Review

Functional Variation in Dipteran Gut Bacterial Communities in Relation to Their Diet, Life Cycle Stage and Habitat

Rebekka Sontowski 1,2,* and Nicole M. van Dam 1,2

1 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany; nicole.vandam@idiv.de
2 Institute for Biodiversity, Friedrich-Schiller University, Dornburger Str. 159, 07743 Jena, Germany
* Correspondence: rebekka.sontowski@idiv.de

Received: 4 June 2020; Accepted: 11 August 2020; Published: 17 August 2020

Simple Summary: Like in many other organisms, the guts of insects are full with many different bacteria. These bacteria can help their hosts to overcome toxic diets or can boost their resistance to pathogens. We were curious to learn which factors determine the composition of gut bacterial communities (GBCs) in true flies and mosquitoes, which belong to the order Diptera. We searched for research papers reporting on GBCs in these insects. Using these published data, we investigated whether the GBCs are species-specific, or whether they are determined by the diet, life stage or environment of the host insect. We found that the GBCs in larvae and adults of the same insect species can be very different. Insects on similar diets did not necessarily show similar GBCs. This made us conclude that GBCs are mostly life stage-specific. However, we found that the number of data papers we could use is limited; more data are needed to strengthen our conclusion. Lastly, novel DNA technologies can show ‘who is there’ in GBCs. At the same time, we lack knowledge on the exact function of gut bacteria. Obtaining more knowledge on the function of GBCs may help to design sustainable pest control measures.

Abstract: True flies and mosquitoes (Diptera) live in habitats and consume diets that pose specific demands on their gut bacterial communities (GBCs). Due to diet specializations, dipterans may have highly diverse and species-specific GBCs. Dipterans are also confronted with changes in habitat and food sources over their lifetime, especially during life history processes (molting, metamorphosis). This may prevent the development of a constant species- or diet-specific GBC. Some dipterans are vectors of several human pathogens (e.g., malaria), which interact with GBCs. In this review, we explore the dynamics that shape GBC composition in some Diptera species on the basis of published datasets of GBCs. We thereby focus on the effects of diet, habitats, and life cycle stages as sources of variation in GBC composition. The GBCs reported were more stage-specific than species- or diet-specific. Even though the presence of GBCs has a large impact on the performance of their hosts, the exact functions of GBCs and their interactions with other organisms are still largely unknown, mainly due to the low number of studies to date. Increasing our knowledge on dipteran GBCs will help to design pest management strategies for the reduction of insecticide resistance, as well as for human pathogen control.

Keywords: bacteria; insect–microbe interaction; host symbiosis; development; food source; malaria; pathogen vectors; pest management
1. Introduction

True flies and mosquitoes (Diptera) are both a blessing and a curse for humans. On the one hand, several dipterans, such as hoverflies (Syrphidae) and humbleflies (Bombyliidae), fulfill important ecosystem services as pollinators [1,2]. On the other hand, some species are agricultural pests or vectors of human diseases, such as the Mediterranean fruit fly (Tephritidae) or the tsetse fly (Hippoboscidae) [3,4]. Diptera is one of the most species-rich insect orders with an estimated number of 155,000 species spread around the world [5]. The large number of species and ecological functions emerged because dipterans adapted to a wide range of ecological niches. To exploit a wide diversity of niches, various species adapted to harsh and noxious environments or food sources that are inaccessible to other species [6,7]. This led to the emergence of many feeding strategies within this large insect order, which contains specialist feeding on plant tissues, pollen, nectar, vertebrate tissues, blood, carrion, feces, or other invertebrates. Each of these specialists face specific challenges with regards to the digestibility and detoxification of their diets.

To understand adaptations of Diptera to specific niches and diets, we should also consider their microbiomes, which mainly comprise bacteria. This consortium of microorganisms is an intrinsic part of the “holobiont”. A holobiont includes the host organism and all associated microorganisms and their interactions [8]. Microbes can be mutualists (of both host and microbe benefit), parasites (harmful to host), or commensals (neutral to host). The outcomes of host–microbe interactions shape the composition of microbial communities in hosts and can shape hosts’ performance [9,10]. Host performance can be particularly enhanced when their gut microbiome supports nutrient acquisition and the detoxification of diets [11,12].

Gut microbiomes include all bacteria, fungi, protists, and viruses living in the digestive tract of macroorganisms. In this review, we focus on bacteria and refer to them as gut bacterial communities (GBCs). GBCs provide nutrients, digest recalcitrant food sources, detoxify noxious compounds in the diet, and fend off harmful organisms [13–15]. On the basis of the high rate of food specialization within the Diptera, we expect a mutual adaptation between hosts and their gut microbiome. This mutual adaptation would result in a species-specific and stable GBC, especially in specialists. However, Diptera are holometabolic insects; in other words, they go through distinct developmental stages including complete metamorphosis in the pupal stage. Complete metamorphosis is frequently coupled with diet shifts and changes in environmental conditions. Mosquito larvae, for example, live in aquatic habitats and feed on algae, whereas the adults live in terrestrial habitats and feed on nectar and, in females only, on blood [16,17]. These changes pose specific requirements to GBCs and likely result in a variable GBC composition over the life cycle of a single insect [18]. In addition to the composition, GBC diversity may also be of importance. A highly diverse GBC colonization of the gut may help dipterans to meet the different requirements posed by different life stages as well as help them to adapt to a wide range of (variable) environments. A high GBC diversity may be especially advantageous for generalists, which are confronted with a broad range of food sources and toxins. Studies on GBC in another order of holometabolic insects, the lepidoptera, presented controversial results. Some bacteria seem to be diet-specific [19], whereas other studies revealed no correlation with food sources [20]. Finally, dipterans are often vectors for vertebrate pathogens, for instance pathogens that cause malaria or dengue fever [21,22]. These vertebrate pathogens may shift GBC composition and density [23].

In this review, we report the current status quo and explore the relevance of variation in GBCs in Diptera. Using published datasets, we examine to what extent we can draw conclusions about species-specific GBCs. In addition, GBCs may vary within single dipteran species over different life stages, which come with shifts in diet or environmental conditions. Additionally, we will discuss the importance and effects of single bacterial species on GBC composition and the interactions of dipteran GBCs with human pathogens.
2. GBCs Are Supporting Diptera to Exploit Specific Niches and Food Sources

Dipterans exploit a wide variety of diets, including plant tissues (such as pollen and nectar), vertebrate tissues, blood, feces, carrion, and invertebrates. These diets range from nearly sterile, such as blood, to diets that support enriched microbial communities, such as carrion or feces. At the same time, dipteran diets may contain chemical defenses, antibiotics, or indigestible compounds. Dipterans feeding on living hosts, such as herbivores, mosquitoes, or predators, will be confronted with the immune responses of their host. The utilization of recalcitrant resources and the colonization of these specific niches require special adaptations. Establishing interactions with pre-adapted microorganisms will facilitate the adaptation process. Microbes have occupied a large variety of exceptional habitats for millions of years before macroorganisms started to colonize our planet [24]. Dipterans successfully acquired some of these microbes, most notably of the genera Wolbachia and Wigglesworthia, as symbionts [25–28] These endosymbionts can affect host reproduction, immunity, nutrient status, and fitness, including the sex ratio of the offspring [9,25,29]. It is estimated that ancestors of tsetse flies were already infected with Wigglesworthia glossinidia-related bacteria approximately 50–100 million years ago [13]. Wolbachia infections in Drosophila simulans and Culex pipiens are dated about 58–66 million years ago [30]. In addition to these endosymbionts, insects carry a rich variety of other microbes inside and outside their bodies. A hotspot of microbes in insect bodies is their gut. Gut microbes provide important metabolic functions, including the digestion of food, the synthesis of micronutrients, and the conversion of recalcitrant resources, such as polysaccharides, to carbohydrates that can be absorbed by the gut [31]. For instance, the olive fruit fly Bactrocera oleae (Rossi) is a specialist on olive fruits. Olives contain a high proportion of indigestible proteins [32]. The gut bacterium Pseudomonas savastanoi hydrolyses the indigestible proteins in olive fruit flesh and converts them to amino acids [12]. After removing the GBCs via antibiotic treatments from the olive fruit fly adults, the larvae failed to reach the pupal stage in unripe olive fruits [12,33]. The “sterile” larvae were unable to digest unripe olives or non-hydrolyzed proteins [12]. Hagen [12] suggested that P. savastanoi hydrolyses the olive proteins and synthesizes the amino acids threonine and methionine. Both amino acids were not detected in the olive fruits but are essential for host development. In general, unripe fruits contain low levels of nutrients. This makes unripe fruits unattractive for herbivores. Olive fruit flies solve this challenge through a symbiotic interaction with the bacterium Candidatus Erwinia dacicola. This beneficial bacterium either directly serves as an amino acid and protein source, or it increases the protein digestibility in unripe olives [33]. Another fruit fly, the Mediterranean fruit fly, or Medfly, Ceratitis capitata (Wiedemann), is unable to acquire sufficient nitrogen from its diet, which mainly comprises soft fruits. This fly established symbioses with several nitrogen-fixing gut microbes that provide nitrogen to their host [34].

