Insect Lipid Metabolism in the Presence of Symbiotic and Pathogenic Viruses and Bacteria

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Abstract

Insects, like most animals, have intimate interactions with microorganisms that can influence the insect host's lipid metabolism. In this chapter, we describe what is known so far about the role prokaryotic microorganisms play in insect lipid metabolism. We start exploring microbe-insect lipid interactions focusing on endosymbionts, and more specifically the gut microbiota that has been predominantly studied in *Drosophila melanogaster*. We then move to an overview of the work done on the common and well-studied endosymbiont *Wolbachia pipientis*, also in interaction with other microbes. Taking a slightly different angle, we then look at the effect of human pathogens, including dengue and other viruses, on the lipids of mosquito vectors. We extend the work on human pathogens and include interactions with the endosymbiont *Wolbachia* that was identified as a natural tool to reduce the spread of mosquito-borne diseases. Research on lipid metabolism of plant disease vectors is up and coming and we end this chapter by highlighting current knowledge in the field.

Keywords: Phospholipids; Cholesterol; Fatty acids; Triacylglycerols; Fat content; *Spiroplasma; Serratia*; Diapause; *Aedes aegypti; Culex pipiens;* Phytophagous insects; *Wolbachia pipientis*

1 Introduction

All insects harbor a diverse and extensive microbial community, referred to as the microbiota (i.e., the assemblage of microorganisms -bacteria, fungi, viruses, archaea, and protists- associated with a defined host or environment; Berg et al., 2020). The diversification and evolution of insects are closely tied to their symbiotic interactions with microorganisms that may be mutualistic, commensal, or parasitic (Cornwallis et al., 2023; Janson et al., 2008). Bacterial symbionts represent the largest part of the microbiota that can be located either on the surface of the host's body, i.e., ectosymbionts, or reside inside the host's body, i.e., endosymbionts. In insects, endosymbionts are primarily present in the gut or in specialized cells called bacteriocytes (Baumann et al., 2006). Insects can also function as vectors for disease-causing microbes, such as dengue virus (DENV) transmitted by mosquitoes causing dengue fever in humans or plant viruses transmitted by phloem-sucking insects that can have a large effect on crops (e.g., beet, turnip etc…). Both symbiotic and pathogenic microorganisms can have substantial effects on many different aspects of the host's biology.

Symbiotic bacteria are known for a plethora of effects on insect hosts. The insect microbiota, for example, can affect *i)* the host's immune system and protection against various predators, parasites, disease vectors or pathogens; *ii)* communication and behavior among individuals of the same or from different species; *iii)* host mating preferences and reproductive systems; *iv)* host life histories and fitness-related traits (e.g., development, lifespan, fecundity); and *v)* host resilience to environmental disturbances (e.g., pesticides) (Douglas, 2015; Engel and Moran, 2013; Engl and Kaltenpoth, 2018; Zhang et al., 2022). Notwithstanding these important functions, the provisioning of essential nutrients for the insect host seems to be a primary task of gut microorganisms. Many microorganisms provide nutrients that the insect cannot synthesize, such as amino acids, B vitamins or sterols (Douglas, 2015). The bacterial endosymbiont *Buchnera aphidicola*, for example, is of primary importance for aphid development and adult life by providing essential amino acids, and in return aphids provide a stable and nutrient-rich environment (Douglas et al., 2001). Another well-known insect endosymbiont, *Wolbachia pipientis*, has also been shown to supply B vitamins to its host, the bedbug *Cimex lectularius* (Hosokawa et al., 2010; Newton and Rice, 2020). Nutrient provisioning by bacteria can compensate for nutrient-poor diets, aids the digestion of recalcitrant food components (e.g., degradation of cellulose in plant cell walls), and supply essential amino acids, metabolic compounds, or nutrients (Engel et al., 2012; Hu et al., 2018; Jing et al., 2020; Russell et al., 2014; Sannino et al., 2018; Tokuda et al., 2018).

Regarding nutritional interactions, symbiotic bacteria were already found to have a major impact on lipid metabolism in humans (Xu et al., 2022). For example, changes in gut bacterial communities are related to metabolic diseases, such as obesity, cardiovascular disease, and type 2 diabetes (Depommier et al., 2019; Liu et al., 2021; Wang et al., 2022). Relatively little is known, however, about the role played by symbiotic microorganisms in insect lipid metabolism. Considering how microorganisms affect key metabolic interactions is important, because more than 10% of insect species rely on obligate bacterial symbionts for survival or reproduction, and many more microorganisms are facultatively associated with insects (Hilgenboecker et al., 2008; Sazama et al., 2017; Weinert et al., 2015; Wernegreen, 2002). In contrast, recent work on human pathogens, mainly DENV, has revealed major lipid

metabolic adjustments in the insect vector incited by the virus that are of importance for viral propagation (Chotiwan et al., 2018; Perera et al., 2012; Tongluan et al., 2017).

Lipids have also been implicated in immune responses of insects, which has already been reviewed extensively (Wrońska et al., 2023; Barletta et al., 2016), and falls beyond the scope of this chapter. We set out to unite research aimed at understanding the role of prokaryotic symbiotic or pathogenic microorganisms on insect lipid metabolism. We focus on prokaryotes, i.e., bacteria and viruses, to be able to set forth and identify commonalities and differences in the ways insect host/vector lipid metabolism is affected.

2 The impact of symbiotic microorganisms on host insect lipid metabolism

Influence of the gut microbiota

There is growing evidence that the gut microbiota plays a key role in the regulation of insect fat storage. Most studies to date have focused on the impact of gut microbiota and microbe interactions on fat metabolism of the vinegar fly *Drosophila melanogaster* (Figure 1), which is an emerging model system in the field (Douglas, 2019; Erkosar et al., 2013). Generally, *D. melanogaster* deprived of the entire microbiota (i.e., axenic/germ-free individuals) had a higher triacylglycerol content than individuals with microbiota (Huang & Douglas, 2015; Newell & Douglas, 2014; Wong et al., 2014; but see Ridley et al., 2012 and Henry et al., 2020 who found no difference in fat content between axenic and control *D. melanogaster flies*). The lack of bacteria that usually utilize host gut nutrients could explain the higher triacylglycerol content in axenic *D. melanogaster* flies. Overall, the multitude of studies comparing axenic and microbiota-containing *D. melanogaster* show a range of different results on triacylglycerol content, which can be explained by variation in host-related factors, such as host sex and feeding rate, as well as composition of the diet (e.g., sugar:yeast ratio, nutrient-poor or rich diet) and how these factors interact with the microbiota and each other (Huang & Douglas, 2015; McMullen et al., 2020; Wong et al., 2014).

Interspecific bacterial interactions can lead to substantial differences in triacylglycerol content of *D. melanogaster,* where both laboratory and wild populations have low-diversity gut microbiota. The *D. melanogaster* gut microbiota is commonly dominated by bacteria in the family *Acetobacteraceae* (mainly represented by the genus *Acetobacter*) and the order *Lactobacillales* (mainly represented by the genus *Lactobacillus*) (Adair et al., 2018; Chandler et al., 2011, 2012; Wong et al., 2011). Both mutualistic and antagonistic associations between *Acetobacter* and *Lactobacillus* have been found in *D. melanogaster*, depending on the bacterial species involved (Consuegra et al., 2020; McMullen et al., 2020; Sommer and Newell, 2019). The impact of bacterial taxa (i.e., a total of five *Acetobacter* and *Lactobacillus* species) on *D. melanogaster* triacylglycerol content was assessed by comparing single-, dual- or multi-species infections (compared to both axenic and conventional flies) (Newell and Douglas, 2014). Combinations of bacterial taxa and corresponding triacylglycerol content showed that *1)* dual-microbe infected *D. melanogaster* individuals generally had a lower triacylglycerol content than axenic and single-microbe infected individuals; *2)* bacterial effects on *D. melanogaster* triacylglycerol levels are microbe-specific and dependent on interactions, e.g., mono-infection by *Lactobacillus brevis* and *L. plantarum* did not lead to different triacylglycerol levels, only

in interaction with *Acetobacter* were levels significantly lower; and *3)* bacterial interactions are essential to restore the natural insect phenotype (i.e., similar to untreated flies). Newell & Douglas (2014) also highlighted that *Acetobacter tropicalis* abundance is promoted by the colonization of *L. brevis* in *D. melanogaster*. High *A. tropicalis* cell density, in turn, decreased fly triacylglycerol content in a dose-dependent manner (Newell and Douglas, 2014). *Drosophila melanogaster* triacylglycerol content is thus mediated by the composition of the gut microbiota, bacterial abundance, and bacterial interactions.

The capacity of some bacteria, such as *Acetobacter* or *Lactobacillus*, to reduce *D. melanogaster* fat content (confirmed by Bozkurt et al., 2023) has been attributed to several, not mutually exclusive, processes. First, the bacteria can reduce host triacylglycerol levels via the consumption of dietary glucose, e.g., *Lactobacillus* produces lactate via the consumption of glucose, the latter being a substrate for fatty acid and subsequent fat synthesis in insects (Huang and Douglas, 2015; Sommer and Newell, 2019). Second, microorganisms can modulate host nutritional signaling pathways. For example, the increased production of acetic acid by *Acetobacter pomorum,* in response to the production of lactate by *Lactobacillus*, was shown to increase *D. melanogaster*'s insulin levels resulting in reduced adult fat content (Shin et al., 2011). Third, *Lactobacillus* can modulate the TOR (Target of Rapamycin) signaling pathway that also affects insulin signaling (Storelli et al., 2011). Fourth, metabolic models predicted a high release rate of succinate from *Drosophila* gut bacteria (Ankrah et al., 2021), impacting the citric acid cycle by reducing citrate levels available for fatty acid synthesis (Zhang et al., 2022).

Bacteria can also interact with other microorganisms, such as fungi, affecting triacylglycerol levels differently depending on the interactions considered (Bozkurt et al., 2023; McMullen et al., 2020). For example, Bozkurt et al., 2023 showed a positive correlation between the abundances of *A. persici*, *A. pomorum* and Basidiomycota in *D. melanogaster*, as well as a negative correlation between these microbial taxa and triacylglycerol levels. In contrast, the fungus *Hanseniaspora uvarum* (order *Saccharomycetales*), also part of the *D. melanogaster* gut microbiota (Chandler et al., 2012), showed antagonistic interactions with *L. brevis* and *A. fabarum*. When *H. uvarum* is present, there is a negative effect on the abundance of *L. brevis*. The abundance of both *H. uvarum* and *A. fabarum* decreases when present together (McMullen et al., 2020). For the *H. uvarum*-*A. fabarum* interaction, a negative correlation was also observed between *D. melanogaster* triacylglycerol content and acetic acid that varied significantly with the presence of both *A. fabarum* and *H. uvarum*, consistent with previous studies (Newell and Douglas, 2014; Sommer and Newell, 2019). *Drosophila melanogaster* associated with both *A. fabarum* and *H. uvarum* displayed high acetic acid levels, but interestingly, triacylglycerol levels were also significantly elevated in flies only infected by the yeast *H. uvarum* (compared with axenic flies). *Hanseniaspora uvarum* was hypothesized to be another producer of acetic acid, as was found also for other fungi (Bueno et al., 2020; Jolly et al., 2014). Interactions between *A. fabarum* and *H. uvarum* could modulate the concentration of acetic acid, reducing triacylglycerol synthesis (McMullen et al., 2020). Taken together, these results demonstrate the key role of the gut microbiota and microbial fermentation products, such as acetic acid, on the nutritional status of *Drosophila*, particularly with respect to fat accumulation.

In species other than *Drosophila,* only little progress has been made so far, and contrasting results have been reported regarding insect fat metabolism and fat content. In the

aphid *Acyrthosiphon pisum*, axenic individuals showed increased triacylglycerol levels, in line with findings in *D. melanogaster* (Rahbé et al., 1994). In contrast, lower fat content was reported for adults of three fruit fly species, *Ceratitis capitata*, *Bactrocera tryoni*, and *Anastrepha fraterculus,* following antibiotic treatment (Ben-Yosef et al., 2008; Goane et al., 2022; Nguyen et al., 2021). Similar to findings in *D. melanogaster*, the fat content of the other fruit flies was affected by interactions between microbiota, diet, and sex (Ben-Yosef et al., 2008; Nguyen et al., 2021).

In the fruit fly *B. dorsalis*, a genomic study comparing gene expression of antibiotic-treated and control individuals revealed upregulation of *i)* fatty acid synthesis genes (e.g., fatty acid synthase (*fas*), acetyl-CoA carboxylase), *ii)* genes encoding triacylglycerol catabolism (e.g., lipases, fatty acid hydroxylase), and *iii)* downregulation of genes involved in fatty acid beta-oxidation (e.g., enoyl-CoA hydratase), suggesting a general increase of free fatty acids in the axenic insect (Xie et al., 2023). Downregulation of genes involved in lipid storage (i.e., vitellogenin) and transport (i.e., lipophorins), as well as a decrease in lipid content of the host's fat body have also been reported in *Aedes aegypti* axenic mosquitoes (Romoli et al., 2021). It has remained unclear how and why the expression of fatty acid and triacylglycerol metabolic genes changes depending on gut microbiota presence. One proposed hypothesis is that lipolysis facilitated by endosymbiotic bacteria increases the availability of different lipid types for the insect host. When no bacteria are present, the host insect is forced to start synthesizing different lipid types, while reducing fat storage (due to lower quantities of available precursors (Goane et al., 2022)).

