non-fly-derived bacteria will help to determine if core genome components, auxiliary components, or both components of bacterial genomes are important to fly–bacterial interactions.

Microbe Evolution in Hosts

Evolutionary analyses of bacteria are a promising avenue for elucidating fly–microbe interactions. The approaches can take two different forms: studies of naturally-occurring microbes and experimental evolution of microbial populations in the laboratory. Both approaches have been under-utilized to understand fly–microbe interactions in general, and within blow flies in particular. We highlight areas where evolutionary approaches can be applied to improve our understanding of the effects of bacteria on blow fly behavior and physiology.

Evolutionary analyses can reveal the long-term relationships between hosts and bacteria, as well as environmental factors that affect survival and reproduction of host-associated bacteria. When bacteria coevolve with their hosts for millions of years, the phylogenetic relationships of host and bacterial species are congruent (Munson et al. 1991, Page 2003, Moeller et al. 2016). Discordant evolutionary relationships of hosts and bacteria can then be used to reveal adaptations of a bacterial species to a particular host diet or

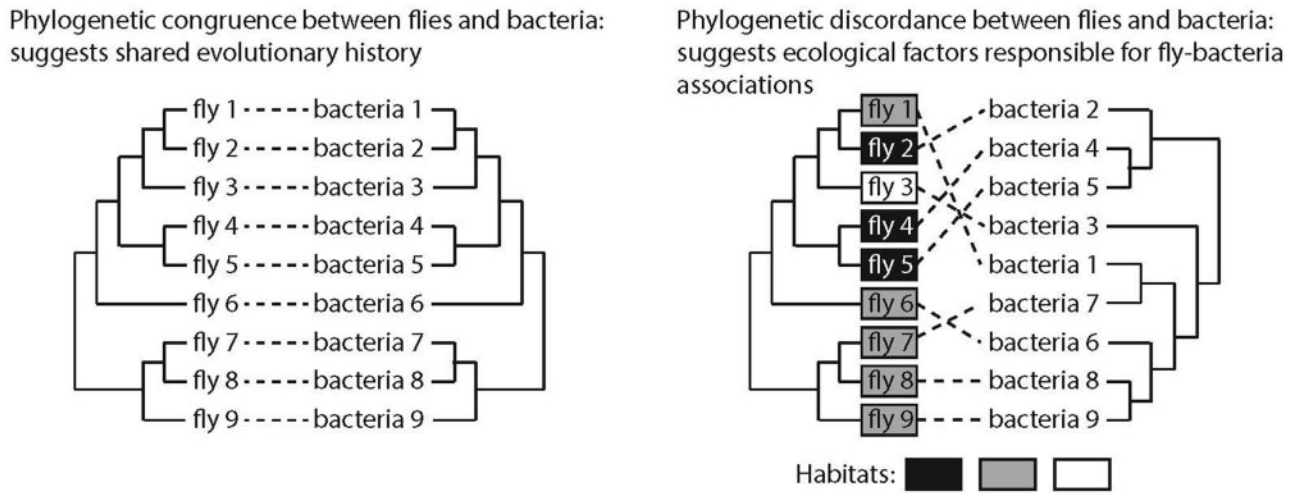

Fig. 5. Comparisons of phylogenies of host flies and their associated bacteria can inform our understanding of the habitats in which flies live. Phylogenies show the evolutionary relationships of nine fly species and bacterial samples taken from each of the nine flies. On the left, the phylogenies of the flies and bacteria are in perfect congruence, demonstrating that the bacteria have evolved within each species and without horizontal transmission between species. On the right, the phylogenies of the flies and bacteria are discordant, suggesting that fly habitat is a better predictor of bacterial association than evolutionary ancestry. Three candidate habitat categories are defined for each fly species based on the evolutionary relationships of their associated bacteria.

life history (Fig. 5). For example, the gut microbial communities in mammals and insects are associated with the host diet, and not necessarily congruent with the phylogenetic relationships of the hosts (Muegge et al. 2011, Yun et al. 2014). As described above, the preferred substrate for oviposition differs across blow fly species, and we therefore expect blow fly species that oviposit in the same substrate to have associated microbial communities that resemble each other. In addition, the microbial communities associated with blow fly species whose larvae are blood-feeding parasites of birds (*Protocalliphora* sp.) should differ from blow flies whose larvae develop in carrion or flesh (Sabrosky et al. 1989).

Comparisons of host-associated bacteria across blow fly species have tremendous potential to resolve cryptic variation and similarities in life history strategies across species. While the larval habitats of blow flies are easily characterized, the breadth of adult diet choice and greater adult mobility can expose the flies to bacteria whose origin is more difficult to determine. For example, a female bias in visits to oviposition sites could result in sexually dimorphic microbial communities within blow fly adults (Mohr and Tomberlin 2014), which may translate into variation in the bacterial communities of their offspring and in traits dependent on bacteria. Adult females will also use a variety of nutrient resources for oogenesis, ranging from pollen (Brodie et al. 2015) to carrion, which will differ considerably in their microbe contents (Fridman et al. 2012, Metcalf et al. 2013, Shade et al. 2013, Aleklett et al. 2014, Pechal et al. 2014). Differential resource use may also enable fly-associated bacteria to exchange genes with bacteria from a diverse pool of community members (Akhtar et al. 2009, Crippen and Poole 2009), which may alter bacterial phenotypes compared to more commonly studied "standard" strains of the same bacterial species. Thus, *E. coli* from a fly may not contain the same gene content as common laboratory strains that are well studied but raised continuously in monoculture, and cannot be expected to demonstrate the same traits as their "domesticated" conspecifics. This logic is also at play in *P. mirabilis* strains described above, where the clinical strain that was originally sequenced was not considered a sufficient reference genome to study self-recognition, as the strain used to study that system exhibited a different gene content compared to the clinical strain (Sullivan et al. 2013). Community analyses from a broad sampling of blow flies and their larval substrates could therefore identify specific aspects of fly behavior, diet, and life history that affect the microbial communities found across species. These approaches could also produce candidate bacterial species that affect blow fly behavior and physiology in a sex-, diet-, or life-stage-specific manner.

Culture-dependent and DNA sequencing approaches have revealed which bacteria taxa are associated with blow fly larvae and adults (see above), and genome sequencing has identified inter-strain differences between blow fly associated and other *P. mirabilis* strains (Yuan et al. 2016). However, we do not know which specific genetic changes in *Proteus* and other bacteria genomes are responsible for successful colonization of blow fly salivary glands and digestive tract. Laboratory experiments in which microbes evolve within hosts are especially revealing of the aspects of host physiology that have the greatest effects on bacterial survival (Brockhurst and Koskella 2013, Hoang et al. 2016). For example, *E. coli* populations in the guts of laboratory mice evolve higher growth rates and reduced motility (Giraud et al. 2008, Lee et al. 2010). Evolution experiments involving bacteria within blow fly larvae and adults therefore have tremendous potential to reveal specific bacterial traits that are adaptive to the fly digestive tract or salivary glands. Sequencing of bacteria strains that evolved within blow flies would allow for the identification of specific mutations that promote colonization, and the targeting of the products of those genes could reduce the ability of pathogenic bacteria to be vectored by blow flies. In addition, host-specific adaptation should result in an evolved bacteria strain that affects fly physiology differently than the ancestral strain. Aside from immune-related genes (see below), little is known about specific fly traits that regulate bacterial proliferation. It would thus be informative of fly traits involved in bacterial interactions to compare the phenotypes of blow flies fed ancestral and derived strains of host-evolved bacteria.

Comparative Genomics of Blow Flies to Study Ecological Interactions Between Bacteria and Hosts

Genome sequences of blow flies will also improve our understanding of interactions with bacteria and the specific aspects of fly physiology responsible for regulating bacteria. For example, comparisons of gene content and sequence evolution in the genomes of related fly species can reveal long-term fly–bacteria interactions. The fly innate immune system includes effector proteins that suppress bacteria growth or kill microbes, such as antimicrobial peptides (AMPs). These AMPs are promising candidates for treatment of bacteria infections, especially those that are resistant to existing antibiotics (Bexfield et al. 2004, Cerovsky et al. 2010, Poppel et al. 2015). The genes encoding effector proteins vary in copy number across *Drosophila* sp. and they have expanded along the house fly lineage (Sackton et al. 2007, Scott et al. 2014, Sackton et al. 2016). In addition, genes encoding immune system proteins involved in recognizing bacteria are more likely to evolve under positive Darwinian selection in *Drosophila* and have also experienced a copy number expansion in house flies (Sackton et al. 2007, Scott et al. 2014, Sackton et al. 2016). Both of these patterns suggest that the fly immune system evolves in an arms race to suppress bacterial infections, rapidly changing to keep up with a diverse community of rapidly evolving bacteria. These observations make blow flies an ideal system to study fly interactions with bacteria, given their associations with numerous bacteria taxa.