Next to recalcitrant resources, several insects are confronted with toxic compounds in their environments and/or diets, especially when they are confronted with novel or changed environments. GBCs are able to support some hosts through the detoxification of plant allelochemicals, pesticides, drugs, and reactive oxygen species (ROS). For instance, the larvae of the Chironomid Chironomus javanus can live in heavy metal-contaminated environments. Ch. javanus larvae produce the enzymes glutathione S-transferase (GST) and metallothionein (MT) [35]. GSTs make heavy metals less toxic by making them more water-soluble. This enables the larvae to excrete the heavy metals quickly [35]. MTs bind several metals and transport them to the cytosol. There they are stored separately from fundamental organelles. This process prevents essential organelles from being damaged by the heavy metals [36]. Another study showed that chironomid larvae harbor several adapted bacteria in their guts (e.g., Shewanella decolorationis, Chromobacterium aquaticum), which are able to detoxify lead and chromium [37]. The authors also found that larvae without the bacteria S. decolorationis or C. aquaticum had a lower survival rate in heavy metal environments compared with those with these bacteria. This suggest that these bacteria protect the chironomid larvae against toxic metals [37]. The fact that specific bacteria can improve survival in heavy metal environments may indicate that the GSTs and MTs needed for the detoxification are provided by these bacteria. Herbivorous dipterans have to deal
with other natural toxins; plant tissues commonly contain plant defense compounds, which the insects ingest when feeding. GBCs can be confronted with these compounds, depending on the gut structure of the insect and their spatial distribution in the gut (foregut, midgut, or hindgut). Several studies showed that members of GBCs possess detoxification mechanisms, which may be beneficial to the GBCs as well as their hosts [15,38].

One of the oldest examples of gut microbes detoxifying defense compounds in their host’s diet was reported in olive fruit flies ([12]). Next to the indigestible proteins mentioned above, young olive fruits contain bitter-tasting defense allelochemicals, such as the phenolic compound oleuropein [32]. This makes them unpalatable to humans unless they are curated in water or brine. Experimental studies showed that oleuropein and derivatives thereof can deter herbivores, including ovipositing female olive fruit flies [39]. Oleuropein also reduces the availability of dietary proteins and lysine in unripe olives. Unripe olives in particular contain a high amount of oleuropein [33]. That makes unripe olives unpalatable for most insects. The olive fruit fly larvae can still feed on these fruits because they form a symbiotic relationship with their gut bacterium Ca. Erwinia dacicola. It is not exactly known how these bacteria overcome the effects of oleuropein, which also has antimicrobial and antifungal effects [40,41]. Presumably, this beneficial microbe degrades or binds oleuropein, or it uses enzymes resistant to the inhibitory effects of oleuropein to digest unripe olives [33].

The cabbage root fly (Delia radicum L.) is another example of an insect herbivore hosting GBCs that can detoxify plant defenses. Larvae of D. radicum are specialists in feeding on roots of species in the family Brassicaceae [42]. Just like the leaves, the roots contain glucosinolates, a class of anti-herbivore defense compounds typical for the Brassicaceae [43]. Upon damage, for example by D. radicum larvae, glucosinolates are mixed with the enzyme myrosinase. This causes the conversion of the glucosinolates in several breakdown products, including the toxic and deterrent isothiocyanates [44]. The glucosinolate 2-phenylethyl is one of the prominent glucosinolates in the roots of Brassicacea [45]. When root flies are feeding on the plant, phenylethyl-isothiocyanate is formed [46,47]. Several Gammaproteobacteria that can detoxify phenylethyl-isothiocyanate were isolated from the guts of cabbage root fly larvae [48]. Several of these bacteria possess a saxA gene encoding for a hydrolase that can break down various plant-produced isothiocyanates [49]. Deletion of the saxA gene in one of the gut microbes, Pectobacterium carotivorum, prevented this bacterium from being able to degrade plant material [38]. Whether these gut microbes are essential for the larvae to detoxify their host plant’s defense system and/or their possession of the detoxification enzymes to occupy this specific niche is not clear yet.

A third example of GBCs playing a role in detoxification is provided to blood-feeding dipterans, which also encounter toxic substances. Mosquitos are confronted by various toxic oxidants from blood, especially reactive oxygen species (ROS) and reactive nitrogen oxygen species (RNOS) [50,51]. A mixture of gut microbes, such as Klebsiella, Serratia, Enterobacter, and Pseudomonas, isolated from the blood-feeding insects Anopheles gambiae and Lutzomyia longipalpis, degraded toxic oxidants by producing microbial antioxidants [23,50]. However, GBCs seem not to be essential for all mosquitos to survive on blood meal. Reduction of the GBCs in Aedes aegypti females by different antibiotics had no effect on their survival [52]. However, it reduced the lysis of the red blood cells, digestion of blood proteins, and egg production in this species [52]. This suggests that at least some mosquitos possess mechanisms to overcome the toxic oxidants in blood meals by themselves. Other than the bacteria, which catabolize the oxidants, several mosquitos tolerate oxidants by producing a peritrophic matrix structure in their gut after the ingestion of a blood meal, such as A. aegypti, An. gambiae, Anopheles stephensi, Anopheles labranchiae, and Culex tarsalis [53,54]. This membrane separates the epithelium cells from ingested blood and reduces damage to other organs [53,54]. In waste-feeding Diptera, GBCs affect host performance. Studies in Musca domestica showed that the removal of GBCs reduced the growth and development of this fly species [55,56]. The examples above show that GBCs can be essential to exploring specific habitats and food sources for some dipteran species, such as the olive flies or the chironomid larvae, or in the case of M. domestica, affect their growth and development.
In other dipters, it is not so clear how much the insect depends on the GBCs. These dipteran species may have evolved their own strategies to cope with toxic diets and habitats.

3. Effects of Diet on Differences in Gut Microbial Community Composition among Dipteran Species

Considering the diversity of food sources consumed by dipters, it can be expected that GBCs are equally diverse and differ among fly species, depending on their diet. A recent review comprising 21 insect orders showed that, overall, GBC diversity increased from blood-sucking insects via herbivores and carnivores to omnivores [57]. That may be due to the nearly sterile diets that blood-sucking insects consume, whereas omnivores have to handle a wide spectrum of food sources that themselves may contain rich microbial communities [57].

The question we address here is whether we observe a diet-specific pattern in GBCs in a subset of Diptera with different food sources. We addressed this question by using published GBC datasets on the bacterial phylum level (Figure 1) and more in detail on the genus level (Table S1). We searched the scientific literature for datasets of Diptera and only kept studies that analyzed insects fed on natural food sources (except for *An. gambiae* and *Anopheles culicifacies*) and used culture-independent methods to analyze GBCs. We tried to cover a broad range of food sources and to include different families. The papers were also screened for GBC identification on the genus level. On the basis of our criteria, we selected 16 different studies on 15 different dipteran species and 27 different samples (Table 1). The diets of the dipters ranged from plant material and sugar to zooplankton, waste, vertebrate tissues, and blood (Table 1). The data also included different life stages, partly on single species (five studies, seven species) and data on a single life stage offered natural and artificial diets (two studies, two species). Additionally, we extracted data on the presence of bacterial genera where its function is unknown. Presumably the presence of bacterial genera is associated with different life stages, partly on single species (five studies, seven species) and data on a single life stage offered natural and artificial diets (two studies, two species).

To compare the GBCs, we generated a heatmap with the gplots package 3.0.1.2 [58] in R 3.6.2 [59]. A one-way hierarchical clustering heatmap was generated using the relative abundance of bacterial phyla. Dipteran species were manually sorted according to their food sources. This allowed us to identify similarities and differences in GBCs among the tested dipteran species on the level of bacterial phyla.

**Figure 1.** Gut bacterial community composition in Diptera that is based on the studies in Table 1. Heatmap of gut bacterial composition in dipteran species feeding on various diets (plants, sugar, zooplankton, decomposer, flesh, or blood). Relative abundance (percentage of total) of bacteria is indicated at a phylum level. The life stages of the hosts (a: adult, l: larvae) and their sex (m: male, f: female) are indicated next to the species name.
Table 1. Food sources in Diptera. Overview of food sources in different dipteran species, life stages, and corresponding studies, which were used for detecting diet-specific patterns in dipteran gut microbial communities.

| Species                  | Life Stage | Food Source            | Origin   | Method                                | Reference |
|--------------------------|------------|------------------------|----------|---------------------------------------|-----------|
| Mayetiola destructor     | Adult      | Plants (leaves)        | Laboratory | 16S rRNA (454-pyrosequencing) | [60]      |
| Delia radicum            | Larvae     | Plants (roots)         | Laboratory | 16S rRNA (Ion Torrent)            | [61]      |
| Bactrocera cucurbitae    | Adult      | Plants (fruits)        | Natural  | 16S rRNA (ABI)                      | [62]      |
| Anastrepha serpentina    | Adult      | Plants (fruits)        | Natural  | 16S rRNA (454-pyrosequencing)       | [63]      |
| Hermetia illucens        | Larvae     | Plants (seeds)         | Laboratory | 16S rRNA (454-pyrosequencing)       | [64]      |
| Anopheles gambiae        | Adult      | Sugar solution         | Laboratory | 16S rRNA (454-pyrosequencing)       | [50]      |
| Anopheles culicifacies   | Adult      | Sugar solution         | Laboratory | 16S rRNA (454-pyrosequencing)       | [65]      |
| Aedes aegypti            | Adult      | Blood                  | Laboratory | 16S rRNA (Illumina)                | [66]      |
| Musca domestica          | Adult      | Decomposers (omnivore) | Natural  | 16S rRNA (Illumina, ABI)            | [67,68]   |
|                          | Larvae     | Plants (seeds)         | Laboratory | 16S rRNA (Illumina)              | [69]      |
| Drosophila melanogaster  | Adult      | Decomposers (plants)   | Natural  | 16S rRNA (Illumina)                | [70]      |
| Drosophila suzukii       | Adult      | Plant (fruits)         | Natural  | 16S rRNA (Illumina)                | [71]      |
|                          | Larvae     | Plants (fruits)        | Natural  | 16S rRNA (Illumina)                |            |
| Sarcophaga spp.          | Adult      | Decomposer (omnivores) | Natural  | 16S rRNA (ABI)                     | [72]      |
|                          | Larvae     | Flesh                  |          |                                      |            |
| Stomoxys calcitrans      | Larvae     | Decomposer (omnivore)  | Natural  | 16S rRNA (454-pyrosequencing)       | [73]      |
| Lucilia cuprina          | Adult      | Flesh                  | Laboratory | 16S rRNA (454-pyrosequencing)       | [74]      |
|                          | Larvae     | Flesh                  |          |                                      |            |
| Lucilia sericata         | Adult      | Flesh                  | Laboratory | 16S rRNA (454-pyrosequencing)       | [74]      |
|                          | Larvae     |                       |          |                                      |            |