Gnotobiotic insects (i.e., insects associated with specific bacterial strain(s)) have also been used in systems other than *Drosophila* to decipher the role of bacterial strains on host fat metabolism and fat content. In the red palm weevil *Rhynchophorus ferrugineus*, for example, a significant reduction in triacylglycerol content was reported in germ-free larvae compared to untreated larvae (Habineza et al., 2019). Introduction of the bacterium *Enterobacter cloacae* into germ-free *R. ferrugineus* larvae partially restored triacylglycerol levels, but no effect was found for *Lactococcus lactis* (Habineza et al., 2019). Another study reported that gnotobiotic *Ae. aegypti* mosquitoes associated with *Flavobacterium* or *Paenibacillus* showed higher triacylglycerol levels compared to control mosquitoes, while Enterobacteriaceae and *Lysobacter* had no impact (Giraud et al., 2022). *Enterobacter cloacae* is known to synthetize various carbohydrate-modifying and glycolytic enzymes (e.g., cellulases, trehalases, glucosidases; Habineza et al., 2019), while Flavobacteria are chitinase producers (McBride et al., 2009), suggesting that bacteria other than *Acetobacter* and *Lactobacillus* can play a role in nutrient acquisition of other insect host species.

201

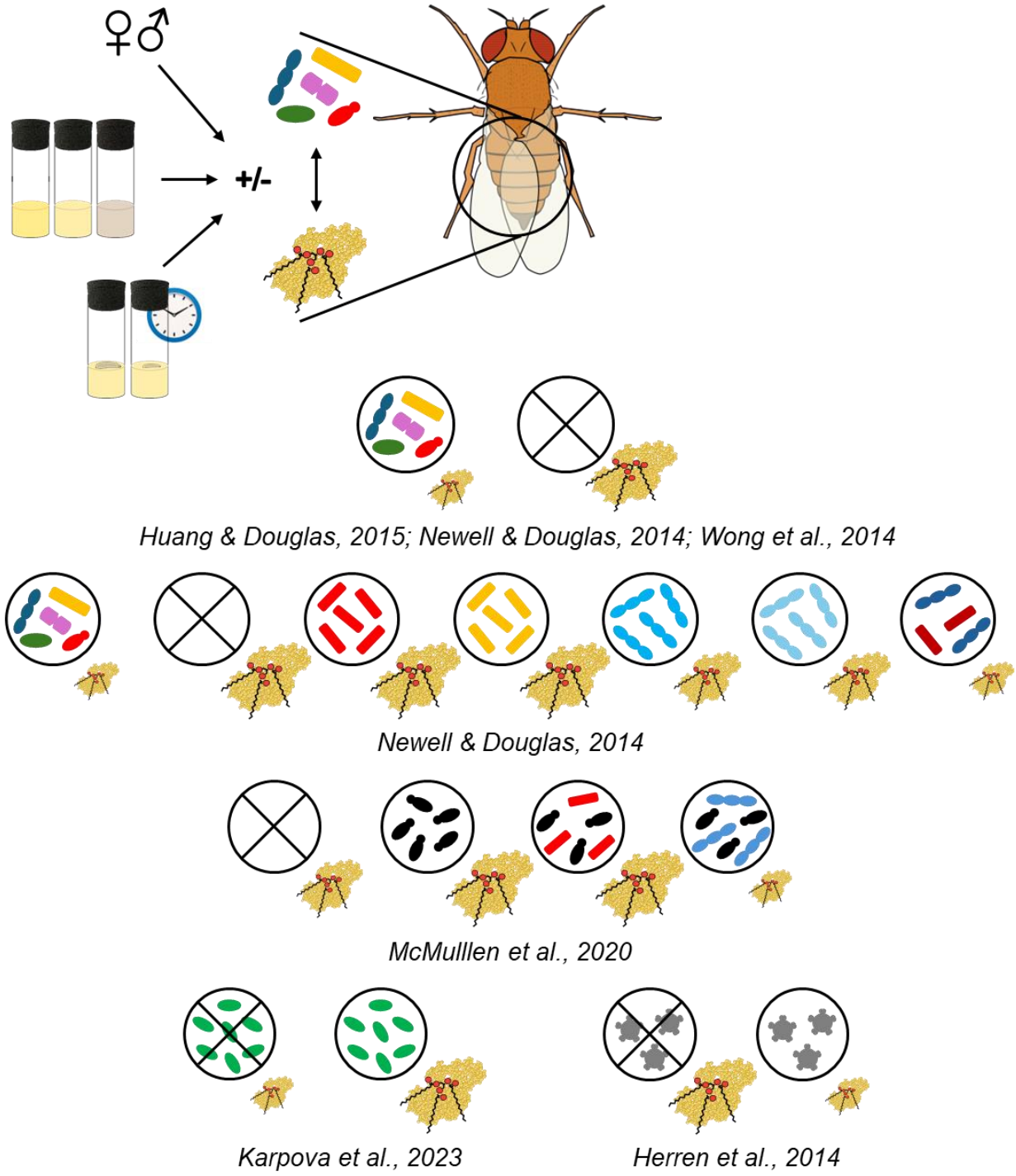

Figure 1. Summary of microbe effects on *Drosophila melanogaster* triacylglycerol levels (i.e., storage lipids). Triacylglycerol levels were compared between axenic (germ-free), mono-infected (bacteria-only or fungi-only), dual-infected (bacteria-bacteria and fungi-bacteria interactions), and conventional flies.

Box 1. The influence of microbes on insect fat accumulation during diapause

Many insects have adapted to seasonal changes and low food availability by entering diapause. Diapause is a genetically and hormonally determined program that depends on various environmental signals (e.g., photoperiod, temperature) allowing insects to anticipate pending unfavorable conditions (Denlinger, 2002; Denlinger et al., 2012). Diapause may occur during any stage of the insect's life cycle (e.g., embryonic, larval, pupal, or adult), depending on the insect species, and is characterized by reduced metabolic and behavioral activity (Hahn and Denlinger, 2011). By delaying development from several weeks up to years, insects can synchronize their life cycle to match with periods suitable for growth, development, and reproduction. In addition to metabolic depression during diapause, insects typically increase energy stores by accumulating fat prior to diapause (Enriquez and Visser, 2023). Fat reserves constitute an important source of energy to meet metabolic demands during and post-diapause (Hahn and Denlinger, 2007).

Only few studies have explored the role of bacterial symbionts on fat accumulation during diapause, despite the importance of facultative and obligatory diapause for many insects (Hahn and Denlinger, 2011). An exception is the work by Didion et al., (2021) that confirmed the critical importance of microbiota for diapause preparation in the mosquito *Culex pipiens*. Diapausing mosquitoes with a low bacterial load had 50% fewer fat reserves, associated with a lower dry mass and lower survival rate. In the parasitic wasp *Nasonia vitripennis*, triacylglycerol levels of diapausing larvae (ranging from 1 to 6 months of diapause) were significantly correlated with changes in microbiota composition (Dittmer and Brucker, 2021). This correlation was rather weak, however, when compared to the effects of temperature and quantities of other nutrient types, such as glycerol or glucose (Dittmer and Brucker, 2021).

Under laboratory conditions, Liu et al., (2016) investigated the link between gut bacterial symbionts and the metabolic shift from protein synthesis to triacylglycerol accumulation in a vegetable pest beetle, *Colaphellus bowringi,* that occurs when females enter diapause. Gut microbiota composition was slightly different between diapausing and non-diapausing individuals: positive correlations were found between diapause preparation and abundances of *Proteobacteria* (e.g., *Serratia* sp., *Sphingomonas* sp.) and *Firmicutes* (e.g., *Lactococcus* sp.), while a negative correlation was found with the abundance of *Bacteroidetes* (e.g., *Flavobacterium* sp.; but see Didion et al., (2021) that found no difference between the microbiota of diapausing and non-diapausing *C. pipiens* mosquitoes). Based on similar findings on the regulation of obesity by microbiota in mammals (Ley et al., 2005), higher abundances of *Proteobacteria* and *Firmicutes* may affect insect fat accumulation. In a more recent study, the endosymbiont *Wolbachia* appeared to reduce the lipid content (estimated by cholesterol amounts) of the parasitoid wasp *Trichogramma brassicae*, leading to a lower percentage of diapausing individuals (Rahimi-Kaldeh et al., 2019).

The above studies highlight an important functional role of the microbiota in insect diapause, although evidence of host-microbiota interactions during insect diapause remains scarce. As diapause is controlled by the insect's endocrine system, the microbiota is expected to interact closely with the host's hormonal signaling pathways. More work is now needed to increase our understanding of how microbe-insect interactions affect diapause, and more generally how microbes affect host fat metabolism under low-temperature stress (Lv et al., 2023; Raza et al., 2020).

The particular case of the endosymbiont *Wolbachia*

Wolbachia pipientis is one of the most widespread heritable bacterial endosymbionts harbored by insects, filarial nematodes, crustaceans, and mites (Serbus et al., 2008), infecting at least 65% of all known insect species (Hilgenboecker et al., 2008; Zug and Hammerstein, 2012). *Wolbachia* is present in host germ line and somatic tissues, such as the fat body, salivary glands or hemolymph (Dobson et al., 1999; Pietri et al., 2016), and can affect a wide variety of the insect host's biological functions. In mutualistic interactions, *Wolbachia* can confer fitness advantages, such as protection against viruses, resistance to heat stress or increasing learning ability, immunity, and life history traits (Arai et al., 2019; Cao et al., 2019; Farahani et al., 2017; Faria et al., 2018; Gruntenko et al., 2017; Maistrenko et al., 2016; Mazzucco et al., 2020). *Wolbachia* can also be parasitic and is perhaps best known for manipulating host reproduction in favor of its vertical transmission and spread within insect populations. *Wolbachia* can reduce population sizes, distort population sex ratios through male-killing or feminization of genetic males, induce parthenogenesis or cause cytoplasmic incompatibility (i.e., mating between individuals differing in *Wolbachia* infection status result in embryonic mortality; Charlat et al., 2001) (Dittmer and Bouchon, 2018; Hurst et al., 1999; Poinsot et al., 2003; Stouthamer et al., 1999).

Host insect nutrient metabolism appears to be strongly influenced by *Wolbachia*. In *D. melanogaster*, *Wolbachia* affects fatty acid profiles, particularly the odd-chain fatty acid fraction (Molloy et al., 2016; Scheitz et al., 2013). Insects cannot synthesize odd-chain fatty acids. In *D. melanogaster* females, odd-chain fatty acids are likely supplied by *Wolbachia*, where odd-chain fatty acids levels are positivively correlated to *Wolbachia* abundance ((Molloy et al., 2016; Scheitz et al., 2013). Odd-chain fatty acids are synthesized and elongated by FAS from the precursor propionic acid (C3), leading to the odd-chain numbers. Odd-chain fatty acids have been found in the insect cuticle and body extracts in a wide variety of insect orders: Diptera (Kaczmarek et al., 2020; Sato et al., 2020), Hymenoptera (Pickett et al., 2000; Stanley-Samuelson et al., 1990), Hemiptera (Bashan et al., 2002; Cakmak et al., 2007a), Coleoptera (Howard and Stanley-Samuelson, 1990; Nikolova et al., 2000), Neuroptera (Cakmak et al., 2007b) and Lepidoptera (Akinnawo and Ketiku, 2000; Gołębiowski et al., 2010). The widespread occurrence among insects demonstrates that odd-chain fatty acids are fairly common components of insect lipids, although the proportion of odd-chain fatty acids is low compared to even-chain fatty acids. Odd-chain fatty acids can have a role in membrane stability and structure, as they have been found in the phospholipid fraction (Howard and Stanley-Samuelson, 1990; Sato et al., 2020). Odd-chain fatty acids have also been found in the triacylglycerol fraction, and used for fat storage (Cakmak et al., 2007a, 2007b). Odd-chain fatty acids can be acquired either by ingestion of symbiotic microorganisms that synthesize them (e.g., bacteria, yeast; Park et al., 2020; Řezanka & Sigler, 2009) or synthesized *de novo.* In *D. melanogaster*, Sato et al., (2020) observed no significant difference in odd-chain fatty acid content between conventional and germ-free flies, suggesting that the microbiota was not involved. Instead, the incorporation of isotopic labels into the odd-chain fatty acids of *D. melanogaster* suggested *de novo* synthesis (Sato et al., 2020).

An increase in triacylglycerols was observed in *D. melanogaster* flies infected either with the *w*MelPlus, *w*Mel, or *w*MelCS45 *Wolbachia* strain compared to uninfected flies

(Karpova et al., 2023). Contrasting results on the effect of *Wolbachia* on host fat metabolism have, however, been reported within and between mosquito species. *Wolbachia* infection led to a decrease in triacylglycerol levels in *Ae. aegypti* (*w*Mel strain) and *Ae. fluviatilis* (*w*Aflu) (Conceição et al., 2021; Koh et al., 2020). Infection of *Ae. aegypti* with *w*Aflu further led to decreased lipid droplet size in the cytoplasm of mosquito cells (Conceição et al., 2021). In *Ae. albopictus*, *w*Mel *Wolbachia* infection decreased diglyceride levels by 32% compared to uninfected mosquitoes, while a 17% increase in triacylglycerols was observed in *w*MelPop-infected mosquitoes (Molloy et al., 2016). Overall, *Wolbachia* effects on various lipid types depend on host and *Wolbachia*-related factors (e.g., host species or genotype, *Wolbachia* strain; (Molloy et al., 2016), as was already shown for other metabolic pathways (e.g., dopamine metabolism; Gruntenko et al., 2017).

In *Drosophila* and several mosquito species, changes in lipid types other than fatty acids and triacylglycerols were observed in the presence of *Wolbachia* (Conceição et al., 2021; Koh et al., 2020; Molloy et al., 2016). In *Ae. albopictus*, *w*Mel and *w*MelPop *Wolbachia* infection resulted in *1)* a decrease in various sphingolipids (mostly ceramides), as well as phosphatidylcholines, phosphatidylethanolamines, and diglycerides, and *2)* an increase in phosphatidylglycerols and phosphatidylinositols in the host (Molloy et al., 2016). *Wolbachia* infection was also shown to differently affect *Ae. albopictus* lipids depending on the *Wolbachia* strain (i.e., either *w*Mel or *w*MelPop). Ceramide levels, for example, decreased 62% in *Ae. albopictus* infected with the *w*Mel *Wolbachia* strain compared to uninfected mosquitoes, while a decrease of only 20% was observed in mosquitoes infected with the *w*MelPop strain (Molloy et al., 2016). A mean decrease in sphingomyelins of 35% was reported in *w*Mel-infected *Ae. albopictus*, while sphingomyelins increased by 28% in *w*MelPop-infected *Ae. albopictus*. *Ae. aegypti* infected with the same *w*Mel *Wolbachia* strain also revealed a reduction of phosphatidylethanolamines and more complex forms of ceramides (e.g., glucosylceramides) (Koh et al., 2020). As sphingolipids and phospholipids play a major structural role in cell membranes (e.g., complex assembly in lipid rafts), depletion of these lipids was hypothesized to affect host membrane fluidity, curvature, and structure. Changes in the host membrane can facilitate *Wolbachia* colonization within the host (Molloy et al., 2016).