The growing number of genome sequences of blow flies and their close relatives will allow for similar comparative analyses of gene content and sequence evolution to elucidate specific aspects of blow fly physiology involved in host–microbe interactions. The expansion of immunity-related genes in the house fly genome suggests that house flies live in a more pathogen-rich environment than *Drosophila*, and require a more diverse immune repertoire to suppress infections (Scott et al. 2014). The selection pressures on the blow fly immune system are expected to differ depending on exposure to microbes, which will depend on larval habitat and adult behavior. Comparisons of immune-related genes across blow fly species and between blow flies and other filth flies could therefore reveal differences in the diversity and abundance of microbial pathogens and other bacteria encountered across the fly life cycle. Additionally, these analyses have the potential to identify cryptic differences in larval and adult habitats between species.

Functional Genomics as a Tool to Understand Blow Fly–Bacteria Interactions

Sequenced blow fly genomes also open up new possibilities for experiments that interrogate the effect of bacteria exposure on fly physiology. Numerous studies on blow flies and their relatives have

demonstrated that the expression of genes encoding AMPs is up-regulated upon exposure to bacteria, but sequenced genomes now allow for the identification of novel recognition and effector components of the immune system and other genes involved in the physiological response to bacteria exposure through genome-wide analyses of gene expression (Joyner et al. 2013, Nayduch et al. 2013, Poppel et al. 2015, Sackton et al. 2016). For example, 47 expressed AMPs were identified in a high throughput RNA sequencing experiment in which *L. sericata* larvae were infected with *P. aeruginosa* and *S. aureus* (Poppel et al. 2015). The activities of 23 of these AMPs were tested against a panel of Gram-positive and -negative bacteria, and their effects varied across AMP–bacteria combinations and depended on which other AMPs were present in the treatment (Poppel et al. 2015). Additional experiments could determine which AMPs (or combinations of AMPs) are best suited to be used as antimicrobial agents for wound therapy or provide targets for pest control measures.

The power of genomic approaches to identify previously uncharacterized components of the blow fly response to bacteria can be increased by comparing multiple species or different strains within a species (Sackton and Clark 2009, Sackton et al. 2010), assaying gene expression in flies carrying mutations in genes involved in host–bacteria interactions (Broderick et al. 2014), or considering different diets, life stages, and sexes (Jacobs et al. 2016). These approaches will only become more feasible as the genomes of additional blow flies are sequenced and the tools for targeted mutagenesis become more tractable in nonmodel organisms (Sander and Joung 2014).

Our understanding of the bacterial perspective of fly–microbe interactions will also benefit from improvements in functional genomics. For example, the effects of specific bacterial gene products on blow fly physiology could be assessed through exposing flies to mutant bacteria or the metabolic products that differ between mutant and wild type bacteria (Ma et al. 2012, Tomberlin et al. 2012, Liu et al. 2016), and measuring blow fly gene expression genome-wide in various tissues. In addition, the molecular nature of phenotypic plasticity in bacteria strains could be interrogated through genome-wide expression analyses of bacteria grown in different conditions (including exposure to flies and even harvested from within flies) to identify novel genes whose expression is associated with variable response to environmental cues. These differentially expressed genes could be candidates for attracting or repelling blow flies. Likewise, expression of bacterial genes when fly-derived species are competing with those that are 1) the same species, but not fly derived, 2) controlled in maggot therapy, 3) differentially competitive within flies (like the *Proteus* – *Salmonella* competition in blow flies studied by Greenberg (1965) and Greenberg et al. (1970)), or 4) in blow fly habitats but are not commonly associated with blow flies, would be useful for understanding the ecology and molecular mechanisms underpinning microbial interactions in the wild.

For example, in mammals we know that compositional and structural shifts of gut microbes and their associated metabolites can affect behaviors, such as neurodevelopmental disorders (Hsiao et al. 2013), and that the nasal cavity, also a rich source of indigenous microbiota, can influence health and disease (Yan et al. 2013). But how much influence do the nasal and gut microbe community structures have on the olfactory capabilities and resulting behavior in animals? Francois et al. (2016) evaluated the development of olfactory epithelium in the nares and nasal cavities of germfree mice. In normal pathogen-free mice, although individual variability exists, they determined that the olfactory epithelium was primarily occupied by Bacteriodetes (15–60%; dominated by *Bacteriodaceae*) and Firmicutes (30–70%; dominated by *Enterococcaceae*, *Lachnospiraceae*, and *Ruminococcaceae*)) and less so by Proteobacteria (5–25%; dominated by *Enterobacteriaceae*) and Actinobacteria (<10% dominated by *Bifidobacteriaceae*). Interestingly, the absence of these gut microbes resulted in a thinner layer of olfactory cilia, a reduced epithelial cell turnover rate and a reduced expression of many genes associated with the olfactory signal transduction pathway. Thus, the microbial community structure may indeed influence the odor sensory capabilities of its host. Flies use the olfactory sensilla and receptors on their antenna and maxillary palps to smell odors and locate resources (Shanbhag et al. 1999, Wasserman and Itagaki 2003). We know that the area around mouthparts can be heavily contaminated with bacteria (Barro et al. 2006). Could the bacteria present around the antennae and fly mouthparts, as well as their gut microbiome, affect development of odor perception organs in the fly? Could raising flies under certain lab conditions or on specific resources, which affect the community structure of the gut microbiome, change adult olfactory capabilities and ultimately the behavior of the fly being investigated? What impact would this have on the olfactory capabilities of wild flies that exist in different ecoregions and are exposed to a differential range of microbes? How much are the host's olfactory capabilities and behavior related to their gut microbial community structure? These are open questions that will be important to study.

Proteomic Responses in Hosts and Microbes

Transcriptomic analyses have potential to identify blow fly genes involved in clearance of bacteria (e.g., AMPs) because those genes are transcriptionally up-regulated upon exposure to microbes. However, genes encoding other components of the fly immune system do not experience changes in expression upon infection, and instead the gene products react to bacterial exposure through modifications to the proteins themselves. For example, information about bacterial infection in flies is communicated through the Toll, IMD, JAK/STAT, and JNK pathways via the cleavage, phosphorylation, and degradation of signaling proteins, which ultimately leads to the up-regulation of effector genes that directly respond to the bacteria (Lemaitre and Hoffmann 2007, Buchon et al. 2014). Proteomic approaches are therefore necessary to characterize the physiological response of blow flies to bacteria mediated by the immune system. Some of these proteomic techniques, including the combination of two dimensional electrophoresis with mass spectrometry (Marouga et al. 2005, Samyn et al. 2006) and iTraq (Dong et al. 2007, Evans et al. 2007), are greatly facilitated by a sequenced genome in order to determine the potential proteins present in the sample. The growing number of blow fly and bacteria genomes in this system will improve our ability to apply proteomic technologies to understand the blow fly response to bacteria as proteomic analyses are enhanced by prior genomic knowledge (Samyn et al. 2006). Applying this approach to different blow fly species exposed to various bacterial taxa and strains will allow for the identification of fly and bacteria species-specific interactions, which are candidate evolved differences across blow flies in response to the variation in substrates in which they develop and diets they consume (see above).

A similar logic applies to the microbial side of the equation, as protein studies have revealed critical aspects of several host–microbe interactions (Shao et al. 2002, Zhang et al. 2005, Chisholm et al. 2006, Lee et al. 2008, Schmidt and Völker 2011), including the role of pathogen proteins in evading immune detection, virulence, communication, and survival. As an example *Yersinia* and *Pseudomonas* virulence is regulated in part by proteases (Shao et al. 2002)—

enzymes that cleave other proteins. Thus, while mRNA studies can provide valuable information regarding interactions between bacteria and hosts, there are also levels of regulation independent of RNA that are equally valuable. As the genomes of blow flies and their microbial associates are produced, these applications will become more feasible—opening up new areas of inquiry.

Metabolic Studies

Metabolic consequences of insect–microbe interactions are important for understanding the mechanisms underpinning them. As an example, this review has demonstrated the importance of indole to a number of blow fly–microbe interactions. This amino acid-derived molecule is also important to bacterial signaling and mammalian regulation of inflammation. In this era where we are learning through studies of bacteria communities that bacteria impact and are associated with a wide array of eukaryotic phenotypes, it will be necessary to step beyond bacterial community structures to the mechanisms underpinning the importance of certain community members on eukaryotic traits. Thus, it will be important to determine if indole is the exception or the rule when attempting to understand interactions among bacteria and eukaryotes (Lee et al. 2015). It will be important to answer the following (and related) questions: Are molecules like indole a common means used by many bacteria to regulate interactions with eukaryotes or is indole a special molecule with properties that are distinct from other metabolites important to the system? Are there many or few metabolites important to fly–microbe interactions? Are regulatory metabolites important to fly interactions distinct to certain clades of bacteria or are they common across taxa? How do flies alter the physiology of bacteria (and vice versa)? The answers to some of these questions can be elucidated, in part, through genomic studies of fly-derived bacteria described above. However, these studies will need to be paired with *in vivo* experimental manipulations to fully appreciate the greater picture of their interactions.