Overall, the dipteran GBCs reported in these 16 studies were dominated by Proteobacteria, Firmicutes, and Bacterioidetes and did not cluster strictly by diet (Figure 1). Within the phylum Proteobacteria, the Gammaproteobacteria were the most abundant inhabitants of the included insect guts, with the taxa Providencia, Morganella, Pseudomonas, and Serratia occurring in nearly all species (Table S1) [61,74,75]. Providencia is known to enable xylan digestion, a common compound of cell walls [76]. The ability to digest xylan is particularly important for arthropod decomposers, especially those living in dead trees or in litter containing a lot of bark. Morganella on the other hand is mainly known as a human pathogen [77]. Even through Morganella is lethal to the Mexican fruit fly (Anastrepha ludens Loew), it occurs in this species as well as the gut of many other fly species [78–80], where its function is unknown. Presumably Morganella is taken up with food. Pseudomonas sp. are also commonly found in dipteran guts (Table S1) [50,60,61,65,73,74]. Several Pseudomonas strains are able to protect their hosts from endopathogenic fungi by producing antimicrobial substances [81].
Pathogenic microorganisms are omnipresent, which makes *Pseudomonas* an important GBC member with potential benefits to their host [82]. The effects of *Serratia* strains, which are frequently found in dipteran guts, can range from lethal to essential in their hosts. Some strains have a strong entomopathogenic effect through the production of chitinases and proteinases, for instance for weevils or *Drosophila* [83,84]. Other strains improve host nutritional status by producing amino acids, or defend their host by enhancing host immunity [85,86]. Both functions enable their hosts to live under challenging conditions.

We expected that species with similar diets also have similar gut microbiome communities. Figure 1 shows that of the dipteran species we considered, only flesh-feeding species had rather uniform GBCs at the phylum level. On lower taxonomic levels, several bacterial genera, such as *Carnobacterium*, *Psychrobacillus*, or *Empedobacter*, were almost exclusively detected in the carnivorous insects studied herein (Table S1). Carnivores and insects feeding on waste also share several microbial taxa (Table S1). *Proteus* seems to be a diet-specific genus of Diptera that feeds on flesh and waste (Table S1); it dominated in the larvae of the flesh flies we considered here, and it was also present in their diet and their parasitoids (*Nasonia vitripennis* (Walker) and *N. giraulti* (Darling)) [87]. Some *Proteus* strains, for instance *Proteus vulgaris* and *Proteus mirabilis*, produce antimicrobial compounds, that are active against *Echerichia coli* and *Staphylococcus aureus* [75,88]. This may be beneficial to the hosts, as both waste and flesh contain a high proportion of microbes that can also be pathogenic to the insects. Hosting *Proteus* strains may thus protect these flies against these pathogens, which they commonly encounter in their diet [75].

On the basis of the published data we considered here, we found no confirmation that the GBC composition was mainly driven by host diet. We assume that some Diptera possess mainly generic gut microbes, and only very few bacterial genera are associated with a specific diet. This makes it difficult to extrapolate putative functions with regards to diet specialization on the basis of GBC composition. Although 16S rRNA amplicon sequencing enables culture-independent identification of bacteria, it does not distinguish between living and dead bacteria nor resident and transient species. This lack of information prohibits us to draw strong conclusions on the relation between GBCs and diet specialization. Variation in sample collection, such as from dissected guts or surface-sterilized insects, and data processing may be additional sources of variation beyond our control, which may have prohibited us from finding a clearer signature of food source in GBCs.

4. Additional Sources of Variation in Dipteran GBC

A second hypothesis we tested is whether GBCs vary per species. On the phylum level, we found no evidence that host species determined the GBC. On the genus level, *Anopheles gambiae* harbors a few species-specific bacteria in its GBC (Table S1). For instance, *Elisabethkingia* was only found in *Anopheles*, independent of the life cycle stage and food source (Table S1) [89]. A reason that the GBCs do not cluster per species in Figure 1 may be that dipterans are confronted with a large number of variable factors in their life. First, as holometabolic insects, they pass through several distinct life stages. In many cases, transitions from one life stage into another come along with shifts in diet, habitat, and behavior [90–92]. These changes may result in GBC transitions within a life cycle [93]. Moreover, the GBC data used for the analyses may also vary because they are from different origins (natural or lab cultures), or are taken from different populations. In addition, GBCs are influenced by interactions within the GBC and with other microorganisms, such as microbes on the food or the aforementioned endosymbionts. Each of these factors might prohibit that we find strong indications for a stable, species- and/or diet-specific GBC in the dipteran species analyzed.

4.1. Variations in GBCs during the Life Cycle

Throughout their life, dipterans come in contact with a large diversity of microbes. A subset of these microbes can be included in their guts. These microbes can be acquired via the parents (vertical transmission) or via the environment (horizontal transmission) [94,95]. Vertical transmission ensures
that symbiotic microbes that are essential for survival are successfully transferred. In other words, the progeny benefits from “inheriting” advantageous microbe–host interactions from their parents, which have become established over a long period of time. Moreover, habitat and/or diet changes within a life cycle are common in the Diptera. Microbes that are no longer relevant can be removed from the community, thereby preventing their use of resources without providing benefits. Horizontal transmission may be advantageous when there are strong shifts in habitats or feeding habits between life stages. GBCs may result from a combination of horizontal and vertical transmission [96]. Due to the large numbers and diversity of microbes and the different transmission possibilities (vertical and horizontal), dipteran GBCs can be very diverse. To cover their needs, Diptera maintain specific conditions in their digestive tract that can “filter out” specific bacteria.

Vertical transfer of GBCs from adults to eggs was found in tephritid fruit flies and A. aegypti [97–99]. The microbial community on the egg surface was mostly derived from the adult’s gastrointestinal tracts. The adults transfer the microbes by smearing feces on the egg shells after oviposition [37]. The few studies there have shown that dipteran eggs have a low density, but a high diversity of bacteria (Figure 2) [100,101]. Some freshly hatched larvae consume the egg shell and thus inoculate their guts with these microbes [93]. The tsetse fly, a blood-feeding species, directly transfers obligate symbionts to the larvae. The larvae hatch in the mother, where they are nourished with special glands [102]. Beside proteins, lipids, and amino acids, the “mother milk” also contains bacteria [103]. In this way, the parental GBC co-determines the GBC of their offspring. However, only a subset of the parental gut microbes may be able to establish in the offspring. For example, in the eggs of a chironomid species, the dominant bacteria belong to Proteobacteria, whereas Firmicutes dominate in larvae [37]. This might be due to morphological and hormonal changes, as well as drastic changes in diets and environmental conditions between their life stages [37]. In the case of mosquitos, the larvae live in the water where they feed on algae and zooplankton (Figure 1) [104,105]. Male and female adults feed on nectar or honeydew, whereas only females of most species need a blood meal to produce eggs [106].

In addition, molting and pupation may be barriers to the smooth transition of GBCs to the next life stage. During the larval stage, dipterans pass through two to five molts [107]. The cuticles of the fore- and hindgut are regenerated after each molting process, meaning that the old ones are shed with a large part of the resident microbiome. Consequently, gut microbial composition and abundance vary among larval instars [108,109]. Total bacterial abundance can increase throughout the larval stages and peak in the last instar, just before the last defecation (Figure 2) [108,110,111]. There may be a positive correlation of larval stage and bacterial abundance. Larvae in later instars have probably been confronted with a wide variety of microbes, much more so than younger larvae.

At the end of the last instar, microbial abundance can strongly decrease. In this stage, the majority of gut microbes and digested food material are removed with defecation and additional excretion processes [111]. The microbial communities may be shed to allow metamorphosis to take place.

Metamorphosis is initiated by specific physiological and environmental conditions (body size, mass, specific hormone ratio) [112–115]. During metamorphosis, dipteran larvae replace nearly all their organs and tissues [107].

Most of the larval gut cells (fore- and hindgut) are released into the body cavity, and new structures are formed from cells of the fore- and hindgut [93,107,111]. In this process, the larval midgut is almost completely recycled. Larval midgut cells and GBCs are sloughed and degenerated. The remaining larval cells, remnant nutrition, and possibly also some surviving gut microbes remain in the lumen, and in several fly species may form the meconium [97,116,117]. In the parasitic spotted flesh fly Wohlfahrtia magnifica (Schiner), the abundance of gram-negative bacteria was particularly reduced after pupation [75]. This decrease correlated with the expression of several antimicrobial protein genes. These proteins are predicted to inhibit bacterial growth over metamorphosis, which may be another reason for the low bacterial abundances commonly found in pupae and freshly emerged adults [50,93,111]. Importantly, pupae do not feed, which excludes diet as a source of bacterial uptake. Even through eggs and pupae are non-feeding stages, the two life stages do not necessarily share the
same microbial community patterns. In blow flies, Actinobacteria and Acidobacteria prevailed in eggs, whereas Flavobacteriaceae and Bacilliales dominated in pupae [74]. This means that over the entire life cycle, bacterial communities can be very variable. A few bacteria seem to be present in all life stages. A study about GBCs in *Drosophila suzukii* found a similar abundance of a few bacteria in larvae and adults, such as *Tatumella punctate*; however, the abundance of several other gut bacteria also differed in both life stages [71]. We assume that the GBCs follow the needs of each life stage to a certain extent, rather than being species-specific.

**Figure 2.** Schematic representation of the changes in gut bacterial density and diversity over the course of the life cycle including egg, larval, pupal, and adult life stages that is based on the current literature reporting on seven species (Table S1). In green: relative gut bacterial community (GBC) density, in blue: relative GBC diversity. Relative changes in gut bacterial diversity and densities are represented by the different shapes and numbers of microbes in a Petri dish. (Picture: Jennifer Gabriel).