Variation in lipid levels may be related to the dependency of *Wolbachia* on the host insect for lipids. A genome sequencing study indeed revealed that a *Wolbachia* strain (*w*Mel) associated with *D. melanogaster* lost many key metabolic pathways, including pathways for fatty acid and cholesterol metabolism (Wu et al., 2004). Cholesterol is the dominant sterol in most insects, and a vital component for cell membrane stability, hormone regulation, and insect development (Behmer & Nes, 2003; Jing & Behmer, 2020). *Wolbachia* thus depends completely on the host to supply fatty acids and cholesterol for its survival and proliferation (Caragata et al., 2017; Zhang et al., 2021). Like some other intracellular bacteria, *Wolbachia* resides in a host-derived vacuole (Cho et al., 2011) within tissues of insects (Dobson et al., 1999; Hughes et al., 2011; Pietri et al., 2016). *Wolbachia* is restricted to the host's Golgi-related vesicles near the endoplasmic reticulum, a site of active nutrient synthesis (Cho et al., 2011). Close positioning next to a lipid-enriched organelle allows *Wolbachia* to acquire nutrients, such as amino acids or lipids, by subverting, modifying (e.g., lipid composition), and redistributing the endoplasmic reticulum of the host to colonize the host cell at a high density (Fattouh et al., 2019).

Only few studies have so far examined the hypothesis that an essential requirement for lipids leads *Wolbachia* to manipulate host lipid metabolism. In adult *Ae. aegypti* infected by the *w*Mel and *w*MelPop *Wolbachia* strain, a decrease of 25.6% and 27.7% in total cholesterol levels was observed, respectively. A reduction in total cholesterol level suggests that *Wolbachia* may use host cellular lipids (Caragata et al., 2014). *Wolbachia* seems to compete for host cholesterol, a pattern already reported for other intracellular bacteria (e.g., *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Brucella abortus*; Lin & Rikihisa, 2003; Watarai et al., 2002). With *Wolbachia* being located in Golgi-related vesicles, where high membrane biogenesis and cholesterol sequestration typically occur, the bacterium has direct access to nutrients metabolized by the insect host (Cho et al., 2011; Howe and Heinzen, 2006).

Recent studies highlighted that *Wolbachia* can affect gene expression of host metabolic pathways, including fat metabolism. *Wolbachia* first seems to act on the host's insulin/insulin-like-growth factor pathway (Currin-Ross et al., 2021; Ikeya et al., 2009). Whether *Wolbachia* actively regulates the insulin signaling pathway, however, remains a matter of debate, as both positive and negative regulation have been reported (Currin-Ross et al., 2021; Ikeya et al., 2009). Moreover, genes underlying host fatty acid synthesis (e.g., *fas*) were further found to be upregulated in *Wolbachia*-infected *D. melanogaster* larval stages (*w*Mel *Wolbachia* strain; Zheng et al., 2011), as well as in adult *D. melanogaster* (*w*Mel; Dou et al., 2021) and mosquitoes (*w*Mel and *w*MelPop; Rancès et al., 2012; Wimalasiri-Yapa et al., 2023), suggesting a role for *Wolbachia* in modulating the expression of host genes involved in fat metabolism.

Endosymbionts other than *Wolbachia* can also alter host fat metabolism

Some endosymbionts appear to compete with the host insect for lipids. In *Spiroplasma poulsonii*-infected *D. melanogaster* flies, for example, a significant decrease in circulating lipids, specifically diglycerides and sterols, was reported in host hemolymph (compared to *S. poulsonii*-free flies) (Herren et al., 2014). The bacterium *S. poulsonii* subverts and utilizes diglycerides contained in host hemolymph lipoprotein particles (i.e., an important hemolymph lipid carrier; Sieber & Thummel, 2012) prior to the arrival of diglycerides at the fat body, resulting in lower triacylglycerol levels (as triacylglycerol synthesis and storage in the fat body largely depends on host hemolymph diglycerides) (Herren et al., 2014). Proliferation of *S. poulsonii* was also found to be limited by the availability of host hemolymph lipids (Herren et al., 2014). The use of host lipids by *S. poulsonii* was confirmed in a parasitic wasp, *Leptopilina boulardi,* parasitizing *D. melanogaster*. Parasitic wasps depend on a single host insect to complete development and obtain sufficient nutritional resources to fuel life (see Chapter 16). The presence of *S. poulsonii* led to direct competition with *L. boulardi* for *D. melanogaster* hemolymph lipids (Paredes et al., 2016). In the *D. melanogaster*-*S. poulsonii*-*L. boulardi* interaction, competition for lipids underlies the protective role of *S. poulsonii* for *D. melanogaster* larvae by reducing developmental success of the parasitic wasp (Paredes et al., 2016).

Other endosymbionts, such as *Serratia*, are beneficial to the insect by enhancing host fatty acid metabolism. *Serratia symbiotica*-infected aphids (*A. pisum*), for example, up-regulated the expression of genes involved in fatty acid and fat synthesis, such as *fas* and diacylglycerol-o-acyltransferase, resulting in higher triglyceride levels in the aphid fat body

(Zhou et al., 2021). In the silkworm *Bombyx mori* fed with the symbiont *Bacillus subtilis*, changes in insect gut microbiota composition were correlated with shifts in glycerophospholipid and sphingolipid composition in the host's hemolymph (Li et al., 2022). The abundance of *Enterococcus* was, for example, negatively correlated with some lysophosphatidylcholines and lysophosphatidylethanolamines and positively correlated with some phosphocholines, suggesting a role of *Enterococcus* in the glycerophospholipid metabolism of the host *B. mori*.

3 Interference of disease-vector lipid metabolism by human pathogenic microbes

Mosquito-vector lipid metabolism upon infection with human pathogenic viruses

Arthropod-borne viruses (arboviruses), such as DENV, West Nile virus, Chikungunya virus and Zika virus (ZIKV), can cause major health problems for humans with hundreds of millions of infections leading to serious diseases and deaths (Bhatt et al., 2013; Fauci and Morens, 2016; Guzman et al., 2010). Like many other viruses, the DENV cycle is initiated with the attachment of the virus to a targeted host cell through the interaction between viral surface proteins and receptor molecules on the host cell surface (Cruz-Oliveira et al., 2015). The internalization of the virus within the infected cell involves receptor-mediated endocytosis (Mosso et al., 2008). Viral genomic RNA is then released into the cytoplasm of the host cell and translated into proteins required for RNA replication and viral particle assembly (Vial et al., 2021). Virus replication is dependent on three cellular pathways: autophagy (e.g., degradation of substrates, such as proteins or lipid droplets), actin polymerization and remodeling (e.g., vesicular trafficking), and fatty acid biosynthesis (Tongluan et al., 2017). Arboviruses are enveloped by lipids derived from the insect vector, leading the scientific community to hypothesize that viral entry, replication, assembly, and release occur in the host's cellular membranes. This led to a surge of studies on the ways in which viruses can manipulate insect vector lipid metabolism.

The fundamental role insect vector lipids play in the virus life cycle has mainly been investigated using flaviviruses, including DENV (see Ratnayake et al., 2023 and Vial et al., 2021 for recent reviews; but see Liu et al., 2021 for an investigation of the mechanisms by which FAS is affected following Classical Swine Fever virus infection). Analysis of the vector's fatty acid biosynthesis pathway revealed that the FAS enzyme is essential for DENV replication (Perera et al., 2012; Tongluan et al., 2017). DENV infection induces upregulation of the *fas* gene leading to *de novo* fatty acid synthesis, and relocalization of the enzyme FAS to sites of DENV replication (Tongluan et al. 2017). Alterations in *de novo* fatty acid synthesis and the role played by various *Ae. aegypti* fatty acid synthases (AaFAS) were recently investigated by Chotiwan et al., (2022). Seven distinct orthologues of human *fas* were identified, five of which produced transcripts. In females, only aaFAS1 showed high expression in both sugar-fed and blood-fed females, where diet does not seem to play a substantial role. To better understand the role played by the other *fas* orthologues, Chotiwan et al., (2022) knocked down aaFAS1 to determine if aaFAS2, aaFAS3, and aaFAS5 transcription could compensate for significantly reduced aaFAS1 transcription. While the other aaFAS's showed a two-fold increase in transcription, aaFAS1 transcription remained higher following knockdown, suggesting that the other aaFAS's may not be able to compensate for aaFAS1

function. Knockdown of aaFAS1 further led to a reduction of DENV replication in both mosquito Aag2 cell line and midguts, suggesting that aaFAS1 is required for DENV replication.

Interestingly, in mosquitoes, a significant increase in fat content was observed during the early stages of DENV infection, especially with higher abundances of glycerolipids, including mono-, di- and triglycerides, as well as other lipid types, such as glycerophospholipids, sphingolipids or sterols (Chotiwan et al., 2018; Perera et al., 2012). Mosquito (i.e., *Aedes* sp.) fat content subsequently decreased after a few days. Increased *de novo* fatty acid synthesis, as well as increased transport of stored fat, suggests that these processes may be required for virus replication, dissemination, and survival during the initial stages of infection (Chotiwan et al., 2018; Perera et al., 2012).

Newly synthesized lipids are redistributed to sites of viral replication, mainly near the insect vector's endoplasmic reticulum membrane. Incorporation of different lipid types can then modify vector membrane structure, i.e., fluidity, permeability, and curvature, altering the functionality of the endoplasmic reticulum to the benefit of virus replication (Vial et al., 2021). DENV translation, replication and assembly indeed require vector cell endoplasmic reticulum membranes that could affect the synthesis of phospholipids, critical cell membrane components. Vial et al., (2019) used high-resolution mass spectrometry to understand how phospholipid metabolism is affected in *Ae. aegypti* cells, midguts, and whole mosquitoes at various times post-infection. Phospholipidomics first revealed that aminophospholipids, including phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylserine (PS), increased at the beginning of the DENV viral cycle, but decreased as time passed. Acylglycerol phosphate acyltransferase (AGPAT) is the rate-limiting enzyme involved in the synthesis of phospholipids (generating phosphatidic acid, a precursor for more complex phospholipids). In *Ae. aegypti,* five AGPAT isoforms were identified, with AGPAT1 being downregulated upon DENV infection (at different times depending on the level of organization, either cell, tissue or whole organism). Vial et al., (2019) then set out to test whether AGPAT1 regulation is involved in the reconfiguration of the phospholipidome. RNA interference on mosquito cells, used to temporarily knock down *agpat1* and thus mimicking DENV infection, revealed an increase in aminophospholipids. Knockdown of *agpat1* indeed also increased DENV production. The instrumental role of *agpat1* for phospholipid remodeling was confirmed by supplementation of ethanolamine in cells with knocked down *agpat1* expression. Ethanolamine is used in the synthesis of PEs and the presence of ethanolamine in the mosquito cell medium partially restored the observed increase in aminophospholipids in DENV-infected cells. In mosquitoes, knockdown of *agpat1* led to an increase in DENV infection through the consumption or redirection of aminophospholipids.

In a follow-up study, Vial et al., (2020) set out to determine how DENV reconfigures aminophospholipids in mosquitoes, but also how aminophospholipid reconfiguration affects virus proliferation. In the first set of experiments, Vial et al., (2020) knocked down several genes involved in *de novo* phospholipid synthesis and monitored changes in the phospholipidome. In addition, DENV-infected mosquito Aag2 cells were supplemented with phospholipid precursors to partly restore *de novo* synthesis. Newly synthesized phospholipids were indeed found to be antiviral, but DENV can inhibit *de novo* synthesis and initiate phospholipid remodeling to modulate and create a more proviral environment. In a stable isotope tracing experiment using different labeled precursors, Vial et al., (2020) then showed

that DENV induces remodeling early on during infection (0-24 hours), after which *de novo* phospholipid synthesis takes place. To test the negative effect of *de novo* phospholipid synthesis *in vivo,* mosquitoes were fed an infected blood meal with increased levels of phospholipid precursors. When fed lower precursor levels, DENV was able to increase phospholipid reconfiguration for its own benefit, but reconfiguration was not sufficient at higher precursor concentrations. When DENV-induced remodeling is inhibited by *de novo* phospholipid synthesis, viral replication (rather than attachment, internalization, or translation) is reduced. Phopholipids were also found to be the main lipid type affected when *Ae. albopictus* cells were infected with ZIKV (Melo et al., 2016).