Repeating previous experiments, augmented with modern chemical and biological techniques, to enhance our metabolic understanding of blow fly interactions with bacteria would be helpful. For example, in the 1960s and 1970s Bernard Greenberg and his colleagues studied the competitive dynamics of bacteria associated with flies (Greenberg 1973). One particular focus was the competition between *Proteus* and *Salmonella* within filth flies (Greenberg 1965, 1968, 1969, Greenberg and Klowden 1972). In this system, the impact of specific bacteria on the health and success of the fly (and vice versa) becomes important. Therefore, they conducted gnotobiotic experiments on *M. domestica* and *L. sericata* to determine the impact of *P. mirabilis* (as compared to a mixed bacterial community) on the persistence of *Salmonella* in flies, finding that exclusion by *P. mirabilis* was more effective in *L. sericata* than in house flies (Greenberg et al. 1970). This strategy is critical in blow fly studies, as these flies are associated with numerous bacterial species (Singh et al. 2015). Therefore, it is necessary to break the impact of a fly associated community into its individual components in order to dissect the role of specific taxa in community functions. Recently, this strategy has also been used to dissect the impact of specific bacteria on blow fly life history (Crooks et al. 2016). This approach is feasible in blow flies as many of their bacterial associates can be cultured.

Now it is possible to conduct these experiments and investigate molecular changes in bacteria and flies associated with gnotobiotic flies as compared to sterile and nonsterile flies at a level of detail that was not possible when Greenberg and his colleagues were conducting their experiments (Graf 2016). This can be done at the mRNA and proteomic levels described above. However, metabolic shifts can also be studied. It is possible to assay basic metabolic responses of flies and bacteria with any number of commercially available panels and tests, such as Biolog plates (Garland and Mills 1991, Garland 1997, Smalla et al. 1998, Choi and Dobbs 1999, Dobranic and Zak 1999, Classen et al. 2003, Bochner et al. 2011), which can be used to evaluate metabolic profiles of both bacteria and eukaryotes. Given the connections between bacterial signaling molecules and essential amino acids, the metabolic profiles of both bacteria and flies will be useful in dissecting their interactions. Some work has already been done with this tool in carrion ecology (Pechal et al. 2013), using plates that included molecules found to be of importance in previous blow fly studies (Ma et al. 2012, Tomberlin et al. 2012). However, individual blow flies and bacteria are small components of that complex system, making it difficult to assign metabolic responses to specific bacteria. Further, and more specific, dissection of blow fly interactions with bacteria using these and similar tools will be useful in determining and differentiating among hypotheses regarding the mechanisms regulating them.

In addition, mass spectrometry (MS) and other chemical analysis techniques can be used to investigate the specific impacts of the presence or absence of key bacteria noted in this review on metabolic responses of flies. Nano-scale ion MS has been used to evaluate metabolic roles of specific bacteria in vivo (Lechene et al. 2007, Li et al. 2008). In the blow fly system, it is also useful to observe advances in the study of the chemistry of decomposition. Forensic studies of the VOCs found during decomposition are commonly conducted, as signatures of human and animal decomposition are useful in death investigations (Vass et al. 2002, Statheropoulos et al. 2005). This literature demonstrates a complex array of products produced during decomposition, including known fly attractants—many of which are chemically similar to other components of the system and include many components produced by the numerous microbes associated with decomposing remains. In order to more fully dissect the chemical signatures of decomposing remains, two dimensional (through the use of double gas chromatography separation steps) MS has been used to enhance the distinctions among similar molecules (Stadler et al. 2012, Perrault et al. 2015). Such advanced chemical analysis approaches will also be useful in gnotobiotic dissection of the fly–bacteria interactions in relation to and independent of decomposition processes. As noted above the mutation of a single *P. mirabilis* gene impacts *L. sericata* biology, yet the chemical impact of that mutation alters the concentrations of numerous molecules produced by the bacterium, including several known and potentially novel fly attractants derived from essential amino acids (Ma et al. 2012, Tomberlin et al. 2012, Liu et al. 2016). The potential impact of such bacterial mutations on internal interactions with hosts could also be numerous. Therefore, to fully appreciate the impacts of mutations within a species or metabolic shifts due to presence of different bacteria on the complex metabolite repertoire of eukaryotes, it will be useful to employ modern chemical analyses to screen for novel components regulating blow fly–microbe interactions. Doing so will assist in breaking down the roles of particular bacteria in blow fly interactions. Expanding the approach to mixtures of key species and whole communities will allow for the determination of the degree to which single species versus communities are important to such interactions.

Future Endeavors

Technological and conceptual advances have set the stage for furthering our understanding of how blow flies interact with bacteria and other microbes. The ability to sequence and assemble genomes

rapidly and cheaply has opened the door to developing a more comprehensive genetic, physiological, and metabolic framework for the types of studies described above that surpass the community sampling information provided by the small number of 16S rDNA sequencing studies described in the previous section. These proposed advances can be determined through genomic studies of both the microbes in this system and the flies. Pairing such studies with organismal studies of behavior and physiology will deepen our understanding of how blow flies interact with bacterial symbionts.

In conclusion, due to their close association with decomposing organic matter, blow flies also have close associations with numerous bacteria that thrive in these environments. Research has shown that bacteria are the initial colonizers of such material, and their degradation of these materials results in the production of VOCs that attract blow flies to colonize the material. Additionally, numerous bacteria species have been identified from the flies themselves, and laboratory studies indicate some bacteria are required for normal immature development, while other species enhance development. These findings hold true for other groups of flies such as mosquitoes (Diptera: Culicidae) and black soldier flies (Diptera: Stratiomyidae), suggesting that blow flies might be a good model system for studying such interactions. The bacterium *P. mirabilis* is commonly found throughout numerous systems, and a compound it produces, indole, is rapidly being identified as a compound that has enormous effects on the behavior of many higher animals.

From basic research aimed at understanding the underlying mechanisms by which these two groups of organisms interact, numerous areas of application can arise. These systems can be mimicked and manipulated to develop trapping mechanisms for filth flies in agricultural settings. The mechanisms by which quorum-sensing compounds such as indole are produced have implications in medicine, as these result in pathogenicity in many bacteria species that affect human health. The response of blow flies to bacterially-derived VOCs in terms of resource location can advance forensic entomology, allowing researchers to determine how, why, and when blow flies are attracted to a carrion resource. These applications represent only the beginning of this exciting area of research and the establishment of blow fly interactions with bacteria as a model system for studying the effects and impacts of microbe–insect interactions and interkingdom communication.

Acknowledgment

A.M.T. and J.K.T. would like to thank Texas A&M University and Texas A&M AgriLife Research for providing support to develop this manuscript. We thank Robert Alaniz at Texas A&M for helpful discussions regarding gnotobiotic research and Natalie Gasz at Deakin University for reviewing this manuscript. AMT is funded by the National Institute of Justice grant 2015-DN-BX-K020. JKT, TLC, AMT, and BS were also funded by the National Institute of Justice grant 2010-DN-BX-K243. Points of view in this document are those of the authors do not necessarily represent the official position or policies of the U.S. Department of Justice.

References Cited

Aak, A., T. Birkemoe, and G. K. Knudsen. 2011. Efficient mass trapping: catching the pest, *Calliphora vicina*, (Diptera, Calliphoridae), of Norwegian stockfish production. J. Chem. Ecol. 37: 924–931.

Ahmad, A., A. Broce, and L. Zurek. 2006. Evaluation of significance of bacteria in larval development of *Cochliomyia macellaria* (Diptera: Calliphoridae). J. Med. Entomol. 43: 1129–1133.

Akhtar, M., H. Hirt, and L. Zurek. 2009. Horizontal transfer of the tetracycline resistance gene tetM mediated by pCF10 among *Enterococcus faecalis* in the house fly (*Musca domestica* L.) alimentary canal. Microb. Ecol. 58: 509–518.

Aleklett, K., M. Hart, and A. Shade. 2014. The microbial ecology of flowers: An emerging frontier in phyllosphere research1. Botany 92: 253–266.

Anderson, G. S., and N. R. Huitson. 2004. Myiasis in pet animals in British Columbia: The potential of forensic entomology for determining duration of possible neglect. Can. Vet. J. 45: 993–998.

Anstead, C. A., P. K. Korhonen, N. D. Young, R. S. Hall, A. R. Jex, S. C. Murali, D. S. Hughes, S. F. Lee, T. Perry, A. J. Stroehlein, et al. 2015. *Lucilia cuprina* genome unlocks parasitic fly biology to underpin future interventions. Nat. Commun. 6: 7344.

Axtell, R. C. 1986. Status and potential of biological control agents in livestock and poultry pest management systems, pp. 1–9. *In* R. S. Patterson and D. A. Rutz (eds.), Biological control of muscoid flies. Entomological Society of America, Miscellaneous Publication 61.

Axtell, R. C., and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annu. Rev. Entomol. 35: 101–126.