Freshly eclosed adults of *A. gambiae* have a nearly sterile gut, as most of the remaining gut microbes are removed with the meconium directly after eclosion [50]. Over the adults’ life time, the density of microbial communities can increases with age (Figure 2) [50,93]. While the microbes increase in numbers, the GBC composition can also change. *Lactobacillus* is dominant in young *Drosophila* flies, and Acetobacteria in older flies [93]. This confirms that GBCs may be mainly stage-specific instead of species-specific in some species. Stage-specific microbes may confer specific functions. The bacterium *Proteus vulgaris* was predominant in larvae and strongly reduced in several adult dipteran species [72]. It was suggested that *Proteus vulgaris* is involved in the digestion of larval food sources, survival in the larval environment, or metamorphosis [72,75]. Both switches in diets between life stages (which may require different GBCs) and new environments/diets (which present a large resource for new microbes)
may generate stage-specific instead of species-specific GBCs in some cases. The inclusion of microbes from new environments/diets upon changing life stage depends on microbial availability and microbial resistance against the defense barriers of the host insect [108]. Furthermore, the “new” microbes have to compete with the established GBCs. Due to the fact that changes in life stages co-occur with diet shifts, the effects of these two factors on GBCs are hard to disentangle.

4.2. Effect of Environment on Dipteran GBCs

Diptera are masters of adaptation. They exploit habitats that contain toxic organic or inorganic compounds or are low in oxygen. Within their lifetime, they may change from aquatic to terrestrial biomes and feed on different food sources. Furthermore, several Diptera, such as *Drosophila*, *Musca*, and *Delia* spp., can be easily reared under laboratory conditions. Each of these environmental factors alone, or in combination, may affect their GBCs.

Dipteran species live in biomes with contrasting environmental conditions. These range from (largely) anaerobic conditions, under water or in the soil, to aerobic aboveground terrestrial ecosystems where high temperatures may be an issue [118–120]. Such strong contrasts may shape GBC compositions. In most other insect orders, GBCs of terrestrial insects were richer in aerobic microbes than those of aquatic insects. This may be also true in Diptera [57], where facultative anaerobic microbes were found in the guts of aquatic stages in Culicidae [98]. Moreover, different life stages of one species can live in different habitats. Mosquitos start their life in water and move to terrestrial habitats in the adult stage [121]. In *A. aegypti*, microbial diversity and abundance changed significantly between both stages. The microbial diversity declined from larvae (74 operational taxonomic unit (OTUs)) to adults feeding on sugar (39 OTUs) [98]. A blood meal reduced the diversity even more to 22 OTUs. The larval GBCs were dominated by *Leucobacter* and *Microbacterium* (Actinobacteria), which were nearly absent in the adults. Adult GBCs were dominated by *Pseudomonas*, *Paenibacillus*, *Aeromonas*, *Aquitalea*, and *Stenotrophomonas* (Gamma- and Betaproteobacteria, Firmicutes). These groups were only found in low numbers in the larvae. The GBCs of females that had a blood meal were dominated by the two bacteria strains *Chryseobacterium* and *Delfia* (both Betaproteobacteria) [98]. Several of these strains turned out to be essential for the larvae to reach the adult stage. Whereas axenic larvae failed to develop beyond the first instar, adding *Acinetobacter*, *Paenibacillus*, *Aeromona*, *Aquitalea*, or *Chryseobacterium* to the axenic larvae enabled them to reach the adult stage [98]. Some of these bacteria produce signal molecules that regulate growth and metabolism, helping them to reach the adult stage [122]. This may explain why the GBC abundance in *Drosophila melanogaster*, *Chrysomya megacephala*, *Bactrocera dorsalis*, and *An. gambiae* decreased from the larval to the adult stage [50,100,101,123].

Another variable factor that dipterans have to handle is temperature. This abiotic factor not only determines developmental time in Diptera [124], but it may also affect GBCs. For instance, both *Wolbachia* and *Acetobacter* live inside *D. melanogaster*. When the host developed at colder temperatures, *Wolbachia* predominated, whereas at higher temperatures *Acetobacter* was more important [125]. This may also be true for other gut bacteria. GBCs may also differ over the seasons, which strongly correlate with shifts in temperature and diets. In green bottle fly GBCs (Calliphoridae), *Staphylococcus* was dominant in spring; *Ignatzschineria* in summer; and *Vagococcus*, *Dysgonomonas*, and an unclassified *Acetobacteraceae* in autumn [126]. The GBC diversity tended to increase from spring (24 OTUs) to autumn (93 OTUs) in these flies. Wei et al. [126] suggested that changes in climatic conditions were the main cause of seasonal variations in fly-associated bacterial communities. However, seasonal changes also come with differences in the environmental microbial communities, including those on food resources. More studies are necessary to show whether GBC variations over seasons are a general pattern in Diptera. Specific manipulative experiments can disentangle the effect of temperature from other factors that change with the season, such as rainfall patterns, UV/sunlight radiation, day length, and the availability of food resources. Such knowledge may also help to predict impacts of global change on dipteran GBCs. On the one hand, GBCs may affect the availability of the host to adapt to global
changes. On the other hand, global change may also affect the occurrence of microbes in the host’s environment and diet [127].

In most cases, both food source and habitat conditions are markedly different between natural and laboratory systems. In laboratory cultures, biotic and abiotic factors vary very little because the rearing conditions are optimized to produce as many individuals as possible [128]. The absence of interactions may lift natural selection pressures, such as enemies, competition with other species, and fluctuating environmental conditions, which normally would shape GBCs. In particular, the lack of microbial cross-infections from other species and/or different diets may restrict fly-associated microbial community development in laboratory cultures. Laboratory-reared *Drosophila* flies indeed possess a less diverse gut microbiome, compared to flies caught in the wild (2.4–5.3 times more OTUs). Moreover, the GBC composition differed; in GBCs of lab-reared flies, *Acetobacter* and *Lactobacillus* dominated, whereas in conspecifics from the field, Proteobacteria in particular were found [93,96,100,129,130]. In contrast, GBCs of wild and laboratory *An. gambiae* strains were rather similar [50]. The same was true for housefly GBCs; essentially the same bacteria species were isolated from the digestive tract of field-collected and laboratory-reared house flies, independent of life stage and collection year [56,131]. On the basis of these few examples, it is too early to conclude whether the GBCs of lab-reared strains are consistently different from conspecifics in their natural habitat. More specific analysis comparing GBCs of lab-reared with field-caught dipterans are needed to address this question.

4.3. Interactions with Other Microorganisms

Apart from the internal and external factors discussed in the previous sections, GBCs may also be affected by interactions with other microbes. Several dipterans vector microbial human pathogens, which may interface with GBCs. In addition, gut microbes may compete among each other as well as with other microbes colonizing their host, such as endosymbionts. Such “host-internal” microbial interactions can both affect and be affected by GBC composition.

Dipterans are well-known vectors of a wide variety of vertebrate diseases, such as malaria and yellow fever [132,133]. The pathogens are mainly bacteria, viruses, or protists, here collectively referred to as human pathogens. Human pathogens interact in different manners with the GBCs of their vectors. First, human pathogens can decrease abundance of particular microbes, thereby altering GBC compositions in some Diptera. For example, the pathogen *Leishmania mexicana* is vectored by sand flies. Its presence in the fly decreases the microbial richness in the insect’s GBC [134]. Several Pseudomonadaceae were reduced in their abundance, whereas Acetobacteraceae became dominant with increasing pathogen densities [134]. Second, dipteran GBCs can affect the development of human pathogens [135]. For many human pathogens, it is essential to replicate or to go through several steps of differentiation in the vector before they can infect humans. Both inhibitory and beneficial effects are reported. GBCs of the mosquitoes *An. stephensi* and *An. albimanus* inhibited the development of the malaria pathogen *Plasmodium* through the activation of general immune system responses in the guts of these mosquitoes [136–138]. The natural common gut bacterium *Enterobacter* produces reactive oxygen species in the midgut of the mosquito *An. gambiae*. The reactive oxygen directly inhibits the development of the malaria pathogen *Plasmodium* [139]. This indicates that GBCs of the vectors can affect the transmission of human pathogens through the inhibition of human pathogen development in their vector. Human pathogens can also benefit from the vector’s GBCs. GBCs of sand flies improved the growth and development of infective stages in the pathogen *Leishmania infantum* [23]. Experimental applications of antibiotics to *L. infantum*-infected sand flies reduced the replication and the development to infectious stages in the vector’s gut, without affecting sand fly fitness [134]. Thus, GBCs can be critical factors for the survival of human pathogens, their differentiation to infective stages, and disease transmission. On the basis of their impact on pathogen life cycles, GBCs provide a potential as biological control for vector transmitted diseases.

Besides human pathogens, other GBC members can also affect GBC composition. Interactions between different GBC members can influence gut microbial co-occurrence in a positive, negative,
Neutral co-occurrence was the most commonly observed interaction within gut microbial taxa in *D. melanogaster*, except for the three following groups [70]. Xanthomonadaceae showed positive co-occurrence effects mainly with taxonomically related strains. *Enterococcus* and *Staphylococcus aureus* showed negative co-occurrence effects mainly with non-closely related strains. Overall, taxa that are interacting with many other bacteria were not the most common ones. It seems that the dominant taxa are not the mayor players in structuring GBCs in *D. melanogaster* [70]. To understand interactions of GBCs with each other and their hosts, we have to gain a better understanding of gut microbe communications and to identify regulatory mediators in GBCs. This could be achieved by analyzing meta-transcriptomes of GBCs challenged with different interaction partners.

5. Conclusions and Future Perspectives

In this review, we explored factors that may determine the composition of GBCs in Diptera. We considered species-related effects, food sources, environmental conditions, life history, and interactions with other microorganisms. On the basis of our findings, diet can partly explain differences in GBCs in the species we could obtain data on, but this applies mostly to species feeding on vertebrate tissues and blood. Changes in food sources are often linked to different life stages. Life cycle transitions may have a strong impact on GBCs due to drastic morphological changes during metamorphosis. Likely, this prevents the development of a strictly species-specific GBC in some dipterans.