Cholesterol appears to be essential for the fusion of the lipid envelope of the viral particle with the vector membranes, allowing DENV release and replication (Blanc et al., 2011; Caragata et al., 2014, 2013; Carro and Damonte, 2013). In *Ae. aegypti*, sterol carrier protein 2 (SCP-2), involved in cholesterol binding and transport, is essential for cellular cholesterol homeostasis and of importance for DENV production (Fu et al., 2015). Knockdown of SCP-2 indeed reduced DENV production in mosquito Aag2 cells. Further studies with mosquito Aag2 cells revealed that DENV reduced protein expression of low-density lipoprotein receptor-related protein 1 (LRP-1), increasing cholesterol levels and stimulating viral replication (Tree et al., 2019). In mosquitoes, however, low-density-lipoproteins contained in human blood inhibited DENV replication during an early stage of viral infection following a blood meal (also for ZIKV; Wagar et al., 2017). Vertebrate lipids thus seem to have contradictory effects on DENV. To test how DENV responds to low levels of vertebrate lipids, Marten et al., (2022) created cell lines mimicking mosquitoes "feeding" on blood (i.e., provided a normal, control, cell culture medium) or not (i.e., a lipid-depleted medium only). Lipid-depleted cells contained less cholesterol, but similar intracellular lipid levels compared to control cells, despite being smaller and showing reduced proliferation. Mosquito cells thus appear to overcome chronic lipid depletion by reducing lipolysis and increasing *de novo* lipid synthesis, including fatty acids synthesis. Similar amounts of DENV were found in both cell lines, meaning that mosquito cellular lipid metabolism compensates for a lipid-depleted environment without affecting DENV infection. Cholesterol was also found to play a critical role in alphavirus (e.g., Semliki Forest virus and Sindbis virus) entry and exit in vector cells (Lu et al., 1999).

Interactions between *Wolbachia*, arboviruses and lipids

During the past decade, considerable progress has been made in developing novel methods to combat the spread of insect disease vectors, including mosquitoes, and consequently virus transmission. A promising strategy is the use of *Wolbachia* to control and limit arboviral transmission in animals, because *Wolbachia* infection can protect against viral infections (Pimentel et al., 2021). For example, *Wolbachia* can significantly reduce viral load, replication, and transmission of several natural pathogenic RNA viruses associated with the *Drosophila* genus (e.g., Nora virus or Drosophila C virus; Teixeira et al., 2008). A similar effect has also been observed for arthropod-borne viruses, such as West Nile virus or Chikungunya virus, with *Wolbachia* presence generally lowering host insect mortality rate (Glaser and Meola, 2010; Hedges et al., 2008; Teixeira et al., 2008). A growing number of studies have, however, suggested that *Wolbachia* can differentially affect viral replication and transmission depending on the insect host species, host strain, and *Wolbachia* strain (Caragata et al., 2013; Hussain et

al., 2013; Reyes et al., 2021). For example, replication of West Nile virus in *Ae. aegypti* mosquitoes is significantly reduced by infection with the *w*MelPop *Wolbachia* strain, but no effect was reported for the *w*Mel strain (Hussain et al., 2013).

Wolbachia blocks viral replication and transmission by priming the host's immune system (Angleró-Rodríguez et al., 2017; Bian et al., 2010; Pan et al., 2018, 2012) and/or competing with the virus for host cellular resources, such as amino acids or lipids (Caragata et al., 2013; Moreira et al., 2009). The hypothesis that modification of host metabolic pathways rather than host immune pathways forms the basis for *Wolbachia* pathogen-blocking abilities finds more empirical support. As both the virus and the bacterium are dependent on host lipids for survival and propagation, there can be extreme competition for host lipids, particularly cholesterol. Caragata et al., (2013) tested the influence of a standard, intermediate, or high cholesterol diet on the ability of *Wolbachia*-infected *D. melanogaster* to resist Drosophila C virus. An increase in cholesterol availability via the enriched diet increased virus replication and reduced the protective effect of *Wolbachia* in a dose-dependent manner. The virus titer was indeed higher in cholesterol-enriched media, leading to earlier death of the flies. An increase in viral replication following cholesterol supplementation was also reported for *Ae. albopictus* and *Ae. aegypti*, suggesting that competition for cholesterol can also play a role in these model systems (Geoghegan et al., 2017; Schultz et al., 2017).

For *Ae. aegypti*, an increase in stored cholesterol (i.e., esterified cholesterol levels) with localized accumulation of lipid droplets in the fat body and a decrease of free cholesterol levels (i.e., potential regulators of lipid transport) were found in *Wolbachia*-infected mosquitoes, suggesting that intracellular cholesterol trafficking may be perturbed (Geoghegan et al., 2017). In *Ae. albopictus*, the abundance of other lipid types, such as sphingolipids, phosphatidylcholines, and diacylglycerols (used by bacteria to enter the cell and activate mechanisms required for bacterial dissemination; Lafont & van der Goot, 2005), also decreased following *Wolbachia* infection in DENV-infected *Ae. albopictus* mosquitoes (Molloy et al., 2016). *Wolbachia* and arboviruses may thus compete for multiple lipid types, not only cholesterol. Gene expression studies support these findings, because several genes involved in fatty acid and lipid metabolism, including *fas, acc* or *sterol-coA desaturase*, were downregulated in the presence of *Wolbachia* (Geoghegan et al., 2017; Teramoto et al., 2019). *Wolbachia*-induced metabolic changes, including increased cholesterol storage near viral replication sites, as well as disruption of vesicular trafficking, may thus reduce energy availability needed for viral replication, thereby blocking viral proliferation and transmission (Geoghegan et al., 2017; Schultz et al., 2018, 2017).

The manner by which *Wolbachia* regulates lipid metabolism in the presence of viruses has remained largely unclear. Haqshenas et al., (2019) revealed, however, downregulation of insulin receptor abundance and phosphorylation levels in *Wolbachia*-infected lines, associated with a reduction of DENV and ZIKV proliferation. Inhibition of the insulin receptor revealed that ZIKV and DENV replication is reduced in a dose-dependent manner, suggesting a key role of insulin receptor kinase activity in virus replication. *Wolbachia* may thus reduce insulin receptor phosphorylation and kinase activity, decreasing virus replication (Haqshenas et al., 2019). Insulin was already linked to the activation of the insect host's immune system (Reyes et al., 2021), but further investigation into the underlying mechanisms is needed. Interestingly,

here cholesterol could also play a role, as cholesterol is known to affect regulation of the insulin-receptor signaling pathway (Sánchez-Wandelmer et al., 2009).

Wolbachia could become a promising tool for regulating arthropod-borne virus transmission (Ant et al., 2023; Ogunlade et al., 2021). Two recent studies have, however, reported that DENV infection in mosquitoes led to a distinct lipid profile when compared to mosquitoes carrying *Wolbachia* (Koh et al., 2020; Manokaran et al., 2020). This could suggest that DENV and *Wolbachia* may use different lipid types and may not be in competition for lipids. Edenborough et al., (2021) suggested that the intra-thoracic DENV infections used in Koh et al., (2020), could inhibit the effects of *Wolbachia* and may not represent the virus-*Wolbachia* relationship in a natural infection (Fraser et al., 2017). A comprehensive view on the impact of *Wolbachia* and interactions with other microorganisms at the cellular and molecular level is now necessary to fully understand the mechanistic basis of *Wolbachia*-arbovirus interference.

4 Plant pathogen effects on insect vector fat metabolism

Plant pathogens represent a major threat to plant populations. In agricultural systems, plant pathogens can reduce yield and affect the quality of agricultural production. Plant pathogens indeed induce significant losses in crops worldwide, representing a major issue for global food security (Fones et al., 2020; Ristaino et al., 2021). Plant viruses can manifest in a variety of symptoms, such as yellowing, spots, necrosis, and distortions of plant structures (Jiang and Zhou, 2023). Most plant viruses depend on insect vectors for their survival and transmission, typically phytophagous hemipterans (e.g., aphids, whiteflies, psyllids, leafhoppers, grasshoppers) that use their piercing, sucking mouthparts to feed on plant sap from which the virus is taken up (Hogenhout et al., 2008; Nault, 1997). The insect vector then transmits the virus by subsequently feeding on sap from healthy plants.

Plant viruses are generally transmitted by insects via three modes: non-persistent, semi-persistent, and persistent (Nault, 1997; Wu et al., 2022). Transmission modes differ in the time during which the insect vector can harbor the virus, ranging from minutes to hours (i.e., non-persistent), days (i.e., semi-persistent), or longer (i.e., persistent; some insects are infected during their entire life and the virus can even be transmitted to insect offspring) (Ng and Falk, 2006). Non-persistent and semi-persistent viruses are mainly retained by the insect vector's stylet and foregut, respectively, while persistent viruses infect insect gut cells and are then released in the hemocoel to invade insect tissues and organs (e.g., salivary glands, reproductive system) (Hogenhout et al., 2008; Ng and Falk, 2006). The persistent mode of transmission is further categorized as propagative or circulative, depending on whether the location of viral replication is in the insect body or not, respectively (Hogenhout et al., 2008).

Plant viruses have a range of effects on insect vectors by modifying, for example, insect-plant preference/choice, population growth, feeding behavior or fitness-related traits that may in turn affect survival and transmission of the virus (Blanc & Michalakis, 2016; Bosque-Pérez & Eigenbrode, 2011; Colvin et al., 2006; Ingwell et al., 2012; Mauck et al., 2012; Stafford et al., 2011). Only little information is available so far on the effects of plant viruses on fat metabolism of insect vectors. Ghodoum Parizipour et al., (2021) investigated the effect of three luteoviruses (i.e., persistent circulative viruses), pea enation mosaic virus (PEMV), bean leafroll virus (BLRV), and barley yellow dwarf virus-PAV (BYDV-PAV) that cause

595 considerable economic losses to cereal and legume fields, on the fatty acid profiles and fat
596 content of the aphid vectors, *A. pisum*, *Aphis fabae*, and *Rhopalosiphum padi*, respectively.
597 Fatty acid profiles differed between infected and uninfected insects in all virus-aphid
598 interactions. In both *A. pisum*-PEMV and *A. fabae*-BRLV interactions, myristic acid (C14:0)
599 quantities increased while an increase in palmitic acid (C16:0) was reported in *A. fabae*-BRLV
600 and *R. padi*-BYDV-PAV associations. An increase of linoleic acid (C18:2), as well as a decrease
601 of capric (C10:0) and oleic acid (C18:1) were also observed in the *A. pisum*-PEMV, *A. fabae*-
602 BRLV, and *R. padi*-BYDV-PAV interactions, respectively, highlighting specific fatty acid
603 changes depending on the virus-aphid interaction. Infection of *A. fabae* individuals by BRLV
604 further led to a reduction of aphid fat content, while no changes in fat content were reported
605 for the other two virus-aphid interactions (Ghodoum Parizipour et al., 2021). In another virus-
606 aphid vector interaction involving the turnip yellows virus (TuYV) (i.e., a persistent circulative
607 virus, one of the most important viruses infecting cultivated Brassicaceae, e.g., lettuce, broccoli
608 etc...) and *Myzus persicae*, virus infection also led to a reduction in fat content (Joffrey et al.,
609 2018).
610 Direct and/or indirect effects of plant viruses have been proposed to explain changes in
611 fatty acid profiles and fat content in *A. fabae* and *M. persicae* infected with BLRV and TuYV
612 respectively. For example, direct immune responses involving lipids, including fatty acids, can
613 protect the insect vector against virus infection (Wrońska et al., 2023). Viruses can also
614 negatively affect plant physiology and quality, decreasing plant biomass and photosynthetic
615 activity, in turn affecting the insect vector (Joffrey et al., 2018). Fat metabolism of the insect
616 vector feeding from the plant sap could be negatively affected due to the lower quantity of
617 nutrients synthetized by the plant (e.g., amino acids). Positive effects of plant virus infection
618 on insect vector fat metabolism have also been reported. The white-backed planthopper
619 *Sogatella furcifera*, vector of the southern rice black-streaked dwarf virus (i.e., a persistent,
620 propagative virus) showed a significant increase in myristic (C14:0), oleic (C18:1), and
621 palmitoleic acid (C16:1) levels in infected individuals (Zhang et al., 2018). Moreover, the small
622 brown planthopper *Laodelphax striatellus,* infected by the maize Iranian mosaic virus (i.e., a
623 persistent propagative virus), harbored more fat that uninfected individuals (Moeini and
624 Tahmasebi, 2019). Effects of the maize Iranian mosaic virus on *L. striatellus* fat content was
625 further found to be stage- (i.e., nymph or adult) and sex-specific, where adults and females
626 accumulated more fat. Lipids, including fatty acids, play a key role during viral replication
627 (Konan and Sanchez-Felipe, 2014; Lorizate and Krausslich, 2011). For persistent propagative
628 viruses, viral replication occurs in the insect tissues/organs; hence increasing and/or modifying
629 insect fat content and fatty acid levels during infection would allow the virus to use insect lipids
630 for replication and dissemination. Finally, an increase of fat storage generally improves insect
631 fitness (Arrese & Soulages, 2010; Scheifler et al., 2024 Chapter 16, Box 1), allowing the insect
632 to colonize new host plants and, thereby, improve virus transmission.
633 Plant pathogens other than viruses were also found to affect fat metabolism of insect
634 vectors, including the bacterial pathogen associated with citrus greening disease, *Candidatus*
635 *Liberibacter asiaticus* (CLAS), for which the Asian citrus phyllid *Diaphorina citri* is the main
636 vector. A proteomic study on *D. citri* adults, infected by CLAS, reported an upregulation of
637 proteins involved in fatty acid beta-oxidation (e.g., enoyl-coA hydratase, acyl-CoA
638 dehydrogenase; Ramsey et al., 2015), while another study found upregulation of *fas* and

vitellogenin (i.e., proteins involved in lipid transport) upon infection (Kruse et al., 2018). No change in fatty acid composition was observed between uninfected and infected *D. citri* adults, yet more palmitoleic (C16:1), palmitic (C16:0), linoleic (C18:2), and stearic acid (C18:0) were found in infected nymphs compared to infected adults, suggesting that variation in fatty acid composition is stage-specific (Killiny and Jones, 2018). There are thus contrasting results for fat metabolic responses of *D. citri*. Taken together, insect vector fat metabolic responses to plant pathogens are highly dependent on the insect vector-pathogen-host plant interaction considered.