Barker, H. S., J. W. Snyder, A. B. Hicks, S. P. Yanoviak, P. Southern, B. K. Dhakal, G. R. Ghimire, and M. R. Couturier. 2014. First case reports of *Ignatzschineria* (*Schineria*) *indica* associated with myiasis. J. Clin. Microbiol. 52: 4432–4434.

Barnes, K. M., and D. E. Gennard. 2011. The effect of bacterially-dense environments on the development and immune defences of the blowfly *Lucilia sericata*. Physiol. Entomol. 36: 96–100.

Barnes, K. M., and D. E. Gennard. 2013. Rearing bacteria and maggots concurrently: A protocol using *Lucilia sericata* (Diptera: Calliphoridae) as a model species. Appl. Entomol. Zool. 48: 247–253.

Barnes, K. M., D. E. Gennard, and R. A. Dixon. 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. Bull. Entomol. Res. 100: 635–640.

Barraud, N., M. V. Storey, Z. P. Moore, J. S. Webb, S. A. Rice, and S. Kjelleberg. 2009. Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. Microb. Biotechnol. 2: 370–378.

Barro, N., S. Aly, O. C. Tidiane, and T. A. Sababenedjo. 2006. Carriage of bacteria by proboscises, legs, and feces of two species of flies in street food vending sites in Ouagadougou, Burkina Faso. J. Food Prot. 69: 2007–2010.

Barton Browne, L. B. 1958. The choice of communal oviposition sites by the Australian sheep blowfly *Lucilia cuprina*. Aust. J. Zool. 6: 241–247.

Baudry, E., J. Bartos, K. Emerson, T. Whitworth, and J. H. Werren. 2003. *Wolbachia* and genetic variability in the birdnest blowfly *Protocalliphora sialia*. Mol. Ecol. 12: 1843–1854.

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

Bexfield, A., Y. Nigam, S. Thomas, and N. A. Ratcliffe. 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 Infect. 6: 1297–1304.

Bishopp, F. C., J. D. Mitchell, and D. C. Parman. 1917. Screw-worm and other maggots affecting animals. US Department of Agriculture Farmer's Bulletin 857.

Blaak, H., R. A. Hamidjaja, A.H.A.M.V. Hoek, L. D. Heer, A.M.D.R. Husman, and F. M. Schets. 2014. Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. Appl. Environ. Microbiol. 80: 239–246.

Blenkiron, C., P. Tsai, L. A. Brown, V. Tintinger, K. J. Askelund, J. A. Windsor, and A. R. Phillips. 2015. Characterisation of the small RNAs in the biomedically important green-bottle blowfly *Lucilia sericata*. PLoS ONE 10: e0122203.

Blueman, D., and C. Bousfield. 2012. The use of larval therapy to reduce the bacterial load in chronic wounds. J. Wound Care 21: 244–253.

Boatright, S. A., and J. K. Tomberlin. 2010. Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae). J. Med. Entomol. 47: 917–923.

Bochner, B. R., M. Siri, R. H. Huang, S. Noble, X.-H. Lei, P. A. Clemons, and B. K. Wagner. 2011. Assay of the multiple energy-producing pathways of mammalian cells. PLoS ONE 6: e18147.

Boulay, J., C. Betremieux, V. Hédouin, and D. Charabidzé. 2015. A first insight into the scanning behaviour of the presocial blow fly larvae. Physiol. Entomol. 40: 317–324.

Boulay, J., J.-L. Deneubourg, V. Hédouin, and D. Charabidzé. 2016. Interspecific shared collective decision-making in two forensically important species. Proc. R. Soc. B. 283: 20152676.

Broce, A. B. 1985. Myiasis-producing flies, pp. 83–100. *In* R. E. Williams and R. D. Hall (eds.), Livestock Entomology. Wiley Press, New York, NY.

Brockhurst, M. A., and B. Koskella. 2013. Experimental coevolution of species interactions. Trends Ecol. Evol. 28: 367–375.

Broderick, N. A., N. Buchon, and B. Lemaitre. 2014. Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. mBio 5: e01117–e01114.

Brodie, B. S., T. Babcock, R. Gries, A. Benn, and G. Gries. 2016. Acquired smell? Mature females of the common green bottle fly shift semiochemical preferences from feces feeding sites to carrion oviposition sites. J. Chem. Ecol. 42: 40–50.

Brodie, B. S., R. Gries, A. Martins, S. VanLaerhoven, and G. Gries. 2014. Bimodal cue complex signifies suitable oviposition sites to gravid females of the common green bottle fly. Entomol. Exp. Appl. 153: 114–127.

Brodie, B. S., M. A. Smith, J. Lawrence, and G. Gries. 2015. Effects of floral scent, color and pollen on foraging decisions and oocyte development of common green bottle flies. PLoS ONE 10: e0145055.

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

Brown, A., A. Horobin, D. G. Blount, P. J. Hill, J. English, A. Rich, P. M. Williams, and D. I. Pritchard. 2012. Blow fly *Lucilia sericata* nuclease digests DNA associated with wound slough/eschar and with *Pseudomonas aeruginosa* biofilm. Med. Vet. Entomol. 26: 432–439.

Buchon, N., N. Silverman, and S. Cherry. 2014. Immunity in *Drosophila melanogaster* - from microbial recognition to whole-organism physiology. Nat. Rev. Immun. 14: 796–810.

Burkepile, D. E., J. D. Parker, C. B. Woodson, H. J. Mills, J. Kubanek, P. A. Sobecky, and M. E. Hay. 2006. Chemically mediated competition between microbes and animals: Microbes as consumers in food webs. Ecology 87: 2821–2831.

Bushland, R. C. 1960. Screwworm research and eradication. Adv. Vet. Sci. 6: 1–18.

Byrd, J. H., and J. C. Allen. 2001. The development of the black blow fly, *Phormia regina* (Meigen). Forensic Sci. Int. 120: 79–88.

Byrd, J. H., and J. F. Butler. 1996. Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. J. Med. Entomol. 33: 901–905.

Byrd, J. H., and J. L. Castner. 2010. Forensic entomology: The utility of arthropods in legal investigations. 2nd edn. CRC Press, Boca Raton, FL.

Caballero, M., G. Hernandez, F. Poudevigne, and I. Ruiz-Martinez. 1996. Isolation and identification of bacteria associated with the screwworm fly *Cochliomyia hominivorax*, Coquerel and its myiasis. Ann. NY Acad. Sci. 791: 248–254.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7: 335–336.

Cardarelli, L., C. Saak, and K. A. Gibbs. 2015. Two proteins form a heteromeric bacterial self-recognition complex in which variable subdomains determine allele-restricted binding. mBio. 6: e00251-15.

Cazander, G., K. E. van Veen, L. H. Bouwman, A. T. Bernards, and G. N. Jukema. 2009a. The influence of maggot excretions on PAO1 biofilm formation on different biomaterials. Clin. Orthop. Relat. Res. 467: 536–545.

Cazander, G., K. E. van Veen, A. T. Bernards, and G. N. Jukema. 2009b. 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. J. Tissue Viability 18: 80–87.

Cazander, G., M. C. van de Veerdonk, C. M. Vandenbroucke-Grauls, M. W. Schreurs, and G. N. Jukema. 2010. Maggot excretions inhibit biofilm formation on biomaterials. Clin. Orthop. Relat. Res. 468: 2789–2796.

Cazander, G., M. W. J. Schreurs, L. Renwarin, C. Dorresteijn, D. Hamann, and G. N. Jukema. 2012. Maggot excretions affect the human complement system. Wound Rep. Regen. 20: 879–886.

Cazander, G., D. I. Pritchard, Y. Nigam, W. Jung, and P. H. Nibbering. 2013. Multiple actions of *Lucilia sericata* larvae in hard-to-heal wounds: Larval secretions contain molecules that accelerate wound healing, reduce chronic inflammation and inhibit bacterial infection. BioEssays 35: 1083–1092.

Cerovsky, V., J. Zdarek, V. Fucik, L. Monincová, Z. Voburka, and R. Bém. 2010. Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. Cell. Mol. Life Sci. 67: 455–466.

Chaudhury, M. F., J. B. Welch, and L. Alfredo Alvarez. 2002. Response of fertile and sterile screwworm (Diptera: Calliphoridae) flies to bovine blood inoculated with bacteria originating from screwworm infested animal wounds. J. Med. Entomol. 39: 130–134.

Chaudhury, M. F., S. R. Skoda, A. Sagel, and J. B. Welch. 2010. Volatiles emitted from eight wound-isolated bacteria differentially attract gravid screwworms (Diptera: Calliphoridae) to oviposit. J. Med. Entomol. 47: 349–354.

Chaudhury, M. F., A. Sagel, and S. R. Skoda. 2012. Evaluation of artificial larval rearing media waste as oviposition attractant for New World screwworm (Diptera: Calliphoridae). J. Med. Entomol. 49: 293–298.

Chaudhury, M. F., J. J. Zhu, A. Sagel, H. Chen, and S. R. Skoda. 2014. Volatiles from waste larval rearing media attract gravid screwworm flies (Diptera: Calliphoridae) to oviposit. J. Med. Entomol. 51: 591–595.