Our review revealed several factors that limit our ability to understand the role of GBCs in Diptera. The main limiting factor is the low number of studies. Even for the order of Diptera, which contains over 150,000 species, the GBCs of only a handful of species have been studied in sufficient detail. Moreover, the studies we analyzed focused mainly on species with economic impact or vectors of human pathogens. More dipteran species, and within each species more life stages, must be analyzed before we can draw firm conclusions how GBC patterns relate to dipteran diversity. A second limiting factor is the classification of bacteria. In general, bacteria are differentiated according to their genetic and phenotypic similarity [140]. However, genetic similarity is often based on a single gene, the 16S rRNA, for both the commonly used OTU and amplicon sequence variant (ASV), which does not necessarily reflect similarity at the whole-genome level. In addition, horizontal gene transfer and mutations occur relatively frequently in bacteria. Such minor changes in the genome can have strong effects on the metabolism. Bacteria with genetic and phenotypic similarity but functional differences are therefore usually divided into separate strains. It is possible that the same ASVs occur in the guts of different insects, but that these ASVs have different functions. This “hidden” level of bacterial diversity prohibits firm statements on bacterial functional diversity.

Despite the increased interest of GBCs in macroorganisms, a large gap remains, namely, the function of the entire GBC versus single members therein. Massive parallel sequencing approaches combined with bioinformatics, collectively called metagenomics, allow us to compare GBCs and identify differences in compositions among species, life stages, and diets in much more detail. However, such comparisons only reveal who is there, and not which function the GBC, or single species therein, might have. Metatranscriptomic analyses identify which genes are up- or down-regulated in a GBC, for example, in response to toxins or environmental changes. This helps to identify possible functions, for instance a main detoxification enzyme, present in the GBC. However, metagenomics and -transcriptomic analyses only generate hypotheses on which function could be important. Preferably, they should be combined with manipulative experiments, knocking out a specific function after which insect performance is also assessed. This approach could also help to identify potential targets for pest management. Targeting stage-specific gut microbes seems a promising control strategy to interfere with the insect’s life cycle. This may prohibit the development of the critical stage or sex, for example, the emergence of female mosquitoes that vector human pathogens. In order to develop such strategies, more specific knowledge about GBCs and its role in each of the host’s life cycle stages are needed.
In addition, we lack understanding of interactions within GBCs or between GBCs and their host. Within GBCs, many interactions may occur; among bacterial species competing for a niche, and among bacteria, fungi, and viruses. To better understand these processes, we would recommend the use of synthetic GBCs that could be fed to sterile insects [141]. These insects could be exposed to different conditions, and the resulting GBCs as well as the host performance could be measured. This would allow us to identify drivers and passengers in GBCs or help to identify specific functions provided by the host. We can draw on tools from biodiversity science and restoration ecology to analyze GBC interaction webs and identify key organisms [142]. Ecosystem service analyses may predict connections among GBC members and identify their functional roles [143]. Possible features to use in GBC models are nutritional data, detoxification mechanisms, and the ability to produce antibiotics by single community members. These data can be obtained from metagenomic or -transcriptomic analyses. The exact signals involved in microbe–microbe communication and how they affect GBC structures are still largely unknown. Integrating metabolomic analyses and molecular diagnostic approaches (e.g., Raman spectroscopy) would be a possible approach to fill this gap.

By studying dipteran GBCs, we will also increase understanding the ecology of the insect hosts and their adaptation to recalcitrant diets and habitats. We recognized that only very few studies report on GBCs in Diptera, particularly in relation with food sources. This makes it very difficult to draw conclusions about diet specificity of GBCs. In order to do so, we would need phylogenetically controlled GBC composition data, for instance screening a single genus with multiple feeding strategies or specialization levels. In addition, having multiple replicates of the same feeding strategies over distant lineages, such as Culicidae vs. Tabanidae, would improve our knowledge on the role of GBCs in habitat adaptations. The same is true for the dynamics of GBCs during the life cycle. Only a handful of studies analyze the GBCs of life cycle stages; studies comparing the fate of GBCs over life cycles of several species are even rarer. More research is needed, especially experimental studies, to understand host–GBC interactions and dynamics within GBCs. Utilizing approaches from other disciplines facilitates the development of new concepts and will help to test current hypotheses on the function of dipteran GBCs.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4450/11/8/543/s1,
Table S1: Presence or absence of gut bacteria on the genus level in selected dipteran species, life stages and food source, Table S2: Density and diversity of gut microbes in seven Diptera species.

Author Contributions: R.S. and N.M.v.D. developed the concept, R.S. prepared the draft version, N.M.v.D. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the German Research Foundation (DFG) Collaborative Research Center 1127 ChemBioSys and DFG funding to the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig (FZT 118).

Acknowledgments: We thank Anja Worrich from the Umweltforschungszentrum (UFZ) for her comments on an earlier version of the manuscript and Jennifer Gabriel for designing Figure 2. We thank also the five unknown reviewers for their comments which improved our manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Kastinger, C.; Weber, A. Bee-flies (Bombylius spp., Bombyliidae, Diptera) and the pollination of flowers. Flora 2001, 196, 3–25. [CrossRef]
2. Bates, A.J.; Sadler, J.P.; Fairbrass, A.J.; Falk, S.J.; Hale, J.D.; Matthews, T.J. Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. PLoS ONE 2011, 6, e23459. [CrossRef] [PubMed]
3. White, I.M.; Elson-Harris, M.M. Fruit Flies of Economic Significance: Their Identification and Bionomics; CAB International: Wallingford, UK, 1992.
4. Alam, U.; Medlock, J.; Brelsfoord, C.; Pais, R.; Lohs, C.; Balmard, S.; Carnogursky, J.; Heddi, A.; Takac, P.; Galvani, A.; et al. Wolbachia symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly Glossina morsitans. PLoS Pathog. 2011, 7, e1002415. [CrossRef] [PubMed]
5. Stork, N.E. How many species of insects and other terrestrial arthropods are there on earth? *Annu. Rev. Entomol.* **2018**, *63*, 31–45. [CrossRef]

6. Postma, J.F.; VanNugteren, P.; De Jong, M.B.B. Increased cadmium excretion in metal-adapted populations of the midge *Chironomus riparius* (Diptera). *Environ. Toxicol. Chem.* **1996**, *15*, 332–339. [CrossRef]

7. Schneider, M.; Wunder, C.; Reuss, E.; Toennes, S.W.; Mebs, D. Evading plant defence: Infestation of poisonous milkweed fruits (Asclepiadaceae) by the fruit fly *Dacus silicultatus* (Diptera: Tephritidae). *Toxicon* **2017**, *139*, 13–19. [CrossRef]

8. Rosenberg, E.; Zilber-Rosenberg, I. Symbiosis and development: The hologenome concept. *Birth Defects Res. Part C Embryo Today Rev.* **2011**, 93, 56–66. [CrossRef]

9. Hagen, K.S. Dependence of the olive fly, *Dacus oleae*, larvae on symbiosis with *Pseudomonas savastanoi* for the utilization of olive. *Nature* **1966**, *209*, 423–424. [CrossRef]

10. Hultmark, D.; Weidmann, K.; Eriksson, M. Life in wood: Predation and symbiosis in the wood-boring fly *Diptera: Rhagoletis cerasi*. *Proc. R. Soc. Lond. B Biol. Sci.* **1997**, *264*, 1011–1016. [CrossRef]

11. Hultmark, D.; Weidmann, K. Life in wood: Predation and symbiosis in the wood-boring fly *Diptera: Rhagoletis cerasi*. *Proc. R. Soc. Lond. B Biol. Sci.* **1997**, *264*, 1011–1016. [CrossRef]

12. Tirados, I.; Costantini, C.; Gibson, G.; Torr, S.J. Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: Implications for vector control. *Med. Vet. Entomol.* **2006**, *20*, 425–437. [CrossRef]

13. Zheng, L.; Crippen, T.L.; Singh, B.; Zhang, Y.; Mebs, D. Evading plant defence: Infestation of poisonous milkweed fruits (Asclepiadaceae) by the fruit fly *Dacus silicultatus* (Diptera: Tephritidae). *Toxicon* **2017**, *139*, 13–19. [CrossRef]

14. McLaughlin, K.; Burkot, T.R.; Oscar, J.; Beebe, N.W.; Russell, T.L. Defining the larval habitat: Abiotic and biotic parameters associated with *Anopheles aarauti* productivity. *Malar. J.* **2019**, *18*, 7. [CrossRef]

15. Simón, H.M. Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed different diets. *Environ. Entomol.* **2011**, *40*, 1111–1122. [CrossRef]

16. Whitaker, M.R.L.; Salzman, S.; Sanders, J.; Kaltenpoth, M.; Pierce, N.E. Microbial communities of Lycaenid butterflies do not correlate with larval diet. *Front. Microbiol.* **2016**, *7*, 13. [CrossRef]

17. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef]

18. Diaz-Albiter, H.; Sant’Anna, M.R.; Genta, F.A.; Dillon, R.J. Reactive oxygen species-mediated immunity against *Leishmania mexicana* and *Serratia marcescens* in the phlebotomine sand fly *Lutzomyia longipalpis*. *J. Biol. Chem.* **2012**, *287*, 23995–24003. [CrossRef] [PubMed]

19. Pinto-Tomás, A.A.; Sittenfeld, A.; Uribe-Lora, L.; Chavarría, F.; Mora, M.; Janzen, D.H.; Goodman, R.M.; Zaballos, J.; Simon, H.M. Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed on different diets. *Environ. Entomol.* **2011**, *40*, 1111–1122. [CrossRef]

20. Whittington, M.R.L.; Salzman, S.; Sanders, J.; Kaltenpoth, M.; Pierce, N.E. Microbial communities of Lycaenid butterflies do not correlate with larval diet. *Front. Microbiol.* **2016**, *7*, 13. [CrossRef]

21. Simon, H.M. Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed different diets. *Environ. Entomol.* **2011**, *40*, 1111–1122. [CrossRef]

22. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef]

23. Torsvik, V.; Øvreås, L. Microbial diversity, life strategies, and adaptation to life in extreme soils. In *Microbiology of Extreme Soils*; Springer: Berlin/Heidelberg, Germany, 2008; pp. 15–43.