Another topic that has received some attention is the impact of plant viruses and plant physiology and quality on higher trophic levels. Many parasitoids infect vectors of plant pathogens, and virus infection is expected to affect parasitoid performance. Joffrey et al., (2018) studied the effects of TuYV on a plant-aphid-parasitoid interaction, involving the aphid *M. persicae* and the parasitoid *Aphidius colemani*. Reduced photosynthetic activity and lower biomass in TuYV-infected plants led to a decrease in both body size and fat content of *M. persicae* adults. Smaller and leaner aphid adults used as hosts for the parasitoid *A. colemani* led to concomitant decreases in adult parasitoid body size, fat content, and fitness (i.e., lower egg numbers) (Joffrey et al., 2018). No differences were found in host and parasitoid body size and fat content in the aphid *A. fabae,* the parasitoid *Lysiphlebus fabarum* on beets infected with Beet yellows virus (Albittar et al., 2019). Fat storage is particularly important for parasitoids, because most species do not accumulate fat as adults (Visser et al., 2010, Visser, Le Lann et al., 2023; Scheifler et al., 2024 Chapter 16). When the amount or quality of fat that can be carried over from the host is reduced due to plant pathogens, there might be negative consequences for parasitoids, a level higher up the trophic food chain. The complexity of these interactions should be studied more carefully to anticipate potential issues in agricultural systems both due to plant disease and complications in biocontrol.

5 Conclusions and future perspectives

Considering the gut microbiota, research on *D. melanogaster* has revealed that individual microbe effects on lipid metabolism appear to be strongly influenced by the metabolic activities of other co-occurring microbes. The complexity of these interactions and their impact on lipid metabolism in general must, therefore, be studied using community-based approaches (rather than mono or dual-infections; Gurung et al., 2019). Furthermore, microbiota composition differs between the sexes in several insect species, suggesting different types of interactions between male and female insect hosts and their respective microbiota (Chen et al., 2016; Fransen et al., 2017; Tang et al., 2012). Metabolic and physiological differences or requirements between the sexes could also explain why interactions between gut microbiota and host fat metabolism are sex-specific, e.g., females require more resources for egg production, mainly lipids. Future work should consider how diet composition and host-related traits, such as genotype and sex, can affect the resident microbiota (Newell and Douglas, 2014; Ridley et al., 2012). Such analyses could then be extended to other insect species.

Microbes also seem to play a role in insect recognition and communication. Hertaeg et al., (2021) recently showed that endosymbiotic bacteria can alter the cuticular hydrocarbon (CHC, derived from long-chain fatty acids) composition in the aphid *A. fabae*. CHC profiles

682 depend on the host insect's genetic background, as well as the endosymbiont strain present,
683 which in turn impacts aphid interactions with other insects, such as ants (Hertaeg et al., 2021).
684 We are only beginning to understand the role microbes play in lipid metabolism of insect hosts,
685 but lipid-mediated traits, such as chemical communication can further affect interspecific
686 insect-insect interactions, also in species other than *A. fabae*.
687 *Wolbachia* can have widely different effects on the insect host, including lipid
688 metabolism; hence *Wolbachia*-insect interactions remain complex to interpret. Modifications
689 of insect fat metabolism and other lipid types appear to depend on insect species, insect host-
690 related traits, and *Wolbachia* strain (Koh et al., 2020; Molloy et al., 2016). Factors other than
691 *Wolbachia* presence should be considered when studying the impact of *Wolbachia* on host fat
692 metabolism, for example, *Wolbachia* density that can vary in response to biotic (Padde et al.,
693 2023; Pascar et al., 2023; Serbus et al., 2008) and abiotic factors (e.g., temperature; Padde et
694 al., 2023; Mouton 2004). We know very little about the mechanism by which *Wolbachia* can
695 modulate insect host fat metabolism. If we want to uncover more about the intricate interplay
696 between *Wolbachia* and insect metabolism, one could investigate lipid-related gene
697 transcription in both the insect host and the *Wolbachia* strain under study. Such a gene-based
698 approach allows for finding correlative data on regulatory and target genes used or exploited
699 by both interacting partners. Once candidate gene regulators and targets have been identified,
700 gene knockdown approaches, such as RNA interference or CRISPR-Cas9 can be used to find
701 a functional link leading to lipid-related phenotypic effects.
702 Studying the nutritional interplay between symbionts and insect hosts, particularly
703 lipids, is also highly relevant for preventing and managing major public health threats,
704 including vector-borne viruses such as DENV and Chikungunya virus. *Wolbachia* is a
705 promising tool for regulating insect disease vector transmission (Ant et al., 2023; Ogunlade et
706 al., 2021) as *Wolbachia* competes with viruses for multiple host lipid types (Geoghegan et al.,
707 2017; Molloy et al., 2016). A comprehensive overview of the role of fat in *Wolbachia* virus-
708 blocking mechanisms is needed to promote efficient and sustainable virus control in
709 mosquitoes.
710 Intricate biochemical work on the way in which pathogenic arboviruses manifest within
711 insect mosquito vectors has led to major advancements in our understanding of lipid-virus-
712 mosquito interactions (Vial et al., 2021, 2020, 2019). Viruses critically rely on an array of
713 different lipid types, including fatty acids, phospholipids, and cholesterol, each fulfilling a
714 discrete function for different viral stages. Research on plant pathogen effects on vector lipid
715 metabolism has so far led to varying results, and if lipids are affected, only relatively simple
716 estimates of bulk fat content have been estimated. Lipid effects on vectors could thus be due to
717 indirect effects of infected plants or be a consequence of the viral infection itself. We propose
718 that the research field concerned with plant pathogen-vector interactions draws parallels with
719 the work on pathogenic arboviruses, as the mechanisms by which viruses manipulate and
720 utilize host insect vector lipids may be similar. The use of isotope tracing, precursor
721 supplementation and genomic interference mechanisms may increase the resolution with which
722 plant pathogen effects can be studied in insect vectors.
723 Research on the effects of microbes on insect lipid metabolism is up and coming, and
724 we can expect microbes to play unexpected roles in host insect metabolism. The nutritional
725 role lipids play for host insects, microbes or both often remains to be fully elucidated. The

repeated evolution of endosymbioses has led to recurrent environmental compensation, where resource provisioning by the insect host has led to genome reduction and trait loss in microbes (Ellers et al., 2012). The loss of fatty acid synthesis pathways in *Wolbachia* is an excellent example of an evolved evolutionary dependence on an insect host (Wu et al., 2004). We can hypothesize that intricate mechanisms to optimize the host environment have evolved in lipid-dependent endosymbionts, for example by stimulating the synthesis of fatty acids or other lipid types by the host. When considering interactions between coexisting microbes, dependence can also evolve when a microbial species provides a common resource, or public good, that is exploited by the community of microbes, also referred to as the Black Queen Hypothesis (Morris, 2015). No examples have yet come to light regarding lipids as a public good of microbial origin, but nutrient metabolic interactions could be investigated using recently developed tools, such as NetMet, to predict the metabolic capacities of interacting microbes (Tal et al., 2020). Alternatively, microbes can provide certain nutrients or precursors that are required by the host insect. A well-known example is vitamin B, where different variants are produced by a range of microbes associated with distinct insect species (Serrato-Salas and Gendrin, 2023). Regarding various lipids, some microbes, including *Wolbachia*, can synthesize biotin, a co-factor required for acetyl coenzyme A, which is a central intermediary precursor for fatty acid synthesis. We have yet to explore how the synthesis of lipid precursors contributes to lipid dynamics between insect host and symbiont(s).

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References

Adair KL, Wilson M, Bost A, Douglas AE. Microbial community assembly in wild populations of the fruit fly *Drosophila melanogaster*. ISME J 2018;12:959–72. https://doi.org/10.1038/s41396-017-0020-x.

Akinnawo O, Ketiku AO. Chemical composition and fatty acid profile of edible larva of *Cirina forda* (Westwood). Afr J Biomed Res 2000;3:93–6.

Albittar L, Ismail M, Lohaus G, Ameline A, Visser B, Bragard C, et al. Bottom-up regulation of a tritrophic system by beet yellows virus infection: consequences for aphid-parasitoid foraging behaviour and development. Oecologia 2019;191:113–25. https://doi.org/10.1007/s00442-019-04467-0.

Angleró-Rodríguez YI, MacLeod HJ, Kang S, Carlson JS, Jupatanakul N, Dimopoulos G. *Aedes aegypti* molecular responses to zika virus: modulation of infection by the Toll and Jak/Stat immune pathways and virus host factors. Front Microbiol 2017;8:2050. https://doi.org/10.3389/fmicb.2017.02050.

764 Ankrah NYD, Barker BE, Song J, Wu C, McMullen JG, Douglas AE. Predicted metabolic
765 function of the gut microbiota of *Drosophila melanogaster*. mSystems 2021;6:e01369-20.
766 https://doi.org/10.1128/mSystems.01369-20.

767 Ant TH, Mancini MV, McNamara CJ, Rainey SM, Sinkins SP. *Wolbachia*-virus interactions
768 and arbovirus control through population replacement in mosquitoes. Pathog Glob Health
769 2023;117:245–58. https://doi.org/10.1080/20477724.2022.2117939.

770 Arai H, Hirano T, Akizuki N, Abe A, Nakai M, Kunimi Y, et al. Multiple infection and
771 reproductive manipulations of *Wolbachia* in *Homona magnanima* (Lepidoptera: Tortricidae).
772 Microb Ecol 2019;77:257–66. https://doi.org/10.1007/s00248-018-1210-4.

773 Arrese EL, Soulages JL. Insect fat body: energy, metabolism, and regulation. Annu Rev
774 Entomol 2010;55:207–25. https://doi.org/10.1146/annurev-ento-112408-085356.

775 Bashan M, Akbas H, Yurdakoc K. Phospholipid and triacylglycerol fatty acid composition of
776 major life stages of sunn pest, *Eurygaster integriceps* (Heteroptera: Scutelleridae). Comp
777 Biochem Physiol B Biochem Mol Biol 2002;132:375–80. https://doi.org/10.1016/S1096-
778 4959(02)00045-3.

779 Baumann P, Moran NA, Baumann L. Bacteriocyte-associated endosymbionts of insects. In:
780 Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. The Prokaryotes,
781 New York: Springer; 2006, p. 403–38. https://doi.org/10.1007/0-387-30741-9_16.

782 Ben-Yosef M, Jurkevitch E, Yuval B. Effect of bacteria on nutritional status and reproductive
783 success of the Mediterranean fruit fly *Ceratitis capitata*. Physiol Entomol 2008;33:145–54.
784 https://doi.org/10.1111/j.1365-3032.2008.00617.x.

785 Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, et al. Microbiome
786 definition re-visited: old concepts and new challenges. Microbiome 2020;8:103.
787 https://doi.org/10.1186/s40168-020-00875-0.

788 Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global
789 distribution and burden of dengue. Nature 2013;496:504–7.
790 https://doi.org/10.1038/nature12060.

791 Bian G, Xu Y, Lu P, Xie Y, Xi Z. The endosymbiotic bacterium *Wolbachia* induces resistance
792 to dengue virus in *Aedes aegypti*. PLoS Pathog 2010;6:e1000833.
793 https://doi.org/10.1371/journal.ppat.1000833.

794 Blanc M, Hsieh WY, Robertson KA, Watterson S, Shui G, Lacaze P, et al. Host defense against
795 viral infection involves interferon mediated down-regulation of sterol biosynthesis. PLoS Biol
796 2011;9:e1000598. https://doi.org/10.1371/journal.pbio.1000598.

797 Blanc S, Michalakis Y. Manipulation of hosts and vectors by plant viruses and impact of the
798 environment. Curr Opin Insect Sci 2016;16:36–43. https://doi.org/10.1016/j.cois.2016.05.007.

799 Bosque-Pérez NA, Eigenbrode SD. The influence of virus-induced changes in plants on aphid
800 vectors: insights from luteovirus pathosystems. Virus Res 2011;159:201–5.
801 https://doi.org/10.1016/j.virusres.2011.04.020.

802 Bozkurt B, Terlemez G, Sezgin E. Basidiomycota species in *Drosophila* gut are associated with
803 host fat metabolism. Sci Rep 2023;13. https://doi.org/10.1038/s41598-023-41027-2.

804 Bueno E, Martin KR, Raguso RA, Mcmullen JG, Hesler SP, Loeb GM, et al. Response of wild
805 spotted wing *Drosophila* (*Drosophila suzukii*) to microbial volatiles. J Chem Ecol
806 2020;46:688–98. https://doi.org/10.1007/s10886-019-01139-4.

807 Cakmak O, Bashan M, Bolu H. The fatty acid compositions of predator *Piocoris luridus*
808 (Heteroptera: Lygaeidae) and its host *Monosteria unicostata* (Heteroptera: Tingidae) reared on
809 almond. Insect Sci 2007a;14:461–6. https://doi.org/10.1111/j.1744-7917.2007.00174.x.

810 Cakmak O, Bashan M, Satar A. Total lipid and fatty acid compositions of *Lertha sheppardi*
811 (Neuroptera: Nemopteridae) during its main life stages. Biologia 2007b;62:774–80.
812 https://doi.org/10.2478/s11756-007-0147-8.

813 Cao LJ, Jiang W, Hoffmann AA. Life history effects linked to an advantage for *w*Au *Wolbachia*
814 in *Drosophila*. Insects 2019;10:126. https://doi.org/10.3390/insects10050126.

815 Caragata EP, Pais FS, Baton LA, Silva JBL, Sorgine MHF, Moreira LA. The transcriptome of
816 the mosquito *Aedes fluviatilis* (Diptera: Culicidae), and transcriptional changes associated with
817 its native *Wolbachia* infection. BMC Genomics 2017;18:6. https://doi.org/10.1186/s12864-
818 016-3441-4.

819 Caragata EP, Rancès E, Hedges LM, Gofton AW, Johnson KN, O'Neill SL, et al. Dietary
820 cholesterol modulates pathogen blocking by *Wolbachia*. PLoS Pathog 2013;9:e1003459.
821 https://doi.org/10.1371/journal.ppat.1003459.

822 Caragata EP, Rancès E, O'Neill SL, McGraw EA. Competition for amino acids between
823 *Wolbachia* and the mosquito host, *Aedes aegypti*. Microb Ecol 2014;67:205–18.
824 https://doi.org/10.1007/s00248-013-0339-4.