Chaudhury, M. F., J. J. Zhu, and S. R. Skoda. 2015. Response of *Lucilia sericata* (Diptera: Calliphoridae) to screwworm oviposition attractant. J. Med. Entomol. 52: 527–531.

Chaudhury, M. F., J. J. Zhu, and S. R. Skoda. 2016. Bacterial volatiles attract gravid secondary screwworms (Diptera: Calliphoridae). J. Econ. Entomol. 109: 947–951.

Chisholm, S. T., G. Coaker, B. Day, and B. J. Staskawicz. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. Cell 124: 803–814.

Choi, K.-H., and F. C. Dobbs. 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. J. Microbiol. Meth. 36: 203–213.

Classen, A. T., S. I. Boyle, K. E. Haskins, S. T. Overby, and S. C. Hart. 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. FEMS Microbiol. Ecol. 44: 319–328.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. Ribosomal database project: Data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42: D633–D642.

Collins, R. E., and P. G. Higgs. 2012. Testing the infinitely many genes model for the evolution of the bacterial core genome and pangenome. Mol. Biol. Evol. 29: 3413–3425.

Concha, C., E. J. Belikoff, B. L. Carey, F. Li, A. H. Schiemann, and M. J. Scott. 2011. Efficient germ-line transformation of the economically important pest species *Lucilia cuprina* and *Lucilia sericata* (Diptera, Calliphoridae). Insect Biochem. Mol. Biol. 41: 70–75.

Concha, C., F. Li, and M. J. Scott. 2010. Conservation and sex-specific splicing of the doublesex gene in the economically important pest species *Lucilia cuprina*. J. Genet. 89: 279–285.

Costerton, J. W. 2007. The Biofilm Primer. Springer, Berlin, New York.

Cragg, J. B. 1956. The olfactory behaviour of *Lucilia* species (Diptera) under natural conditions. Ann. Appl. Biol. 44: 467–477.

Crippen, T. L., and T. L. Poole. 2009. Conjugative transfer of plasmid-located antibiotic resistance genes within the gastrointestinal tract of lesser mealworm larvae, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Foodborne Pathog. Dis. 6: 907–915.

Crooks, E. R., M. T. Bulling, and K. M. Barnes. 2016. Microbial effects on the development of forensically important blow fly species. Forensic Sci. Inter. 266: 185–190.

Davis, T. S., T. L. Crippen, R. W. Hofstetter, and J. K. Tomberlin. 2013. Microbial volatile emissions as insect semiochemicals. J. Chem. Ecol. 39: 840–859.

Dethier, V. G. 1947. Chemical Insect Attractants and Repellents. The Blakiston Company, Philadelphia, PA.

DeVaney, J. A., G. W. Eddy, E. M. Ellis, and J. H. Harrington Jr. 1973. Attractancy of inoculated and incubated bovine blood fractions to screwworm flies (Diptear: Calliphoridae): Role of bacteria. J. Med. Entomol. 10: 591–595.

Dobranic, J. K., and J. C. Zak. 1999. A microtiter plate procedure for evaluating fungal functional diversity. Mycologia 91: 756–765.

Dong, M., H. Liu, S. Allen, S. Hall, S. Fisher, T. Hazen, J. Geller, M. Singer, L. Yang, J. Jin, et al. 2007. Methodological refinements in iTRAQ reagent-based tagless strategy of identification and purification of soluble protein complexes in bacteria. Mol. Cell. Pro. 6: 23–23.

Drzewiecka, D. 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb. Ecol. 72: 741–758.

Ebert, D. 2013. The epidemiology and evolution of symbionts with mixed-mode transmission. Annu. Rev. Ecol. Evol. Syst. 44: 623–643.

Eddy, G. W., J. A. DeVaney, and B. D. Handke. 1975. Response of adult screwworm (Diptera-Calliphoridae) to bacteria incubated bovine blood in olfactometer and oviposition tests. J. Med. Entomol. 12: 379–381.

Emmens, R. L., and M. D. Murray. 1982. The role of bacterial odours in oviposition by *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), the Australian sheep blowfly. Bull. Entomol. Res. 72: 367–375.

Emmens, R. L., and M. D. Murray. 1983. Bacterial odors as oviposition stimulants for *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), the Australian sheep blowfly. Bull. Entomol. Res. 73: 411–416.

Erdmann, G. R., and S.K.W. Khalil. 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). J. Med. Entomol. 23: 208–211.

Esser, J. R. 1991. Biology of *Chrysomya megacephala* (Diptera: Calliphoridae) and reduction of losses caused to the salted-dried fish industry in south-east Asia. Bull. Entomol. Res. 81: 33–42.

Evans, F. F., M. J. Raftery, S. Egan, and S. Kjelleberg. 2007. Profiling the secretome of the marine bacterium *Pseudoalteromonas tunicata* using amine-specific isobaric tagging (iTRAQ). J. Proteome Res. 6: 967–975.

Fine, P. E. 1975. Vectors and vertical transmission: An epidemiologic perspective. Ann. NY Acad. Sci. 266: 173–194.

Förster, M., S. Klimpel, H. Mehlhorn, K. Sievert, S. Messler, and K. Pfeffer. 2007. Pilot study on synanthropic flies (e.g. *Musca*, *Sarcophaga*, *Calliphora*, *Fannia*, *Lucilia*, *Stomoxys*) as vectors of pathogenic microorganisms. Parasitol. Res. 101: 243–246.

Förster, M., K. Sievert, S. Messler, S. Klimpel, and K. Pfeffer. 2009. Comprehensive study on the occurrence and distribution of pathogenic microorganisms carried by synanthropic flies caught at different rural locations in Germany. J. Med. Entomol. 46: 1164–1166.

Francois, A., D. Grebert, M. Rhimi, M. Mariadassou, L. Naudon, S. Rabot, and N. Meunier. 2016. Olfactory epithelium changes in germfree mice. Sci. Rep. 6: 24687.

Fridman, S., I. Izhaki, Y. Gerchman, and M. Halpern. 2012. Bacterial communities in floral nectar. Environ. Microbiol. Rep. 4: 97–104.

Garland, J. L. 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24: 289–300.

Garland, J. L., and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57: 2351–2359.

Gibbs, K. A., M. L. Urbanowski, and E. P. Greenberg. 2008. Genetic determinants of self identity and social recognition in bacteria. Science 321: 256–259.

Giraud, A., S. Arous, M. De Paepe, V. Gaboriau-Routhiau, J. C. Bambou, S. Rakotobe, A. B. Lindner, F. Taddei, and N. Cerf-Bensussan. 2008. Dissecting the genetic components of adaptation of *Escherichia coli* to the mouse gut. PLoS Genet. 4: e2.

Grabbe, R. R., and J. P. Turner. 1973. Screwworm attractants: Isolation and identification of organic compounds from bacterially inoculated and incubated blood. Folia Entomol. Mex. 25: 120–121.

Graf, J. 2016. Lessons from digestive-tract symbioses between bacteria and invertebrates. Annu. Rev. Microbiol. 70: 375–393.

Graham, J. P., L. B. Price, S. L. Evans, T. K. Graczyk, and E. K. Silbergeld. 2009. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci. Total Environ. 407: 2701–2710.

Greenberg, B. 1965. Flies and disease. Sci. Am. 213: 92–99.

Greenberg, B. 1968. Model for destruction of bacteria in the midgut of blow fly maggots. J. Med. Entomol. 5: 31–38.

Greenberg, B. 1969. *Salmonella* suppression by known populations of bacteria in flies. J. Bacteriol. 99: 629–635.

Greenberg, B. 1973. Biology and disease transmission. Princeton University Press, Princeton, NJ.

Greenberg, B., and M. Klowden. 1972. Enteric bacterial interactions in insects. Am.. J. Clin. Nut. 25: 1459–1466.

Greenberg, B., J. A. Kowalski, and M. J. Klowden. 1970. Factors affecting the transmission of *Salmonellla* by flies: Natural resistance to colonization and bacterial interference. Infect. Immun. 2: 800–809.

Gupta, A., M. Dharne, A. Rangrez, P. Verma, H. Ghate, M. Rohde, M. Patole, and Y. Shouche. 2011. *Ignatzschineria indica* sp. nov. and *Ignatzschineria ureaclastica* sp. nov., isolated from adult flesh fly (Diptera: Sarcophagidae). Int. J. Syst. Evol. Microbiol. 61: 1360–1369.

Hall, D. G. 1948. The blowflies of North America. Thomas Say Foundation, Lafayette, Indiana.

Hall, M.J.R., R. L. Wall, and J. R. Stevens. 2016. Traumatic myiasis: A neglected disease in a changing world. Annu. Rev. Entomol. 61: 159–176.

Hammack, L. 1991. Oviposition by screwworm flies (Diptera: Calliphoridae) on contact with host fluids. J. Econ. Entomol. 84: 185–190.