24. Torsvik, V.; Øvreås, L. Microbial diversity, life strategies, and adaptation to life in extreme soils. In *Microbiology of Extreme Soils*; Springer: Berlin/Heidelberg, Germany, 2008; pp. 15–43. [CrossRef] [PubMed]

25. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef]

26. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef]

27. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef] [PubMed]

28. Waterman, S.H.; Gubler, D.J. Dengue fever. *Clin. Dermatol.* **1989**, *7*, 117–122. [CrossRef] [PubMed]
27. Ricci, I.; Cancrini, G.; Gabrielli, S.; D’amelio, S.; Favia, G. Searching for Wolbachia (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): Large polymeerase chain reaction survey and new identifications. J. Med. Entomol. 2002, 39, 562–567. [CrossRef] [PubMed]
28. Ono, M.; Braig, H.R.; Munstermann, L.E.; Ferro, C.; O’Neill, S.L. Wolbachia infections of phlebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 2001, 38, 237–241. [CrossRef]
29. Snyder, A.K.; Deberry, J.W.; Runyen-Janecky, L.; Rio, R.V. Nutrient provisioning facilitates homeostasis between bsete fly (Diptera: Glossinidae) symbionts. Proc. R. Soc. B Biol. Sci. 2010, 277, 2389–2397. [CrossRef]
30. Werren, J.H.; Zhang, W.; Guo, L.R. Evolution and phylogeny of Wolbachia: Reproductive parasites of arthropods. Proc. R. Soc. Lond. Ser. B Biol. Sci. 1995, 261, 55–63.
31. Douglas, A.E. The microbial dimension in insect nutritional ecology. Funct. Ecol. 2009, 23, 38–47. [CrossRef]
32. Daane, K.M.; Johnson, M.W. Olive fruit fly: Managing an ancient pest in modern times. Annu. Rev. Entomol. 2010, 55, 151–169. [CrossRef]
33. Ben-Yosef, M.; Pasternak, Z.; Jurkevitch, E.; Yuval, B. Symbiotic bacteria enable olive flies (Bactrocera oleae) to exploit intractable sources of nitrogen. J. Evol. Biol. 2014, 27, 2695–2705. [CrossRef] [PubMed]
34. Behar, A.; Yuval, B.; Jurkevitch, E. Enterobacteria-mediated nitrogen fixation in natural populations of the fruit fly Ceratitis capitata. Mol. Ecol. 2005, 14, 2637–2643. [CrossRef] [PubMed]
35. Somparn, A.; Iwai, C.; Noller, B. Potential use of acetylcholinesterase, glutathione-s-transferase and metallothionein for assessment of contaminated sediment in tropical chironomid, Chironomus javanus. J. Environ. Biol. 2015, 36, 1355.
36. Amiard, J.-C.; Amiard-Triquet, C.; Barka, S.; Pellerin, J.; Rainbow, P. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 2006, 76, 160–202. [CrossRef] [PubMed]
37. Senderovich, Y.; Halpern, M. The protective role of endogenous bacterial communities in chironomid egg masses and larvae. ISME J. 2013, 7, 2147. [CrossRef] [PubMed]
38. Van den Bosch, T.J.; Niemi, O.; Welte, C.U. Single gene enables plant pathogenic pectobacterium to overcome host-specific chemical defence. Mol. Plant. Pathol. 2019, 21, 349–359. [CrossRef]
39. Lo Scalzo, R.; Scarpati, M.L.; Verzegnassi, B.; Vita, G. Olea europaea chemicals repellent Todacus oleae females. J. Chem. Ecol. 1994, 20, 1813–1823. [CrossRef]
40. Fleming, H.; Walter, W.; Etchells, J. Antimicrobial properties of olevuropein and products of its hydrolysis from green olives. Appl. Environ. Microbiol. 1973, 26, 777–782. [CrossRef]
41. Báidez, A.; Gómez, P.; Del Río, J.; OrtúñO, A. Antifungal capacity of major phenolic compounds of Olea europaea l. Against Phytophthora megasperma dreschsler and Cylindrocarpon destructans (Zinssm.) scholten. Physiol. Mol. Plant. Pathol. 1996, 49, 224–229.
42. Finch, S.; Ackley, C.M. Cultivated and wild host plants supporting populations of the cabbage root fly. Ann. Appl. Biol. 1977, 85, 13–22. [CrossRef]
43. Hopkins, R.J.; van Dam, N.M.; van Loon, J.J. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu. Rev. Entomol. 2009, 54, 57–83. [CrossRef] [PubMed]
44. Bones, A.M.; Rossiter, J.I. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 2006, 67, 1053–1067. [CrossRef] [PubMed]
45. Van Dam, N.M.; Tytgat, T.O.; Kirkegaard, J.A. Root and shoot glucosinolates: A comparison of their diversity, function and interactions in natural and managed ecosystems. Phytochem. Rev. 2009, 8, 171–186. [CrossRef]
46. Crespo, E.; Hordijk, C.A.; de Graaf, R.M.; Samudrala, D.; Cristescu, S.M.; Harren, F.J.; van Dam, N.M. On-line detection of root-induced volatiles in Brassica nigra plants infested with Delia radicum l. Root fly larvae. Phytochemistry 2012, 84, 68–77. [CrossRef] [PubMed]
47. Van Dam, N.M.; Samudrala, D.; Harren, F.J.; Cristescu, S.M. Real-time analysis of sulfur-containing volatiles in Brassica plants infested with root-feeding Delia radicum larvae using proton-transfer reaction mass spectrometry. Aob Plants 2012, 2012. [CrossRef] [PubMed]
48. Welte, C.U.; Rosengarten, J.F.; de Graaf, R.M.; Jetten, M.S. Saxa-mediated isothiocyanate metabolism in phytopathogenic pectobacteria. Appl. Environ. Microbiol. 2016, 82, 2372–2379. [CrossRef]
49. Van den Bosch, T.J.; Tan, K.; Joachimiak, A.; Welte, C.U. Functional profiling and crystal structures of isothiocyanate hydrolases found in gut-associated and plant-pathogenic bacteria. Appl. Environ. Microbiol. 2018, 84, 00478-18. [CrossRef]
50. Wang, Y.; Gilbreath, T.M.; III; Kukutla, P.; Yan, G.; Xu, J. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *PLoS ONE* 2011, 6, e24767. [CrossRef]

51. Huang, Y.; Yu, Y.; Zhan, S.; Tomberlin, J.K.; Huang, D.; Cai, M.; Zheng, L.; Yu, Z.; Zhang, J. Dual oxidase duox and toll-like receptor 3 thr3 in the toll pathway suppress zoonotic pathogens through regulating the intestinal bacterial community homeostasis in *Hermertia illucens*. *PLoS ONE* 2020, 15, e0225873. [CrossRef]

52. Gaio, A.d.O.; Gusmão, D.S.; Santos, A.V.; Berbert-Molina, M.A.; Pimenta, P.F.P.; Lemos, F.J.A. Contribution of midgut bacteria to blood digestion and egg production in *Aedes aegypti* (Diptera: Culicidae) (L.). *Parasites Vect.* 2011, 4, 105. [CrossRef]

53. Houk, E.; Obie, E.; Hardy, J. Peritrophic membrane formation and the midgut barrier to arboviral infection in the mosquito, *Culex tarsalis* coquillett (Insecta, Diptera). *Acta Trop.* 1979, 36, 39–45. [PubMed]

54. Freyvogel, T.A.; Stäubli, W. The formation of the peritrophic membrane in Culicidae. *Acta Trop.* 1965, 22, 118–147. [PubMed]

55. Schmidtmann, E.; Martin, P. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. *J. Med. Entomol.* 1992, 29, 232–235. [CrossRef] [PubMed]

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

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

58. Warnes, M.G.R.; Bolker, B.; Bonebakker, L.; Gentleman, R.; Huber, W. Package ‘gplots’. Various R Programming Tools for Plotting Data. 2016. Available online: https://CRAN.R-project.org/package=gplots (accessed on 1 June 2020).

59. R Core Team. *R: A Language and Environment for Statistical Computing*; Verion 3.6.2; R Foundation for Statistical Computing: Vienna, Austria, 2019.