825 Carro AC, Damonte EB. Requirement of cholesterol in the viral envelope for dengue virus
826 infection. Virus Res 2013;174:78–87. https://doi.org/10.1016/j.virusres.2013.03.005.

827 Chandler JA, Eisen JA, Kopp A. Yeast communities of diverse *Drosophila* species: comparison
828 of two symbiont groups in the same hosts. Appl Environ Microbiol 2012;78:7327–36.
829 https://doi.org/10.1128/AEM.01741-12.

830 Chandler JA, Morgan Lang J, Bhatnagar S, Eisen JA, Kopp A. Bacterial communities of
831 diverse *Drosophila* species: ecological context of a host–microbe model system. PLoS Genet
832 2011;7:e1002272. https://doi.org/10.1371/journal.pgen.1002272.

833 Charlat S, Calmet C, Merçot H. On the *mod resc* model and the evolution of *Wolbachia*
834 compatibility types. Genetics 2001;159:1415–22. https://doi.org/10.1093/genetics/159.4.1415.

835 Chen B, Teh B-S, Sun C, Hu S, Lu X, Boland W, et al. Biodiversity and activity of the gut
836 microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Sci Rep
837 2016;6:29505. https://doi.org/10.1038/srep29505.

838 Cho K-O, Kim G-W, Lee O-K. *Wolbachia* bacteria reside in host Golgi-related vesicles whose
839 position is regulated by polarity proteins. PLoS One 2011;6:e22703.
840 https://doi.org/10.1371/journal.pone.0022703.

Chotiwan N, Andre BG, Sanchez-Vargas I, Islam MN, Grabowski JM, Hopf-Jannasch A, et al. Dynamic remodeling of lipids coincides with dengue virus replication in the midgut of *Aedes aegypti* mosquitoes. PLoS Pathog 2018;14:e1006853. https://doi.org/10.1371/journal.ppat.1006853.

Chotiwan N, Brito-Sierra CA, Ramirez G, Lian E, Grabowski JM, Graham B, et al. Expression of fatty acid synthase genes and their role in development and arboviral infection of *Aedes aegypti*. Parasit Vectors 2022;15:233. https://doi.org/10.1186/s13071-022-05336-1.

Colvin J, Omongo CA, Govindappa MR, Stevenson PC, Maruthi MN, Gibson G, et al. Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. Adv Virus Res 2006;67:419–52. https://doi.org/10.1016/S0065-3527(06)67011-5.

Conceição CC, da Silva JN, Arcanjo A, Nogueira CL, de Abreu LA, de Oliveira PL, et al. *Aedes fluviatilis* cell lines as new tools to study metabolic and immune interactions in mosquito-*Wolbachia* symbiosis. Sci Rep 2021;11:19202. https://doi.org/10.1038/s41598-021-98738-7.

Consuegra J, Grenier T, Akherraz H, Rahioui I, Gervais H, da Silva P, et al. Metabolic cooperation among commensal bacteria supports *Drosophila* juvenile growth under nutritional stress. iScience 2020;23:101232. https://doi.org/10.1016/j.isci.2020.101232.

Cornwallis CK, van 't Padje A, Ellers J, Klein M, Jackson R, Kiers ET, et al. Symbioses shape feeding niches and diversification across insects. Nat Ecol Evol 2023;7:1022–44. https://doi.org/10.1038/s41559-023-02058-0.

Cruz-Oliveira C, Freire JM, Conceição TM, Higa LM, Castanho MARB, Da Poian AT. Receptors and routes of dengue virus entry into the host cells. FEMS Microbiol Rev 2015;39:155–70. https://doi.org/10.1093/femsre/fuu004.

Currin-Ross D, Husdell L, Pierens GK, Mok NE, O'Neill SL, Schirra HJ, et al. The metabolic response to infection with *Wolbachia* implicates the insulin/insulin-like-growth factor and hypoxia signaling pathways in *Drosophila melanogaster*. Front Ecol Evol 2021;9:623561. https://doi.org/10.3389/fevo.2021.623561.

Denlinger DL. Regulation of diapause. Annu Rev Entomol 2002;47:93–122. https://doi.org/10.1146/annurev.ento.47.091201.145137.

Denlinger DL, Yocum GD, Rinehart JP. Hormonal control of diapause. Insect Endocrinology, Elsevier; 2012, p. 430–63. https://doi.org/10.1016/B978-0-12-384749-2.10010-X.

Depommier C, Everard A, Druart C, Plovier H, van Hul M, Vieira-Silva S, et al. Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med 2019;25:1096–103. https://doi.org/10.1038/s41591-019-0495-2.

Didion EM, Sabree ZL, Kenyon L, Nine G, Hagan RW, Osman S, et al. Microbiome reduction prevents lipid accumulation during early diapause in the northern house mosquito, *Culex pipiens pipiens*. J Insect Physiol 2021;134:104295. https://doi.org/10.1016/j.jinsphys.2021.104295.

Dittmer J, Bouchon D. Feminizing *Wolbachia* influence microbiota composition in the terrestrial isopod *Armadillidium vulgare*. Sci Rep 2018;8:6998. https://doi.org/10.1038/s41598-018-25450-4.

Dittmer J, Brucker RM. When your host shuts down: larval diapause impacts host-microbiome interactions in *Nasonia vitripennis*. Microbiome 2021;9:85. https://doi.org/10.1186/s40168-021-01037-6.

Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, et al. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochem Mol Biol 1999;29:153–60. https://doi.org/10.1016/S0965-1748(98)00119-2.

Dou W, Miao Y, Xiao J, Huang D. Association of *Wolbachia* with gene expression in *Drosophila* testes. Microb Ecol 2021;82:805–17. https://doi.org/10.1007/s00248-021-01703-0.

Douglas AE. Simple animal models for microbiome research. Nat Rev Microbiol 2019;17:764–75. https://doi.org/10.1038/s41579-019-0242-1.

Douglas AE. Multiorganismal insects: Diversity and function of resident microorganisms. Annu Rev Entomol 2015;60:17–34. https://doi.org/10.1146/annurev-ento-010814-020822.

Douglas AE, Minto LB, Wilkinson TL. Quantifying nutrient production by the microbial symbionts in an aphid. J Exp Biol 2001;204:349–58. https://doi.org/10.1242/jeb.204.2.349.

Edenborough KM, Flores HA, Simmons CP, Fraser JE. Using *Wolbachia* to eliminate dengue: will the virus fight back? J Virol 2021;95:0220320. https://doi.org/10.1128/JVI.02203-20.

Ellers J, Toby Kiers E, Currie CR, McDonald BR, Visser B. Ecological interactions drive evolutionary loss of traits. Ecol Lett 2012;15:1071–82. https://doi.org/10.1111/j.1461-0248.2012.01830.x.

Engel P, Martinson VG, Moran NA. Functional diversity within the simple gut microbiota of the honey bee. Proc Natl Acad Sci, USA 2012;109:11002–7. https://doi.org/10.1073/pnas.1202970109.

Engel P, Moran NA. The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 2013;37:699–735. https://doi.org/10.1111/1574-6976.12025.

Engl T, Kaltenpoth M. Influence of microbial symbionts on insect pheromones. Nat Prod Rep 2018;35:386–97. https://doi.org/10.1039/C7NP00068E.

Enriquez T, Visser B. The importance of fat accumulation and reserves for insect overwintering. Curr Opin Insect Sci 2023;60:101118. https://doi.org/10.1016/j.cois.2023.101118.

Erkosar B, Storelli G, Defaye A, Leulier F. Host-intestinal microbiota mutualism: "Learning on the fly." Cell Host Microbe 2013;13:8–14. https://doi.org/10.1016/j.chom.2012.12.004.

Farahani HK, Ashouri A, Zibaee A, Abroon P, Alford L, Pierre J-S, et al. Early life nutritional quality effects on adult memory retention in a parasitic wasp. Behav Ecol 2017;28:818–26. https://doi.org/10.1093/beheco/arx042.

Faria VG, Martins NE, Schlötterer C, Sucena É. Readapting to DCV Infection without *Wolbachia*: frequency changes of *Drosophila* antiviral alleles can replace endosymbiont protection. Genome Biol Evol 2018;10:1783–91. https://doi.org/10.1093/gbe/evy137.

Fattouh N, Cazevieille C, Landmann F. *Wolbachia* endosymbionts subvert the endoplasmic reticulum to acquire host membranes without triggering ER stress. PLoS Negl Trop Dis 2019;13:e0007218. https://doi.org/10.1371/journal.pntd.0007218.

Fauci AS, Morens DM. Zika virus in the Americas — Yet another arbovirus threat. N Engl J Med 2016;374:601–4. https://doi.org/10.1056/NEJMp1600297.

Fones HN, Bebber DP, Chaloner TM, Kay WT, Steinberg G, Gurr SJ. Threats to global food security from emerging fungal and oomycete crop pathogens. Nat Food 2020;1:332–42. https://doi.org/10.1038/s43016-020-0075-0.

Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The impact of gut microbiota on gender-specific differences in immunity. Front Immunol 2017;8:754. https://doi.org/10.3389/fimmu.2017.00754.

Fraser JE, De Bruyne JT, Iturbe-Ormaetxe I, Stepnell J, Burns RL, Flores HA, et al. Novel *Wolbachia*-transinfected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotypes. PLoS Pathog 2017;13:e1006751. https://doi.org/10.1371/journal.ppat.1006751.

Fu Q, Inankur B, Yin J, Striker R, Lan Q. Sterol carrier protein 2, a critical host factor for dengue virus infection, alters the cholesterol distribution in mosquito Aag2 cells. J Med Entomol 2015;52:1124–34. https://doi.org/10.1093/jme/tjv101.

Geoghegan V, Stainton K, Rainey SM, Ant TH, Dowle AA, Larson T, et al. Perturbed cholesterol and vesicular trafficking associated with dengue blocking in *Wolbachia*-infected *Aedes aegypti* cells. Nat Commun 2017;8:526. https://doi.org/10.1038/s41467-017-00610-8.

Ghodoum Parizipour MH, Tahmasebi A, Shahriari AG, Khashman M, Hemmati F. Luteoviruses affected energy reserves and fatty acid composition of their aphid vectors. J Phytopathol 2021;169:376–86. https://doi.org/10.1111/jph.12993.

Giraud É, Varet H, Legendre R, Sismeiro O, Aubry F, Dabo S, et al. Mosquito-bacteria interactions during larval development trigger metabolic changes with carry-over effects on adult fitness. Mol Ecol 2022;31:1444–60. https://doi.org/10.1111/mec.16327.

Glaser RL, Meola MA. The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to west nile virus infection. PLoS One 2010;5:e11977. https://doi.org/10.1371/journal.pone.0011977.

Goane L, Salgueiro J, Medina Pereyra P, Arce OEA, Ruiz MJ, Nussenbaum AL, et al. Antibiotic treatment reduces fecundity and nutrient content in females of *Anastrepha fraterculus* (Diptera: Tephritidae) in a diet dependent way. J Insect Physiol 2022;139:104396. https://doi.org/10.1016/j.jinsphys.2022.104396.

Gołębiowski M, Boguś MI, Paszkiewicz M, Stepnowski P. The composition of the free fatty acids from *Dendrolimus pini* exuviae. J Insect Physiol 2010;56:391–7. https://doi.org/10.1016/j.jinsphys.2009.11.009.

Gruntenko NE, Ilinsky YY, Adonyeva N V, Burdina E V, Bykov RA, Menshanov PN, et al. Various *Wolbachia* genotypes differently influence host *Drosophila* dopamine metabolism and survival under heat stress conditions. BMC Evol Biol 2017;17:252. https://doi.org/10.1186/s12862-017-1104-y.

Gurung K, Wertheim B, Falcao Salles J. The microbiome of pest insects: it is not just bacteria. Entomol Exp Appl 2019;167:156–70. https://doi.org/10.1111/eea.12768.

Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: A continuing global threat. Nat Rev Microbiol 2010;8:S7–16. https://doi.org/10.1038/nrmicro2460.

Habineza P, Muhammad A, Ji T, Xiao R, Yin X, Hou Y, et al. The promoting effect of gut microbiota on growth and development of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae) by modulating its nutritional metabolism. Front Microbiol 2019;10:1212. https://doi.org/10.3389/fmicb.2019.01212.

Hahn DA, Denlinger DL. Energetics of insect diapause. Annu Rev Entomol 2011;56:103–21. https://doi.org/10.1146/annurev-ento-112408-085436.

Hahn DA, Denlinger DL. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. J Insect Physiol 2007;53:760–73. https://doi.org/10.1016/j.jinsphys.2007.03.018.

Haqshenas G, Terradas G, Paradkar PN, Duchemin J-B, McGraw EA, Doerig C. A role for the insulin receptor in the suppression of dengue virus and zika virus in *Wolbachia*-infected mosquito cells. Cell Rep 2019;26:529-535.e3. https://doi.org/10.1016/j.celrep.2018.12.068.

Hedges LM, Brownlie JC, O'Neill SL, Johnson KN. *Wolbachia* and virus protection in insects. Science 2008;322:702. https://doi.org/10.1126/science.1162418.

Henry Y, Overgaard J, Colinet H. Dietary nutrient balance shapes phenotypic traits of *Drosophila melanogaster* in interaction with gut microbiota. Comp Biochem Physiol A Mol Integr Physiol 2020;241:110626. https://doi.org/10.1016/j.cbpa.2019.110626.

Herren JK, Paredes JC, Schüpfer F, Arafah K, Bulet P, Lemaitre B. Insect endosymbiont proliferation is limited by lipid availability. eLife 2014;3:e02964. https://doi.org/10.7554/eLife.02964.

Hertaeg C, Risse M, Vorburger C, De Moraes CM, Mescher MC. Aphids harbouring different endosymbionts exhibit differences in cuticular hydrocarbon profiles that can be recognized by ant mutualists. Sci Rep 2021;11:19559. https://doi.org/10.1038/s41598-021-98098-2.