Hammack, L., and G. G. Holt. 1983. Responses of gravid screwworm flies, *Cochliomyia hominivorax*, to whole wounds, wound fluid and a standard blood attractant in olfactometer tests. J. Chem. Ecol. 9: 913–922.

Hammack, L., M. Bromel, F. M. Duh, and G. Gassner. 1987. Reproductive factors affecting responses of the screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae) to an attractant of bacteria origin. Ann. Entomol. Soc. Am. 80: 775–780.

Haskell, N. H., and R. E. Williams. 2008. Entomology & death: A procedural guide. 2nd edn. East Park Printing, Clemson, SC.

Hayward, N. J., T. H. Jeavons, and A. J. Nicholson. 1977. Methyl mercaptan and dimethyl disulfide production from methionine by *Proteus* species detected by head-space gas-liquid chromatography. J. Clin. Microbiol. 6: 187–194.

Hoang, K. L., L. T. Morran, and N. M. Gerardo. 2016. Experimental evolution as an underutilized tool for studying beneficial animal–microbe interactions. Front. Microbiol. 7: 1444.

Hobson, R. P. 1935. Sheep blowfly investigations II. Substances whichi induce *Lucilia sericata* mg. to oviposit on sheep. Ann. Appl. Biol. 22: 294–300.

Hobson, R. P. 1936. Sheep blow fly investigations. III. Observations on the chemotropism of *Lucilia sericata*. Ann. Appl. Biol. 23: 845–851.

Hobson, P. R. 1938. Sheep blow-fly investigations VII. Observations on the development of eggs and oviposition in the sheep blow-fly, *Lucilia sericata* Mg. Ann. Appl. Biol. 25: 573–582.

Hollis, J., F. Knapp, and K. Dawson. 1985. Influence of bacteria within bovine feces on the development of the face fly (Diptera: Muscidae). Environ. Entomol. 14: 568–571.

Hsiao, E. Y., S. W. McBride, S. Hsien, G. Sharon, E. R. Hyde, T. McCue, J. A. Codelli, J. Chow, S. E. Reisman, J. F. Petrosino, et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155: 1451–1463.

Iancu, L., T. Sahlean, and C. Purcarea. 2016. Dynamics of necrophagous insect and tissue bacteria for postmortem interval estimation during the warm season in Romania. J. Med. Entomol. 53: 54–66.

Inglin, R. C., M. J. Stevens, L. Meile, C. Lacroix, and L. Meile. 2015. High-throughput screening assays for antibacterial and antifungal activities of *Lactobacillus* species. J. Microbiol. Meth. 114: 26–29.

Jacobs, C.G.C., S. Steiger, D. G. Heckel, N. Wielsch, A. Vilcinskas, and H. Vogel. 2016. Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. Sci. Rep. 6: 25409.

Jaklic, D., A. Lapanje, K. Zupancic, D. Smrke, and N. Gunde-Cimerman. 2008. Selective antimicrobial activity of maggots against pathogenic bacteria. J. Med. Microbiol. 57: 617–625.

Janzen, D. H. 1977. Why fruits rot, seeds mold, and meat spoils. Am. Natl. 111: 691–713.

Joyner, C., M. K. Mills, and D. Nayduch. 2013. *Pseudomonas aeruginosa* in *Musca domestica* L.: Temporospatial examination of bacteria population dynamics and house fly antimicrobial responses. PLoS ONE 8: e79224.

Klassen, W., and C. F. Curtis. 2005. History of the Sterile Insect Technique, pp. 3–36 *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson (eds.), Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, Netherlands, Dordrecht.

Kotze, A. C., B. M. Hines, N. H. Bagnall, C. A. Anstead, P. Gupta, R. C. Reid, A. P. Ruffell, and D. P. Fairlie. 2015. Histone deacetylase enzymes as drug targets for the control of the sheep blowfly, *Lucilia cuprina*. Int. J. Parasitol. Drugs Drug Resist. 5: 201–208.

Langille, M. G., J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. A. Reyes, J. C. Clemente, D. E. Burkepile, R. L. Vega Thurber, R. Knight, et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31: 814–821.

Le Brun, C., M. Gombert, S. Robert, E. Mercier, and P. Lanotte. 2015. Association of necrotizing wounds colonized by maggots with *Ignatzschineria*-associated septicemia. Emerg. Infect. Dis. 21: 1881–1883.

Lechene, C. P., Y. Luyten, G. McMahon, and D. L. Distel. 2007. Quantitative imaging of nitrogen fixation by individual bacteria within animal cells. Science 317: 1563–1566.

Lee, J., A. Jayaraman, and T. K. Wood. 2007. Indole is an inter-species biofilm signal mediated by SdiA. BMC Microbiol. 7: 42.

Lee, E. Y., D. S. Choi, K. P. Kim, and Y. S. Gho. 2008. Proteomics in gram-negative bacterial outer membrane vesicles. Mass Spectrom. Rev. 27: 535–555.

Lee, S. M., A. Wyse, A. Lesher, M. L. Everett, L. Lou, Z. E. Holzknecht, J. F. Whitesides, P. A. Spears, D. E. Bowles, S. S. Lin, et al. 2010. Adaptation in a mouse colony monoassociated with *Escherichia coli* K-12 for more than 1,000 days. Appl. Environ. Microbiol. 76: 4655–4663.

Lee, C. K., C. W. Herbold, S. W. Polson, K. E. Wommack, S. J. Williamson, I. R. McDonald, and S. C. Cary. 2012. Groundtruthing next-gen sequencing for microbial ecology-biases and errors in community structure estimates from PCR amplicon pyrosequencing. PLoS ONE 7: e44224.

Lee, J.-H., T. K. Wood, and J. Lee. 2015. Roles of indole as an interspecies and interkingdom signaling molecule. Trends Microbiol. 23: 707–718.

Lemaitre, B., and J. Hoffmann. 2007. The host defense of *Drosophila melanogaster*. Annu. Rev. Immunol. 25: 697–743.

Li, T., T.-D. Wu, L. Mazéas, L. Toffin, J.-L. Guerquin-Kern, G. Leblon, and T. Bouchez. 2008. Simultaneous analysis of microbial identity and function using NanoSIMS. Environ. Microbiol. 10: 580–588.

Lipsitch, M., M. A. Nowak, D. Ebert, and R. M. May. 1995a. The population dynamics of vertically and horizontally transmitted parasites. Proc. Biol. Sci. 260: 321–327.

Lipsitch, M., S. Siller, and M. A. Nowak. 1995b. The evolution of virulence in pathogens with vertical and horizontal transmission. Evolution 50: 1729–1741.

Liu, W., M. Longnecker, A. M. Tarone, and J. K. Tomberlin. 2016. Response of *Lucilia sericata* (Diptera: Caliphoridae) to compounds from microbial decomposition of larval resources. Anim. Behav. 115: 217–225.

Lively, C. M., K. Clay, M. J. Wade, and C. Fuqua. 2005. Competitive coexistence of vertically and horizontally transmitted parasites. Evol. Ecol. Res. 7: 1183–1190.

Lysyk, T. J., L. Kalischuk-Tymensen, L. B. Selinger, R. C. Lancaster, L. Wever, and K. J. Cheng. 1999. Rearing stable fly larvae (Diptera: Muscidae) on an egg yolk medium. J. Med. Entomol. 36: 382–388.

Ma, Q., A. Fonseca, W. Liu, A. T. Fields, M. L. Pimsler, A. F. Spindola, A. M. Tarone, T. L. Crippen, J. K. Tomberlin, and T. K. Wood. 2012. *Proteus mirabilis* interkingdom swarming signals attract blow flies. ISME J. 6: 1356–1366.

Mac Aogáin, M., T. R. Rogers, and B. Crowley. 2016. Identification of emergent bla CMY-2 -carrying *Proteus mirabilis* lineages by whole-genome sequencing. New Microbes New Infect. 9: 58–62.

Mackerras, I. M. 1936. The sheep blowfly problem in australia: results of some recent investigations. H.J. Green, Government Printer, Melbourne.

Mann, C. M., S. Barnes, B. Offer, and R. Wall. 2015. Lethal and sub-lethal effects of faecal deltamethrin residues on dung-feeding insects. Med. Vet. Entomol. 29: 189–195.

Marouga, R., S. David, and E. Hawkins. 2005. The development of the DIGE system: 2D fluorescence difference gel analysis technology. Anal. Bioanal. Chem. 382: 669–678.

Maurin, M., J. Delbano, L. Mackaya, H. Colomb, C. Guier, and A. Mandjee. 2007. Human infection with *Schineria* larvae. Emerg. Infect. Dis. 13: 671–673.

McKenzie, J. A., and M. J. Whitten. 1982. Selection for insecticide resistance in the Australian sheep blowfly, *Lucilia cuprina*. Experientia 38: 84–85.

Metcalf, J. L., L. W. Parfrey, A. Gonzalez, C. L. Lauber, D. Knights, G. Ackermann, G. C. Humphrey, M. J. Gebert, W. Van Treuren, D. Berg-Lyons, et al. 2013. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. Elife 2: e01104.