60. Bansal, R.; Hulbert, S.H.; Reese, J.C.; Whitworth, R.J.; Stuart, J.J.; Chen, M.-S. Pyrosequencing reveals the predominance of pseudomonadaceae in gut microbiome of a gall midge. *Pathogens* 2014, 3, 459–472. [CrossRef]

61. Welte, C.U.; de Graaf, R.M.; van den Bosch, T.J.M.; Op den Camp, H.J.M.; van Dam, N.M.; Jetten, M.S.M. Plasmids from the gut microbiome of cabbage root fly larvae encode saxa that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environ. Microbiol.* 2017, 19, 1857–1867. [CrossRef] [PubMed]

62. Hadapad, A.B.; Prabhakar, C.S.; Chandekar, S.C.; Tripathi, J.; Hire, R.S. Diversity of bacterial communities in food waste-reducing larvae of *Hermetia illucens* (Diptera: Tephritidae) based on pyrosequencing. *FEMS Microbiol. Ecol.* 2018, 94, 1–10. [CrossRef] [PubMed]

63. Ventura, C.; Briones-Roblero, C.I.; Hernández, E.; Rivera-Orduña, F.N.; Zuñiga, G. Comparative analysis of the gut bacterial community of four *Anastrepha* fruit flies (Diptera: Tephritidae) based on pyrosequencing. *Curr. Microbiol.* 2018, 75, 966–976. [CrossRef]

64. Jeon, H.; Park, S.; Choi, J.; Jeong, G.; Lee, S.-B.; Choi, Y.; Lee, S.-J. The intestinal bacterial community in the food waste-reducing larvae of *Hermertia illucens*. *Curr. Microbiol.* 2011, 62, 1390–1399. [CrossRef]

65. Sharma, P.; Sharma, S.; Murarya, R.K.; De, T.D.; Thomas, T.; Lata, S.; Singh, N.; Pandey, K.C.; Valecha, N.; Dixit, R. Salivary glands harbor more diverse microbial communities than gut in *Anopheles culicifacies*. *Parasites Vectors* 2014, 7, 235. [CrossRef] [PubMed]

66. Muturi, E.J.; Dunlap, C.; Ramirez, J.L.; Rooney, A.P.; Kim, C.-H. Host blood-meal source has a strong impact on gut microbiota of *Aedes aegypti*. *FEMS Microbiol. Ecol.* 2018, 95. [CrossRef] [PubMed]

67. Bahrndorff, S.; de Jonge, N.; Skovgård, H.; Nielsen, J.L. Bacterial communities associated with houseflies (*Musca domestica* L.) sampled within and between farms. *PLoS ONE* 2017, 12, e0169753. [CrossRef] [PubMed]

68. Gupta, A.K.; Nayduch, D.; Verma, P.; Shah, B.; Ghate, H.V.; Patole, M.S.; Shouce, Y.S. Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS Microbiol. Ecol.* 2012, 79, 581–593. [CrossRef]

69. Zhao, Y.; Wang, W.; Zhu, F.; Wang, X.; Wang, X.; Lei, C. The gut microbiota in larvae of the housefly *Musca domestica* and their horizontal transfer through feeding. *AMB Express* 2017, 7, 147. [CrossRef]

70. Adair, K.L.; Wilson, M.; Bost, A.; Douglas, A.E. Microbial community assembly in wild populations of the fruit fly *Drosophila melanogaster*. *ISME J.* 2018, 12, 959–972. [CrossRef]
71. Chandler, J.A.; James, P.M.; Jospin, G.; Lang, J.M. The bacterial communities of Drosophila suzukii collected from undamaged cherries. PeerJ 2014, 2, e474. [CrossRef]

72. Gupta, A.; Rastogi, G.; Nayduch, D.; Sawant, S.; Bhonde, R.; Shouche, Y. Molecular phylogenetic profiling of gut-associated bacteria in larvae and adults of flesh flies. Med. Vet. Entomol. 2014, 28, 345–354. [CrossRef]

73. Scully, E.; Friesen, K.; Wienhold, B.; Durso, L.M. Microbial communities associated with stable fly (Diptera: Muscidae) larvae and their developmental substrates. Ann. Entomol. Soc. Am. 2017, 110, 61–72. [CrossRef]

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

75. Toth, E.; Hell, E.; Kovács, G.; Borsodi, A.; Marialigeti, K. Bacteria isolated from the different developmental stages and larval organs of the obligate parasitic fly, Wohlfahrtia magnifica (Diptera: Sarcophagidae). Microb. Ecol. 2006, 51, 13–21. [CrossRef] [PubMed]

76. Thomson, J.A. Molecular biology of xylan degradation. FEMS Microbiol. Lett. 1993, 106, 65–82. [CrossRef] [PubMed]

77. Liu, H.; Zhu, J.; Hu, Q.; Rao, X. Morganella morganii, a non-negligent opportunistic pathogen. Int. J. Infect. Dis. 2016, 50, 10–17. [CrossRef] [PubMed]

78. Chaiwong, T.; Srivoramas, T.; Suebsamran, P.; Sukontason, K.; Sanford, M.; Sukontason, K. The blow fly, Chrysomya megacephala, and the house fly, Musca domestica, as mechanical vectors of pathogenic bacteria in northeast Thailand. Trop. Biomed. 2014, 31, 336–346.

79. Shelomi, M.; Wu, M.-K.; Chen, S.-M.; Huang, J.-J.; Burke, C.G. Microbes associated with black soldier fly (Diptera: Stratiomyidae) degradation of food waste. Environ. Entomol. 2020, 49, 405–411. [CrossRef] [PubMed]

80. Salas, B.; Conway, H.E.; Schuenzel, E.L.; Hopperstad, K.; Vitck, C.; Vacek, D.C. Morganella morganii (Enterobacteriales: Enterobacteriaceae) is a lethal pathogen of mexican fruit fly (Diptera: Tephritidae) larvae. Fla. Entomol. 2017, 100, 743–751. [CrossRef]

81. Zhang, F.; Huang, Y.H.; Liu, S.Z.; Zhang, L.; Li, B.T.; Zhao, X.X.; Fu, Y.; Liu, J.J.; Zhang, X.X. Pseudomonas reactans, a bacterial strain isolated from the intestinal flora of Blattella germanica with anti-Beauveria bassiana activity. Environ. Entomol. 2013, 42, 453–459. [CrossRef]

82. Indiragandhi, P.; Anandham, R.; Madhaiyan, M.; Poonguzhali, S.; Kim, G.; Saravanan, V.; Sa, T. Cultivable bacteria associated with larval gut of Prothiofos-resistant, Prothiofos-susceptible and field-caught populations of diamondback moth, Plutella xylostella and their potential for, antagonism towards entomopathogenic fungi and host insect nutrition. J. Appl. Microbiol. 2007, 103, 2664–2675.

83. Flyg, C.; Kenne, K.; Boman, H.G. Insect pathogenic properties of Serratia marcescens: Phage-resistant mutants with a decreased resistance to Cecropia immunity and a decreased virulence to Drosophila. Microbiology 1980, 120, 173–181. [CrossRef]

84. Klein, M.G.; Kaya, H. Bacillus and Serratia species for scarab control. Memórias Do Inst. Oswaldo Cruz 1995, 90, 87–95. [CrossRef]

85. Oliver, K.M.; Degnan, P.H.; Burke, G.R.; Moran, N.A. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol. 2010, 55, 247–266. [CrossRef] [PubMed]

86. Scrsacia, M.; Pazzani, C.; Valentini, F.; Oliva, M.; Russo, V.; D’Addabbo, P.; Porcelli, F. Identification of pigmented Serratia marcescens symbiotically associated with Rhynchophorus ferrugineus olivier (Coleoptera: Curculionidae). MicrobiologyOpen 2016, 5, 883–890. [CrossRef] [PubMed]

87. Brucker, R.M.; Bordenstein, S.R. The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus Nasonia. Science 2013, 341, 667–669. [CrossRef] [PubMed]

88. Erdmann, G.R.; Bromel, M.; Gassner, G.; Freeman, T.P.; Fischer, A. Antibacterial activity demonstrated by culture filtrates of Proteus mirabilis isolated from screwworm (Cochliomyia hominivorax) (Diptera: Calliphoridae) larvae. J. Med. Entomol. 1984, 21, 159–164. [CrossRef]

89. Kämpfer, P.; Matthews, H.; Glaesser, S.P.; Martin, K.; Lodders, N.; Faye, I. Elizabethkingia anophelis sp. Nov., isolated from the midgut of the mosquito Anopheles gambiae. Int. J. Syst. Evol. Microbiol. 2011, 61, 2670–2675.

90. Delettre, Y.R.; Morvan, N.; Tre’Hén, P.; Grootaert, P. Local biodiversity and multi-habitat use in empidoid flies (Insecta: Diptera, Empididae). Biodivers. Conserv. 1998, 7, 9–25. [CrossRef]

91. McLachlan, A.; Ladle, R. Life in the puddle: Behavioural and life-cycle adaptations in the Diptera of tropical rain pools. Biol. Rev. 2001, 76, 377–388. [CrossRef]
92. Liu, X.; Chen, X.; Wang, H.; Yang, Q.; ur Rehman, K.; Li, W.; Cai, M.; Li, Q.; Mazza, L.; Zhang, J.; et al. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS ONE* 2017, 12, e0182601. [CrossRef]

93. Broderick, N.A.; Lemaître, B. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 2012, 3, 307–321. [CrossRef]

94. Schorr, H. Zur verhaltensbiologie und symbiose von *Brachyspella aterrima* först. (Cydnidae, Heteroptera). Z. Für Morphol. Und Ökologie Der Tiere 1957, 45, 561–602. [CrossRef]

95. Kikuchi, Y.; Hosokawa, T.; Fukatsu, T. Insect-microbe mutualism without vertical transmission: A stinkbug acquires a beneficial gut symbiont from the environment every generation. *Appl. Environ. Microbiol.* 2007, 73, 4308–4316. [CrossRef] [PubMed]

96. Chandler, J.A.; Lang, J.M.; Bhatnagar, S.; Eisen, J.; Kopp, A. Bacterial Communities of Diverse *Drosophila* Species: Ecological Context of a Host–Microbe Model System. *PLoS Genet.* 2011, 7, e1002272. [CrossRef] [PubMed]

97. Lauzon, C.; McCombs, S.; Potter, S.; Peabody, N. Establishment and vertical passage of enterobacter (Pantoea) agglomerans and *Klebsiella pneumoniae* through all life stages of the mediterranean fruit fly (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 2009, 102, 85–95. [CrossRef]

98. Coon, K.L.; Vogel, K.J.; Brown, M.R.; Strand, M.R. Mosquitoes rely on their gut microbiota for development. *Mol. Ecol.* 2014, 23, 2727–2739. [CrossRef]

99. Kellner, R.L. The role of microorganisms for eggs and progeny. In *Chemoeckology of Insect Eggs and Egg Deposition*; Blackwell: Berlin, Germany, 2002; pp. 149–167.