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*? - A statistical analysis of current data. FEMS Microbiol Lett 2008;281:215–20. https://doi.org/10.1111/j.1574-6968.2008.01110.x.

Hogenhout SA, Ammar E-D, Whitfield AE, Redinbaugh MG. Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol 2008;46:327–59. https://doi.org/10.1146/annurev.phyto.022508.092135.

995 Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T. *Wolbachia* as a bacteriocyte-associated
996 nutritional mutualist. Proc Natl Acad Sci, USA 2010;107:769–74.
997 https://doi.org/10.1073/pnas.0911476107.

998 Howard RW, Stanley-Samuelson DW. Phospholipid fatty acid composition and arachidonic
999 acid metabolism in selected tissues of adult *Tenebrio molitor* (Coleoptera: Tenebrionidae). Ann
1000 Entomol Soc Am 1990;83:975–81. https://doi.org/10.1093/aesa/83.5.975.

1001 Howe D, Heinzen RA. *Coxiella burnetii* inhabits a cholesterol-rich vacuole and influences
1002 cellular cholesterol metabolism. Cell Microbiol 2006;8:496–507.
1003 https://doi.org/10.1111/j.1462-5822.2005.00641.x.

1004 Hu Y, Sanders JG, Łukasik P, D'Amelio CL, Millar JS, Vann DR, et al. Herbivorous turtle ants
1005 obtain essential nutrients from a conserved nitrogen-recycling gut microbiome. Nat Commun
1006 2018;9:964. https://doi.org/10.1038/s41467-018-03357-y.

1007 Huang J-H, Douglas AE. Consumption of dietary sugar by gut bacteria determines *Drosophila*
1008 lipid content. Biol Lett 2015;11:20150469. https://doi.org/10.1098/rsbl.2015.0469.

1009 Hughes GL, Koga R, Xue P, Fukatsu T, Rasgon JL. *Wolbachia* infections are virulent and
1010 inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles aambiae*. PLoS
1011 Pathog 2011;7:e1002043. https://doi.org/10.1371/journal.ppat.1002043.

1012 Hurst GDD, Jiggins FM, von der Schulenburg JHG, Bertrand D, West SA, Goriacheva II, et al.
1013 Male–killing *Wolbachia* in two species of insect. Proc R Soc Lond B Biol Sci 1999;266:735–
1014 40. https://doi.org/10.1098/rspb.1999.0698.

1015 Hussain M, Lu G, Torres S, Edmonds JH, Kay BH, Khromykh AA, et al. Effect of *Wolbachia*
1016 on replication of West Nile Virus in a mosquito cell line and adult mosquitoes. J Virol
1017 2013;87:851–8. https://doi.org/10.1128/JVI.01837-12.

1018 Ikeya T, Broughton S, Alic N, Grandison R, Partridge L. The endosymbiont *Wolbachia*
1019 increases insulin/IGF-like signalling in *Drosophila*. Proc R Soc Lond B Biol Sci
1020 2009;276:3799–807. https://doi.org/10.1098/rspb.2009.0778.

1021 Ingwell LL, Eigenbrode SD, Bosque-Pérez NA. Plant viruses alter insect behavior to enhance
1022 their spread. Sci Rep 2012;2:578. https://doi.org/10.1038/srep00578.

1023 Janson EM, Stireman JO, Singer MS, Abbot P. Phytophagous insect-microbe mutualisms and
1024 adaptive evolutionary diversification. Evolution 2008;62:997–1012.
1025 https://doi.org/10.1111/j.1558-5646.2008.00348.x.

1026 Jiang T, Zhou T. Unraveling the mechanisms of virus-induced symptom development in plants.
1027 Plants 2023;12:2830. https://doi.org/10.3390/plants12152830.

1028 Jing T-Z, Qi F-H, Wang Z-Y. Most dominant roles of insect gut bacteria: digestion,
1029 detoxification, or essential nutrient provision? Microbiome 2020;8:38.
1030 https://doi.org/10.1186/s40168-020-00823-y.

1031 Joffrey M, Chesnais Q, Spicher F, Verrier E, Ameline A, Couty A. Plant virus infection
1032 influences bottom-up regulation of a plant-aphid-parasitoid system. J Pest Sci 2018;91:361–
1033 72. https://doi.org/10.1007/s10340-017-0911-7.

Jolly NP, Varela C, Pretorius IS. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Res 2014;14:215–37. https://doi.org/10.1111/1567-1364.12111.

Kaczmarek A, Wrońska AK, Kazek M, Boguś MI. Metamorphosis-related changes in the free fatty acid profiles of *Sarcophaga* (Liopygia) *argyrostoma* (Robineau-Desvoidy, 1830). Sci Rep 2020;10:17337. https://doi.org/10.1038/s41598-020-74475-1.

Karpova EK, Bobrovskikh MA, Deryuzhenko MA, Shishkina OD, Gruntenko NE. *Wolbachia* effect on *Drosophila melanogaster* lipid and carbohydrate metabolism. Insects 2023;14:357. https://doi.org/10.3390/insects14040357.

Killiny N, Jones SE. Metabolic alterations in the nymphal instars of *Diaphorina citri* induced by *Candidatus* Liberibacter asiaticus, the putative pathogen of huanglongbing. PLoS One 2018;13:e0191871. https://doi.org/10.1371/journal.pone.0191871.

Koh C, Islam MN, Ye YH, Chotiwan N, Graham B, Belisle JT, et al. Dengue virus dominates lipid metabolism modulations in *Wolbachia*-coinfected *Aedes aegypti*. Commun Biol 2020;3:518. https://doi.org/10.1038/s42003-020-01254-z.

Konan K V, Sanchez-Felipe L. Lipids and RNA virus replication. Curr Opin Virol 2014;9:45–52. https://doi.org/10.1016/j.coviro.2014.09.005.

Kruse A, Ramsey JS, Johnson R, Hall DG, MacCoss MJ, Heck M. *Candidatus* Liberibacter asiaticus minimally alters expression of immunity and metabolism proteins in hemolymph of *Diaphorina citri*, the insect vector of huanglongbing. J Proteome Res 2018;17:2995–3011. https://doi.org/10.1021/acs.jproteome.8b00183.

Lafont F, van der Goot FG. Bacterial invasion via lipid rafts. Cell Microbiol 2005;7:613–20. https://doi.org/10.1111/j.1462-5822.2005.00515.x.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci, USA 2005;102:11070–5. https://doi.org/10.1073/pnas.0504978102.

Li G, Zheng X, Zhu Y, Long Y, Xia X. *Bacillus* symbiont drives alterations in intestinal microbiota and circulating metabolites of lepidopteran host. Environ Microbiol 2022;24:4049–64. https://doi.org/10.1111/1462-2920.15934.

Lin M, Rikihisa Y. *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* lack genes for lipid A biosynthesis and incorporate cholesterol for their survival. Infect Immun 2003;71:5324–31. https://doi.org/10.1128/IAI.71.9.5324-5331.2003.

Liu B-N, Liu X-T, Liang Z-H, Wang J-H. Gut microbiota in obesity. World J Gastroenterol 2021;27:3837–50. https://doi.org/10.3748/wjg.v27.i25.3837.

Liu W, Li Y, Guo S, Yin H, Lei C-L, Wang X-P. Association between gut microbiota and diapause preparation in the cabbage beetle: a new perspective for studying insect diapause. Sci Rep 2016;6:38900. https://doi.org/10.1038/srep38900.

Liu Y-Y, Liang X-D, Liu C-C, Cheng Y, Chen H, Baloch AS, et al. Fatty acid synthase is involved in classical swine fever virus replication by interaction with NS4B. J Virol 2021;95. https://doi.org/10.1128/JVI.00781-21.

Lorizate M, Krausslich H-G. Role of lipids in virus replication. Cold Spring Harb Perspect Biol 2011;3:a004820–a004820. https://doi.org/10.1101/cshperspect.a004820.

Lu YE, Cassese T, Kielian M. The cholesterol requirement for Sindbis virus entry and exit and characterization of a spike protein region involved in cholesterol dependence. J Virol 1999;73:4272–8. https://doi.org/10.1128/JVI.73.5.4272-4278.1999.

Lv W-X, Cheng P, Lei J-J, Peng H, Zang C-H, Lou Z-W, et al. Interactions between the gut micro-community and transcriptome of *Culex pipiens* pallens under low-temperature stress. Parasit Vectors 2023;16:12. https://doi.org/10.1186/s13071-022-05643-7.

Maistrenko OM, Serga S V, Vaiserman AM, Kozeretska IA. Longevity-modulating effects of symbiosis: insights from *Drosophila–Wolbachia* interaction. Biogerontology 2016;17:785–803. https://doi.org/10.1007/s10522-016-9653-9.

Manokaran G, Flores HA, Dickson CT, Narayana VK, Kanojia K, Dayalan S, et al. Modulation of acyl-carnitines, the broad mechanism behind *Wolbachia*-mediated inhibition of medically important flaviviruses in *Aedes aegypti*. Proc Natl Acad Sci, USA 2020;117:24475–83. https://doi.org/10.1073/pnas.1914814117.

Marten AD, Tift CT, Tree MO, Bakke J, Conway MJ. Chronic depletion of vertebrate lipids in *Aedes aegypti* cells dysregulates lipid metabolism and inhibits innate immunity without altering dengue infectivity. PLoS Negl Trop Dis 2022;16:e0010890. https://doi.org/10.1371/journal.pntd.0010890.

Mauck K, Bosque-Pérez NA, Eigenbrode SD, De Moraes CM, Mescher MC. Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Funct Ecol 2012;26:1162–75. https://doi.org/10.1111/j.1365-2435.2012.02026.x.

Mazzucco R, Nolte V, Vijayan T, Schlötterer C. Long-term dynamics among *Wolbachia* strains during thermal adaptation of their *Drosophila melanogaster* hosts. Front Genet 2020;11:482. https://doi.org/10.3389/fgene.2020.00482.

McBride MJ, Xie G, Martens EC, Lapidus A, Henrissat B, Rhodes RG, et al. Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. Appl Environ Microbiol 2009;75:6864–75. https://doi.org/10.1128/AEM.01495-09.

McMullen JG, Peters-Schulze G, Cai J, Patterson AD, Douglas AE. How gut microbiome interactions affect nutritional traits of *Drosophila melanogaster*. J Exp Biol 2020;223:jeb227843. https://doi.org/10.1242/jeb.227843.

Melo CFOR, de Oliveira DN, Lima E de O, Guerreiro TM, Esteves CZ, Beck RM, et al. A lipidomics approach in the characterization of zika-infected mosquito cells: potential targets for breaking the transmission cycle. PLoS One 2016;11:e0164377. https://doi.org/10.1371/journal.pone.0164377.

Moeini P, Tahmasebi A. Maize Iranian mosaic virus infection promotes the energy sources of its insect vector, *Laodelphax striatellus*. J Appl Entomol 2019;143:271–6. https://doi.org/10.1111/jen.12585.

Molloy JC, Sommer U, Viant MR, Sinkins SP. *Wolbachia* modulates lipid metabolism in *Aedes albopictus* mosquito cells. Appl Environ Microbiol 2016;82:3109–20. https://doi.org/10.1128/AEM.00275-16.

Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. Cell 2009;139:1268–78. https://doi.org/10.1016/j.cell.2009.11.042.

Morris JJ. Black Queen evolution: the role of leakiness in structuring microbial communities. Trends Genet 2015;31:475–82. https://doi.org/10.1016/j.tig.2015.05.004.

Mosso C, Galván-Mendoza IJ, Ludert JE, del Angel RM. Endocytic pathway followed by dengue virus to infect the mosquito cell line C6/36 HT. Virology 2008;378:193–9. https://doi.org/10.1016/j.virol.2008.05.012.

Nault LR. Arthropod transmission of plant viruses: a new synthesis. Ann Entomol Soc Am 1997;90:521–41. https://doi.org/10.1093/aesa/90.5.521.

Newell PD, Douglas AE. Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in *Drosophila melanogaster*. Appl Environ Microbiol 2014;80:788–96. https://doi.org/10.1128/AEM.02742-13.

Newton ILG, Rice DW. The Jekyll and Hyde Symbiont: Could *Wolbachia* be a nutritional mutualist? J Bacteriol 2020;202:e00589-19. https://doi.org/10.1128/JB.00589-19.

Ng JCK, Falk BW. Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Annu Rev Phytopathol 2006;44:183–212. https://doi.org/10.1146/annurev.phyto.44.070505.143325.

Nguyen B, Dinh H, Morimoto J, Ponton F. Sex-specific effects of the microbiota on adult carbohydrate intake and body composition in a polyphagous fly. J Insect Physiol 2021;134:104308. https://doi.org/10.1016/j.jinsphys.2021.104308.

Nikolova N, Rezanka T, Nikolova-Damyanova B. Fatty acid profiles of main lipid classes in adult *Chrysomela vigintipunctata* (Scopoli) (Coleopterai: Chrysomelidae). Z Naturforsch C 2000;55:661–6. https://doi.org/10.1515/znc-2000-7-828.

Ogunlade ST, Meehan MT, Adekunle AI, Rojas DP, Adegboye OA, McBryde ES. A review: *Aedes*-borne arboviral infections, controls and *Wolbachia*-based strategies. Vaccines 2021;9:32. https://doi.org/10.3390/vaccines9010032.

Padde JR, Lu Q, Long Y, Zhang D, Hou M, Chen Lu, et al. The impact of environmental and host factors on *Wolbachia* density and efficacy as a biological tool. Decod Infect Trans 2023;1:100006. https://doi.org/10.1016/j.dcit.2023.100006.

Pan X, Pike A, Joshi D, Bian G, McFadden MJ, Lu P, et al. The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti*. ISME J 2018;12:277–88. https://doi.org/10.1038/ismej.2017.174.

Pan X, Zhou G, Wu J, Bian G, Lu P, Raikhel AS, et al. *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. Proc Natl Acad Sci, USA 2012;109:E23-31. https://doi.org/10.1073/pnas.1116932108.