Mingchay, P., A. Sai-Ngam, A. Phumee, P. Bhakdeenuan, K. Lorlertthum, U. Thavara, A. Tawatsin, W. Choochote, and P. Siriyasatien. 2014. *Wolbachia* supergroups A and B in natural populations of medically important filth flies (diptera: muscidae, calliphoridae, and sarcophagidae) in Thailand. Southeast Asian J. Trop. Med. Public Health 45: 309–318.

Moeller, A. H., A. Caro-Quintero, D. Mjungu, A. V. Georgiev, E. V. Lonsdorf, M. N. Muller, A. E. Pusey, M. Peeters, B. H. Hahn, and H. Ochman. 2016. Cospeciation of gut microbiota with hominids. Science 353: 380–382.

Mohr, R. M., and J. K. Tomberlin. 2014. Environmental factors affecting early carcass attendance by four species of blow flies (Diptera: Calliphoridae) in Texas. J. Med. Entomol. 51: 702–708.

Mohr, R. M., and J. K. Tomberlin. 2015. Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance. Int. J. Legal Med. 129: 851–859.

Moran, N. A., and P. Baumann. 2000. Bacterial endosymbionts in animals. Curr. Opin. Microbiol. 3: 270–275.

Morris, M. C., L. W. Morrison, M. A. Joyce, and B. Rabel. 1998. Trapping sheep blowflies with lures based on bacterial cultures. Aust. J. Exp. Agric. 38: 125–130.

Muegge, B. D., J. Kuczynski, D. Knights, J. C. Clemente, A. Gonzalez, L. Fontana, B. Henrissat, R. Knight, and J. I. Gordon. 2011. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970–974.

Mumcuoglu, K. Y., J. Miller, M. Mumcuoglu, M. Friger, and M. Tarshis. 2001. Destruction of bacteria in the digestive tract of the maggot of *Lucilia sericata* (Diptera: Calliphoridae). J. Med. Entomol. 38: 161–166.

Munson, M. A., P. Baumann, M. A. Clark, L. Baumann, N. A. Moran, D. J. Voegtlin, and B. C. Campbell. 1991. Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families. J. Bacteriol. 173: 6321–6324.

Nayduch, D., H. Cho, and C. Joyner. 2013. *Staphylococcus aureus* in the house fly: Temporospatial fate of bacteria and expression of the antimicrobial peptide defensin. J. Med. Entomol. 50: 171–178.

Olsen, A. R. 1998. Regulatory action criteria for filth and other extraneous materials. III. Review of flies and foodborne enteric disease. Reg. Tox. Pharm. 28: 199–211.

Owings, C., C. Spiegelman, A. M. Tarone, and J. K. Tomberlin. 2014. Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. Int. J. Legal Med. 128: 709–717.

Paczkowski, M., S. Nicke, H. Ziegenhagen, and S. Schütz. 2015. Volatile emission of decomposing pig carcasses (*Sus scrofa domesticus* L.) as an indicator for the postmortem interval. J. Forensic Sci. 60: S130–S137.

Page, R. D. 2003. Tangled trees: Phylogeny, cospeciation, and coevolution. University of Chicago Press, Chicago, IL.

Pava-Ripoll, M., R. E. Pearson, A. K. Miller, and G. C. Ziobro. 2012. Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and Listeria monocytogenes associated with the body surfaces and guts of individual filth flies. Appl. Environ. Microbiol. 78: 7891–7902.

Payne, J. A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. Ecology 46: 592–602.

Payne, J. A., E. W. King, and G. Beinhart. 1968. Arthropod succession and decomposition of buried pigs. Nature 219: 1180–1181.

Pearson, M. M., M. Sebaihia, C. Churcher, M. A. Quail, A. S. Seshasayee, N. M. Luscombe, Z. Abdellah, C. Arrosmith, B. Atkin, T. Chillingworth, et al. 2008. Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. J. Bacteriol. 190: 4027–4037.

Pechal, J. L. 2012. Swine carrion as a model system for studying decomposition ecology: the importance of microbial and primary colonizer interactions. Texas A&M University, College Station, TX.

Pechal, J. L., and M. E. Benbow. 2016. Microbial ecology of the salmon necrobiome: Evidence salmon carrion decomposition influences aquatic and terrestrial insect microbiomes. Environ. Microbiol. 18: 1511–1522.

Pechal, J. L., T. L. Crippen, A. M. Tarone, A. J. Lewis, J. K. Tomberlin, and M. E. Benbow. 2013. Microbial community functional change during vertebrate carrion decomposition. PLoS ONE 8: e79035.

Pechal, J. L., T. L. Crippen, M. E. Benbow, A. M. Tarone, S. Dowd, and J. K. Tomberlin. 2014. The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. Int. J. Legal Med. 128: 193–205.

Perotti, M., T. Lysyk, L. D. Kalischuk-Tymensen, L. J. Yanke, and L. B. Selinger. 2001. Growth and survival of immature *Haematobia irritans* (Diptera: Muscidae) is influenced by bacteria isolated from cattle manure and conspecific larvae. J. Med. Entomol. 38: 180–187.

Perrault, K. A., P. H. Stefanuto, B. H. Stuart, T. Rai, J. F. Focant, and S. L. Forbes. 2015. Reducing variation in decomposition odour profiling using comprehensive two-dimensional gas chromatography. J. Sep. Sci. 38: 73–80.

Polvony, D. 1971. Synanthropy, pp. 17–54. *In* G., Bernard (ed.), Flies and diseases: Vol 1: Ecology, classification and biotic interactions. Princeton University Press, Princeton, NJ.

Poppel, A. K., H. Vogel, J. Wiesner, and A. Vilcinskas. 2015. Antimicrobial peptides expressed in medicinal maggots of the blow fly *Lucilia sericata* show combinatorial activity against bacteria. Antimicrob. Agents Chemother. 59: 2508–2514.

Richardson, M. 1966. Studies on the biogenesis of some simple amines and quaternary ammonium compounds in higher plants: Isoamylamine and isobutylamine. Phytochemistry 5: 23–30.

Romero, A., A. Broce, and L. Zurek. 2006. Role of bacteria in the oviposition behaviour and larval development of stable flies. Med. Vet. Entomol. 20: 115–121.

Roudiere, L., H. Jean-Pierre, C. Comte, I. Zorgniotti, H. Marchandin, and E. Jumas-Bilak. 2007. Isolation of *Schineria* sp. from a man. Emerg. Infect Dis. 13: 659–661.

Sabrosky, C. W., G. F. Bennett, and T. L. Whitworth. 1989. Bird blow flies (Protocalliphora) in North America (Diptera: Calliphoridae), with notes on the Palearctic species/Curtis W. Sabrosky, Gordon F. Bennett, and Terry L. Whitworth. Smithsonian Institution Press, Washington, DC.

Sackton, T. B., and A. G. Clark. 2009. Comparative profiling of the transcriptional response to infection in two species of Drosophila by short-read cDNA sequencing. BMC Gen. 10: 259.

Sackton, T. B., B. P. Lazzaro, T. A. Schlenke, J. D. Evans, D. Hultmark, and A. G. Clark. 2007. Dynamic evolution of the innate immune system in Drosophila. Nat. Genet. 39: 1461–1468.

Sackton, T. B., B. P. Lazzaro, and A. G. Clark. 2010. Genotype and gene expression associations with immune function in Drosophila. PLoS Gen. 6: e1000797.

Sackton, T. B., B. Lazarro, and A. G. Clark. 2016. Rapid expansion of immune-related gene families in the house fly. Musca Domestica. http://dx.doi.org/10.1101/068213.

Samyn, B., K. Sergeant, S. Memmi, G. Debyser, B. Devreese, and J. Van Beeumen. 2006. MALDI-TOF/TOF de novo sequence analysis of 2-D PAGE-separated proteins from *Halorhodospira halophila*, a bacterium with unsequenced genome. Electrophoresis 27: 2702–2711.

Sander, J. D., and J. K. Joung. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat. Biotechnol. 32: 347–355.

Sanford, M. R., T. L. Whitworth, and D. R. Phatak. 2014. Human wound colonization by *Lucilia eximia* and *Chrysomya rufifacies* (Diptera: Calliphoridae): Myiasis, perimortem, or postmortem colonization? J. Med. Entomol. 51: 716–719.

Sasaki-Imamura, T., A. Yano, and Y. Yoshida. 2010. Production of indole from l-Tryptophan and effects of these compounds on biofilm formation by *Fusobacterium nucleatum* ATCC 25586. Appl. Environ. Microbiol. 76: 4260–4268.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, et al. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75: 7537–7541.

Schmidt, F., and U. Völker. 2011. Proteome analysis of host–pathogen interactions: Investigation of pathogen responses to the host cell environment. Proteomics 11: 3203–3211.

Schmidtmann, E. T., and P. A. Martin. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J. Med. Entomol. 29: 232–235.