100. Wong, C.N.A.; Ng, P.; Douglas, A.E. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* 2011, 13, 1889–1900. [CrossRef]

101. Wang, X.; Gao, Q.; Wang, W.; Wang, X.; Lei, C.; Zhu, F. The gut bacteria across life stages in the synanthropic fly *Chrysomya megacephala*. *BMC Microbiol.* 2018, 18, 131. [CrossRef]

102. Attardo, G.M.; Lobs, C.; Heddi, A.; Alam, U.H.; Yildirim, S.; Aksoy, S. Analysis of milk gland structure and function in *Glossina morsitans*: Milk protein production, symbiont populations and fecundity. *J. Insect Physiol.* 2008, 54, 1236–1242. [CrossRef]

103. Aksoy, S.; Chen, X.-A.; Hypsa, V. Phylogeny and potential transmission routes of midgut-associated endosymbionts of tsetse (Diptera: Glossinidae). *Insect Mol. Biol.* 1997, 6, 183–190. [CrossRef]

104. Blaustein, L.; Chase, J.M. Interactions between mosquito larvae and species that share the same trophic level. *Annu. Rev. Entomol.* 2007, 52, 489–507. [CrossRef]

105. Howland, L. The nutrition of mosquito larvae, with special reference to their algal food. *Bull. Entomol. Res.* 1930, 21, 431–439. [CrossRef]

106. Capinera, J.L. Encyclopedia of Entomology; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2008.

107. Chapman, R.F.; Chapman, R.F. *The Insects: Structure and Function*; Cambridge University Press: New York, NY, USA, 1998.

108. Bansal, R.; Hulbert, S.; Schemerhorn, B.; Reese, J.C.; Whitworth, R.J.; Stuart, J.J.; Chen, M.-S. Hessian fly-associated bacteria: Transmission, essentiality, and composition. *PLoS ONE* 2011, 6, e23170. [CrossRef] [PubMed]

109. Carrasco, P.; Perez-Cobas, A.E.; van de Pol, C.; Baixeras, J.; Moya, A.; Latorre, A. Succession of the gut microbiota in the cockroach *Blatella germanica*. *Int. Microbiol.* 2014, 17, 99–109. [PubMed]

110. Bakula, M. The persistence of a microbial flora during postembryogenesis of *Drosophila melanogaster*. *J. Invertebr. Pathol.* 1969, 14, 365–374. [CrossRef]

111. Moll, R.M.; Romoser, W.S.; Modrakowski, M.C.; Moncayo, A.C.; Lerdhthusnee, K. Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. *J. Med. Entomol.* 2001, 38, 29–32. [CrossRef]

112. Andres, A.J.; Thummel, C.S. Hormones, puffs and flies: The molecular control of metamorphosis by ecdysone. *Trends Genet.* 1992, 8, 132–138. [CrossRef]

113. Bakker, K. Feeding period, growth, and pupation in larvae of *Drosophila melanogaster*. *Entomol. Exp. Appl.* 1959, 2, 171–186. [CrossRef]

114. Ždárek, J.; Denlinger, D.L. Changes in temperature, not photoperiod, control the pattern of adult eclosion in the tsetse, *Glossina morsitans*. *Physiol. Entomol.* 1995, 20, 362–366. [CrossRef]
115. Srivastava, U.; Gilbert, L.I. The influence of juvenile hormone on the metamorphosis of *Sarcophaga bullata*. *J. Insect Physiol.* **1969**, *15*, 177–189. [CrossRef]

116. Pemberton, C.E.; Willard, H.F. A contribution to the biology of fruit-fly parasites in Hawaii. *J. Agric. Res.* **1918**, *15*, 419–465.

117. Wilson, H. *Opis fletcheri* as a parasite of the melon fly in Hawaii. *J. Agric. Res.* **1920**, *20*, 423–438.

118. Lindegaard, C. Chironomidae (Diptera) of European cold springs and factors influencing their distribution. *J. Kans. Entomol. Soc.* **1995**, *68*, 108–131.

119. Steffan, A.W. Chironomid (Diptera) biocoenoses in Scandinavian glacier brooks. *Can. Entomol.* **2012**, *103*, 477–486. [CrossRef]

120. Lepage, M.; Bourgeois, G.; Brodeur, J.; Boivin, G. Effect of soil temperature and moisture on survival of eggs and first-instar larvae of *Delia radicum*. *Environ. Entomol.* **2012**, *41*, 159–165. [CrossRef] [PubMed]

121. Merritt, R.; Dadd, R.; Walker, E. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* **1992**, *37*, 349–374. [CrossRef]

122. Bjedov, I.; Toivonen, J.M.; Kerr, F.; Slack, C.; Jacobson, J.; Foley, A.; Partridge, L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* **2010**, *11*, 35–46. [CrossRef]

123. Zhao, X.; Zhang, X.; Chen, Z.; Wang, Z.; Lu, Y.; Cheng, D. The divergence in bacterial components associated with bactrocera dorsalis across developmental stages. *Front. Microbiol.* **2018**, *9*, 9. [CrossRef]

124. Tarone, A.M.; Picard, C.J.; Spiegelman, C.; Foran, D.R. Population and temperature effects on *Lucilia sericata* (Diptera: Calliphoridae) body size and minimum development time. *J. Med. Entomol.* **2011**, *48*, 1062–1068. [CrossRef]

125. Moghadam, N.N.; Thorshauge, P.M.; Kristensen, T.N.; de Jonge, N.; Bjedov, I.; Toivonen, J.M.; Kerr, F.; Slack, C.; Jacobson, J.; Foley, A.; Partridge, L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* **2010**, *11*, 35–46. [CrossRef]

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

127. Classen, A.T.; Sundqvist, M.K.; Henning, J.A.; Newman, G.S.; Moore, J.A.M.; Cregger, M.A.; Moorhead, L.C.; Patterson, C.M. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere* **2015**, *6*, 130. [CrossRef]

128. Puggioli, A.; Balestrino, F.; Damiens, D.; De, J.; Jacobson, J.; Foley, A.; Partridge, L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* **2010**, *11*, 35–46. [CrossRef]

129. Cox, C.R.; Gilmore, M.S. Native microbial colonization of *Drosophila melanogaster* and its use as a model of *Enterococcus faecalis* pathogenesis. *Infect. Immun.* **2007**, *75*, 1565–1576. [CrossRef] [PubMed]

130. Corby-Harris, V.; Pontaroli, A.C.; Shimkets, L.J.; Bennetzen, J.L.; Habel, K.E.; Promislow, D.E.L. Geographical distribution and diversity of bacteria associated with natural populations of *Drosophila melanogaster*. *Appl. Environ. Microbiol.* **2007**, *73*, 3470–3479. [CrossRef]

131. Grübel, P.; Hoffman, J.S.; Chong, F.K.; Burstein, N.A.; Mepani, C.; Cave, D.R. Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*. *J. Clin. Microbiol.* **1997**, *35*, 1300–1303. [CrossRef] [PubMed]

132. Kuhn, K.G.; Campbell-Lendrum, D.H.; Davies, C.R. A continental risk map for malaria mosquito (Diptera: Culicidae) vectors in Europe. *J. Med. Entomol.* **2002**, *39*, 621–630. [CrossRef] [PubMed]

133. Huang, Y.-M. *Aedes (stegeomyia) bromeliae* (Diptera: Culicidae), the yellow fever virus vector in east Africa. *J. Med. Entomol.* **1986**, *23*, 196–200. [CrossRef]

134. Kelly, P.H.; Bahar, S.M.; Serafin, T.D.; Ajami, N.J.; Petrov, J.F.; Meneses, C.; Kirby, J.R.; Valenzuela, J.G.; Kish, S.; Wilson, M.E. The gut microbiome of the vector *Lutzomyia longipalpis* is essential for survival of *Leishmania infantum*. *mBio* **2017**, *8*, e01121-16. [CrossRef]

135. Dennison, N.J.; Jupatanakul, N.; Dimopoulos, G. The mosquito microbiota influences vector competence for human pathogens. *Curr. Opin. Insect Sci.* **2014**, *3*, 6–13. [CrossRef]

136. Pumpuni, C.; Beier, M.; Nataro, J.; Guers, L.D.; Davis, J. *Plasmodium falciparum*: Inhibition of sporogonic development in *Anopheles stephensi* by gram-negative bacteria. *Exp. Parasitol.* **1993**, *77*, 195–199. [CrossRef]

137. Gonzalez-Ceron, L.; Santillan, F.; Rodriguez, M.H.; Mendez, D.; Hernandez-Avila, J.E. Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. *J. Med. Entomol.* **2003**, *40*, 371–374. [CrossRef]
138. Dong, Y.; Manfredini, F.; Dimopoulos, G. Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathog.* 2009, 5, e1000423. [CrossRef] [PubMed]

139. Cirimotich, C.M.; Dong, Y.; Clayton, A.M.; Sandiford, S.L.; Souza-Neto, J.A.; Mulenga, M.; Dimopoulos, G. Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science* 2011, 332, 855–858. [CrossRef] [PubMed]

140. Riley, M.A.; Lizotte-Waniewski, M. Population genomics and the bacterial species concept. *Methods Mol. Biol.* 2009, 532, 367–377. [PubMed]

141. O’Banion, B.; O’Neal, L.; Alexandre, G.; Lebeis, S. Bridging the gap between single-strain and community-level plant-microbe chemical interactions. *Mol. Plant. Microbe Interact.* 2019, 33, 124–134. [CrossRef]

142. Gibbs, J.P.; Marquez, C.; Sterling, E.J. The role of endangered species reintroduction in ecosystem restoration: Tortoise–cactus interactions on Española Island, Galápagos. *Restor. Ecol.* 2008, 16, 88–93. [CrossRef]

143. Gagic, V.; Bartomeus, I.; Jonsson, T.; Taylor, A.; Winqvist, C.; Fischer, C.; Slade, E.M.; Steffan-Dewenter, I.; Emmerson, M.; Potts, S.G.; et al. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proc. R. Soc. B Biol. Sci.* 2015, 282, 20142620. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).