Scott, J. G., W. C. Warren, L. W. Beukeboom, D. Bopp, A. G. Clark, S. D. Giers, M. Hediger, A. K. Jones, S. Kasai, C. A. Leichter, et al. 2014. Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. Genome Biol. 15: 466.

Shade, A., P. S. McManus, and J. Handelsman. 2013. Unexpected fiversity during community succession in the apple flower microbiome. mBio 4: e00602-12.

Shanbhag, S. R., B. Müller, and R. A. Steinbrecht. 1999. Atlas of olfactory organs of *Drosophila melanogaster*: 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Ins. Morphol. Embryol. 28: 377–397.

Shao, F., P. M. Merritt, Z. Bao, R. W. Innes, and J. E. Dixon. 2002. A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. Cell 109: 575–588.

Sharpton, T. J. 2014. An introduction to the analysis of shotgun metagenomic data. Front. Plant Sci. 5: 209.

Sherman, R. A., M.J.R. Hall, and S. Thomas. 2000. Medicinal maggots: An ancient remedy for some contemporary afflictions. Annu. Rev. Entomol. 45: 55–81.

Shewell, G. E. 1987. Calliphoridae, pp. 1133–1145 *In* J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, and D. M. Wood (eds), Manual of Nearctic Diptera. Research Branch Agriculture Canda, Ottawa, Canada.

Singh, B., T. L. Crippen, L. Zheng, A. Fields, Z. Yu, Q. Ma, T. Wood, S. Dowd, M. Flores, J. K. Tomberlin, et al. 2015. A metagenomic assessment of the bacteria associated with *Lucilia sericata* and *Lucilia cuprina* (Diptera: Calliphoridae). Appl. Microbiol. Biotechnol. 99: 869–883.

Smalla, K., U. Wachtendorf, H. Heuer, W.-T. Liu, and L. Forney. 1998. Analysis of BIOLOG GN substrate utilization patterns by microbial communities. Appl. Environ. Microbiol. 64: 1220–1225.

Smith, K.G.V. 1986. A Manual of Forensic Entomology. Comstock Publishing Associates, New York.

Spivak, M., D. Conlon, and W. J. Bell. 1991. Wind-guided landing and search behavior in fleshflies and blowflies exploiting a resource patch (Diptera: Sarcophagidae, Calliphoridae). Ann. Entomol. Soc. Am. 84: 447–452.

Stadler, S., P. H. Stefanuto, M. Brokl, S. L. Forbes, and J. F. Focant. 2012. Characterization of volatile organic compounds from human analogue decomposition using thermal desorption coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. Anal. Chem. 85: 998–1005.

Statheropoulos, M., C. Spiliopoulou, and A. Agapiou. 2005. A study of volatile organic compounds evolved from the decaying human body. Forensic Sci. Int. 153: 147–155.

Stensmyr, M. C., I. Urru, I. Collu, M. Celander, B. S. Hansson, and A. M. Angioy. 2002. Rotting smell of dead-horse arum florets - These blooms chemically fool flies into pollinating them. Nature 420: 625–626.

Stouthamer, R., J. A. Breeuwer, and G. D. Hurst. 1999. *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. Annu. Rev. Microbiol. 53: 71–102.

Sullivan, N. L., A. N. Septer, A. T. Fields, L. M. Wenren, and K. A. Gibbs. 2013. The complete genome sequence of *Proteus mirabilis* strain BB2000 reveals differences from the *P. mirabilis* reference strain. Genome Announc PLoS ONE. 1: e00024-13.

Sze, S. H., J. P. Dunham, B. Carey, P. L. Chang, F. Li, R. M. Edman, C. Fjeldsted, M. J. Scott, S. V. Nuzhdin, and A. M. Tarone. 2012. A *de novo* transcriptome assembly of *Lucilia sericata* (Diptera: Calliphoridae) with predicted alternative splices, single nucleotide polymorphisms and transcript expression estimates. Insect Mol. Biol. 21: 205–221.

Thompson, C. R., R. S. Brogan, L. Z. Scheifele, and D. B. Rivers. 2013. Bacterial interactions with necrophagous flies. Ann. Entomol. Soc. Am. 106: 799–809.

Tillyard, R. J., and H. R. Seddon. 1933. The sheep blowfly problem in Australia Report No. 1. Council for Scientific and Industrial Research, Melbourne.

Tomberlin, J. K., and M. E. Benbow. 2015. Forensic Entomology: International Dimensions and Frontiers. CRC Press, Boca Raton, FL.

Tomberlin, J. K., T. L. Crippen, A. M. Tarone, B. Singh, K. Adams, Y. H. Rezenom, M. E. Benbow, M. Flores, M. Longnecker, J. L. Pechal, et al. 2012. Interkingdom responses of flies to bacteria mediated by fly physiology and bacterial quorum sensing. Anim. Behav. 84: 1449–1456.

Toth, E. M., E. Hell, G. Kovacs, A. K. Borsodi, and K. Marialigeti. 2006. Bacteria isolated from the different developmental stages and larval organs of the obligate parasitic fly, *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). Microb. Ecol. 51: 13–21.

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

Valachova, I., P. Takac, and J. Majtan. 2014. Midgut lysozymes of *Lucilia sericata* - new antimicrobials involved in maggot debridement therapy. Insect Mol. Biol. 23: 779–787.

van der Niet, T., D. M. Hansen, and S. D. Johnson. 2011. Carrion mimicry in a South African orchid: Flowers attract a narrow subset of the fly assemblage on animal carcasses. Ann. Bot. 107: 981–992.

Vass, A. A., G. Sega, J. Caton, J. Synstelien, J. Love, J. Skeen, and S. Barshick. 2002. Decomposition chemistry of human remains: A new methodology for determining the postmortem interval. J. Forensic Sci. 47: 542–553.

Warman, G. R., R. D. Newcomb, R. D. Lewis, and C. W. Evans. 2000. Analysis of the circadian clock gene *period* in the sheep blow fly *Lucilia cuprina*. Genet. Res. Camb. 75: 257–267.

Wasserman, S. L., and H. Itagaki. 2003. The olfactory responses of the antenna and maxillary palp of the fleshfly, *Neobellieria bullata* (Diptera: Sarcophagidae), and their sensitivity to blockage of nitric oxide synthase. J. Insect Physiol. 49: 271–280.

Watson, D. W., P. A. Martin, and E. T. Schmidtmann. 1993. Egg yolk and bacteria growth medium for *Musca domestica* (Diptera: Muscidae). J. Med. Entomol. 30: 820–823.

Wei, T., R. Ishida, K. Miyanaga, and Y. Tanji. 2014a. Seasonal variations in bacterial communities and antibiotic-resistant strains associated with green bottle flies (Diptera: Calliphoridae). Appl. Microbiol. Biotechnol. 98: 4197–4208.

Wei, T., K. Miyanaga, and Y. Tanji. 2014b. Persistence of antibiotic-resistant and -sensitive *Proteus mirabilis* strains in the digestive tract of the housefly (*Musca domestica*) and green bottle flies (Calliphoridae). Appl. Microbiol. Biotechnol. 98: 8357–8366.

Wells, J. D., M. L. Goff, J. K. Tomberlin, and H. Kurahashi. 2002. Molecular systematics of the endemic Hawaiian blowfly genus *Dyscritomyia* Grimshaw, (Diptera: Calliphoridae). Med. Entomol. Zool. 53: 231–238.

Whitworth, T. 2006. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proc. Entomol. Soc. Wash. 108: 689–725.

Yan, M., S. J. Pamp, J. Fukuyama, P. H. Hwang, D. Y. Cho, S. Holmes, and D. A. Relman. 2013. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage. Cell Host Microbe. 14: 631–640.

Yuan, Y., Y. Zhang, S. Fu, T. L. Crippen, D. K. Visi, M. E. Benbow, M. S. Allen, J. K. Tomberlin, S. H. Sze, and A. M. Tarone. 2016. Genome sequence of a *Proteus mirabilis* strain isolated from the salivary glands of larval *Lucilia sericata*. Genome Announc. 4: e00672–e00616.

Yun, J. H., S. W. Roh, T. W. Whon, M. J. Jung, M. S. Kim, D. S. Park, C. Yoon, Y. D. Nam, Y. J. Kim, J. H. Choi, et al. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Appl. Environ. Microbiol. 80: 5254–5264.

Zhang, C. G., B. A. Chromy, and S. L. McCutchen-Maloney. 2005. Host-pathogen interactions: A proteomic view. Expert Rev. Proteomics. 2: 187–202.

Zheng, L., T. L. Crippen, B. Singh, A. M. Tarone, S. Dowd, Z. Yu, T. K. Wood, and J. K. Tomberlin. 2013. A survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. J. Med. Entomol. 50: 647–658.

Zurek, L., C. Schal, and D. W. Watson. 2000. Diversity and contribution of the intestinal bacterial community to the development of *Musca domestica* (Diptera: Muscidae) larvae. J. Med. Entomol. 37: 924–928.