Paredes JC, Herren JK, Schüpfer F, Lemaitre B. The role of lipid competition for endosymbiont-mediated protection against parasitoid wasps in *Drosophila*. mBio 2016;7:e01006-16. https://doi.org/10.1128/mBio.01006-16.

Park Y, Ledesma-Amaro R, Nicaud J-M. De novo biosynthesis of odd-Chain fatty acids in *Yarrowia lipolytica* enabled by modular pathway engineering. Front Bioeng Biotechnol 2020;7:484. https://doi.org/10.3389/fbioe.2019.00484.

Pascar J, Middleton H, Dorus S. *Aedes aegypti* microbiome composition covaries with the density of *Wolbachia* infection. Microbiome 2023;11:255. https://doi.org/10.1186/s40168-023-01678-9.

Perera R, Riley C, Isaac G, Hopf-Jannasch AS, Moore RJ, Weitz KW, et al. Dengue virus infection perturbs lipid homeostasis in infected mosquito cells. PLoS Pathog 2012;8:e1002584. https://doi.org/10.1371/journal.ppat.1002584.

Pickett KM, McHenry A, Wenzel JW. Nestmate recognition in the absence of a pheromone. Insectes Soc 2000;47:212–9. https://doi.org/10.1007/PL00001705.

Pietri JE, DeBruhl H, Sullivan W. The rich somatic life of *Wolbachia*. MicrobiologyOpen 2016;5:923–36. https://doi.org/10.1002/mbo3.390.

Pimentel AC, Cesar CS, Martins M, Cogni R. The antiviral effects of the symbiont bacteria *Wolbachia* in insects. Front Immunol 2021;11:626329. https://doi.org/10.3389/fimmu.2020.626329.

Poinsot D, Charlat S, Merçot H. On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: confronting the models with the facts. BioEssays 2003;25:259–65. https://doi.org/10.1002/bies.10234.

Rahbé Y, Delobel B, Febvay G, Chantegrel B. Aphid-specific triglycerides in symbiotic and aposymbiotic *Acyrthosiphon pisum*. Insect Biochem Mol Biol 1994;24:95–101. https://doi.org/10.1016/0965-1748(94)90127-9.

Rahimi-Kaldeh S, Bandani A, Ashouri A. Does *Wolbachia* change diapause and energy reserves of *Trichogramma brassicae* in response to light wavelengths? J Agr Sci Tech 2019;21:1173–82.

Ramsey JS, Johnson RS, Hoki JS, Kruse A, Mahoney J, Hilf ME, et al. Metabolic interplay between the Asian citrus psyllid and its *Profftella* Symbiont: an Achilles' heel of the citrus greening insect vector. PLoS One 2015;10:e0140826. https://doi.org/10.1371/journal.pone.0140826.

Rancès E, Ye YH, Woolfit M, McGraw EA, O'Neill SL. The relative importance of innate immune priming in *Wolbachia*-mediated dengue interference. PLoS Pathog 2012;8:e1002548. https://doi.org/10.1371/journal.ppat.1002548.

Ratnayake OC, Chotiwan N, Saavedra-Rodriguez K, Perera R. The buzz in the field: the interaction between viruses, mosquitoes, and metabolism. Front Cell Infect Microbiol 2023;13:1128577. https://doi.org/10.3389/fcimb.2023.1128577.

Raza MF, Wang Y, Cai Z, Bai S, Yao Z, Awan UA, et al. Gut microbiota promotes host resistance to low-temperature stress by stimulating its arginine and proline metabolism pathway in adult *Bactrocera dorsalis*. PLoS Pathog 2020;16:e1008441. https://doi.org/10.1371/journal.ppat.1008441.

Reyes JIL, Suzuki Y, Carvajal T, Muñoz MNM, Watanabe K. Intracellular interactions between arboviruses and *Wolbachia* in *Aedes aegypti*. Front Cell Infect Microbiol 2021;11:690087. https://doi.org/10.3389/fcimb.2021.690087.

Řezanka T, Sigler K. Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. Prog Lipid Res 2009;48:206–38. https://doi.org/10.1016/j.plipres.2009.03.003.

Ridley E V, Wong AC-N, Westmiller S, Douglas AE. Impact of the resident microbiota on the nutritional phenotype of *Drosophila melanogaster*. PLoS One 2012;7:e36765. https://doi.org/10.1371/journal.pone.0036765.

Ristaino JB, Anderson PK, Bebber DP, Brauman KA, Cunniffe NJ, Fedoroff N V., et al. The persistent threat of emerging plant disease pandemics to global food security. Proc Natl Acad Sci, USA 2021;118. https://doi.org/10.1073/pnas.2022239118.

Romoli O, Schönbeck JC, Hapfelmeier S, Gendrin M. Production of germ-free mosquitoes via transient colonisation allows stage-specific investigation of host–microbiota interactions. Nat Commun 2021;12:942. https://doi.org/10.1038/s41467-021-21195-3.

Russell CW, Poliakov A, Haribal M, Jander G, van Wijk KJ, Douglas AE. Matching the supply of bacterial nutrients to the nutritional demand of the animal host. Proc R Soc Lond B Biol Sci 2014;281:20141163. https://doi.org/10.1098/rspb.2014.1163.

Sánchez-Wandelmer J, Dávalos A, Herrera E, Giera M, Cano S, de la Peña G, et al. Inhibition of cholesterol biosynthesis disrupts lipid raft/caveolae and affects insulin receptor activation in 3T3-L1 preadipocytes. Biochim Biophys Acta Biomembr 2009;1788:1731–9. https://doi.org/10.1016/j.bbamem.2009.05.002.

Sannino DR, Dobson AJ, Edwards K, Angert ER, Buchon N. The *Drosophila melanogaster* gut microbiota provisions thiamine to its host. mBio 2018;9:e00155-18. https://doi.org/10.1128/mBio.00155-18.

Sato A, Ohhara Y, Miura S, Yamakawa-Kobayashi K. The presence of odd-chain fatty acids in *Drosophila* phospholipids. Biosci Biotechnol Biochem 2020;84:2139–48. https://doi.org/10.1080/09168451.2020.1790337.

Sazama EJ, Bosch MJ, Shouldis CS, Ouellette SP, Wesner JS. Incidence of *Wolbachia* in aquatic insects. Ecol Evol 2017;7:1165–9. https://doi.org/10.1002/ece3.2742.

Scheitz CJF, Guo Y, Early AM, Harshman LG, Clark AG. Heritability and inter-population differences in lipid profiles of *Drosophila melanogaster*. PLoS One 2013;8:e72726. https://doi.org/10.1371/journal.pone.0072726.

Schultz MJ, Isern S, Michael SF, Corley RB, Connor JH, Frydman HM. Variable inhibition of zika virus replication by different *Wolbachia* strains in mosquito cell cultures. J Virol 2017;91:339–56. https://doi.org/10.1128/JVI.00339-17.

Schultz MJ, Tan AL, Gray CN, Isern S, Michael SF, Frydman HM, et al. *Wolbachia w*Stri blocks zika virus growth at two independent stages of viral replication. mBio 2018;9:e00738-18. https://doi.org/10.1128/mBio.00738-18.

Serbus LR, Casper-Lindley C, Landmann F, Sullivan W. The genetics and cell biology of *Wolbachia* - host Interactions. Annu Rev Genet 2008;42:683–707. https://doi.org/10.1146/annurev.genet.41.110306.130354.

Serrato-Salas J, Gendrin M. Involvement of microbiota in insect physiology: focus on B vitamins. mBio 2023;14:e02225-22. https://doi.org/10.1128/mbio.02225-22.

Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, et al. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science 2011;334:670–4. https://doi.org/10.1126/science.1212782.

Sieber MH, Thummel CS. Coordination of triacylglycerol and cholesterol homeostasis by *DHR96* and the *Drosophila* LipA homolog *magro*. Cell Metab 2012;15:122–7. https://doi.org/10.1016/j.cmet.2011.11.011.

Sommer AJ, Newell PD. Metabolic basis for mutualism between gut bacteria and its impact on the *Drosophila melanogaster* host. Appl Environ Microbiol 2019;85:e01882-18. https://doi.org/10.1128/AEM.01882-18.

Stafford CA, Walker GP, Ullman DE. Infection with a plant virus modifies vector feeding behavior. Proc Natl Acad Sci, USA 2011;108:9350–5. https://doi.org/10.1073/pnas.1100773108.

Stanley-Samuelson DW, Howard RW, Akre RD. Nutritional interactions revealed by tissue fatty acid profiles of an obligate *Myrmecophilous* predator, *Microdon albicomatus*, and its prey, *Myrmica incompleta*, (Diptera: Syrphidae) (Hymenoptera: Formicidae). Ann Entomol Soc Am 1990;83:1108–15. https://doi.org/10.1093/aesa/83.6.1108.

Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. *Lactobacillus plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metab 2011;14:403–14. https://doi.org/10.1016/j.cmet.2011.07.012.

Stouthamer R, Breeuwer JAJ, Hurst GDD. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annu Rev Microbiol 1999;53:71–102. https://doi.org/10.1146/annurev.micro.53.1.71.

Tal O, Selvaraj G, Medina S, Ofaim S, Freilich S. NetMet: A network-based tool for predicting metabolic capacities of microbial species and their interactions. Microorganisms 2020;8:840. https://doi.org/10.3390/microorganisms8060840.

Tang X, Adler PH, Vogel H, Ping L. Gender-specific bacterial composition of black flies (Diptera: Simuliidae). FEMS Microbiol Ecol 2012;80:659–70. https://doi.org/10.1111/j.1574-6941.2012.01335.x.

Teixeira L, Ferreira Á, Ashburner M. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. PLoS Biol 2008;6:e1000002. https://doi.org/10.1371/journal.pbio.1000002.

Teramoto T, Huang X, Armbruster PA, Padmanabhan R. Infection of *Aedes albopictus* mosquito C6/36 cells with the *w*Melpop strain of *Wolbachia* modulates dengue virus-induced host cellular transcripts and induces critical sequence alterations in the dengue viral genome. J Virol 2019;93:e00581-19. https://doi.org/10.1128/JVI.00581-19.

Tokuda G, Mikaelyan A, Fukui C, Matsuura Y, Watanabe H, Fujishima M, et al. Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. Proc Natl Acad Sci, USA 2018;115:E11996–2004. https://doi.org/10.1073/pnas.1810550115.

Tongluan N, Ramphan S, Wintachai P, Jaresitthikunchai J, Khongwichit S, Wikan N, et al. Involvement of fatty acid synthase in dengue virus infection. Virol J 2017;14:28. https://doi.org/10.1186/s12985-017-0685-9.

Tree MO, Londono-Renteria B, Troupin A, Clark KM, Colpitts TM, Conway MJ. Dengue virus reduces expression of low-density lipoprotein receptor-related protein 1 to facilitate replication in *Aedes aegypti*. Sci Rep 2019;9:6352. https://doi.org/10.1038/s41598-019-42803-9.

Vial T, Marti G, Missé D, Pompon J. Lipid interactions between flaviviruses and mosquito vectors. Front Physiol 2021;12:763195. https://doi.org/10.3389/fphys.2021.763195.

Vial T, Tan W-L, Deharo E, Missé D, Marti G, Pompon J. Mosquito metabolomics reveal that dengue virus replication requires phospholipid reconfiguration via the remodeling cycle. Proc Natl Acad Sci, USA 2020;117:27627–36. https://doi.org/10.1073/pnas.2015095117.

Vial T, Tan W-L, Wong Wei Xiang B, Missé D, Deharo E, Marti G, et al. Dengue virus reduces AGPAT1 expression to alter phospholipids and enhance infection in *Aedes aegypti*. PLoS Pathog 2019;15:e1008199. https://doi.org/10.1371/journal.ppat.1008199.

Visser B, Le Lann C, Den Blanken FJ, Harvey JA, van Alphen JJM, Ellers J. Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. Proc Natl Acad Sci, USA 2010;107:8677–82. https://doi.org/10.1073/pnas.1001744107.

Visser B, Le Lann C, Hahn DA, Lammers M, Nieberding CM, Alborn HT, et al. Many parasitoids lack adult fat accumulation, despite fatty acid synthesis: A discussion of concepts and considerations for future research. Curr Res Insect Sci 2023;3:100055. https://doi.org/10.1016/j.cris.2023.100055.

Wagar ZL, Tree MO, Mpoy MC, Conway MJ. Low density lipopolyprotein inhibits flavivirus acquisition in *Aedes aegypti*. Insect Mol Biol 2017;26:734–42. https://doi.org/10.1111/imb.12334.

Wang L, Wang S, Zhang Q, He C, Fu C, Wei Q. The role of the gut microbiota in health and cardiovascular diseases. Mol Biomed 2022;3:30. https://doi.org/10.1186/s43556-022-00091-2.

Watarai M, Makino S, Michikawa M, Yanagisawa K, Murakami S, Shirahata T. Macrophage plasma membrane cholesterol contributes to *Brucella abortus* infection of mice. Infect Immun 2002;70:4818–25. https://doi.org/10.1128/IAI.70.9.4818-4825.2002.

Weinert LA, Araujo-Jnr E V, Ahmed MZ, Welch JJ. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc R Soc Lond B Biol Sci 2015;282:20150249. https://doi.org/10.1098/rspb.2015.0249.

Wernegreen JJ. Genome evolution in bacterial endosymbionts of insects. Nat Rev Genet 2002;3:850–61. https://doi.org/10.1038/nrg931.

Wimalasiri-Yapa BMCR, Huang B, Ross PA, Hoffmann AA, Ritchie SA, Frentiu FD, et al. Differences in gene expression in field populations of *Wolbachia*-infected *Aedes aegypti* mosquitoes with varying release histories in northern Australia. PLoS Negl Trop Dis 2023;17:e0011222. https://doi.org/10.1371/journal.pntd.0011222.

Wong CAN, Dobson AJ, Douglas AE. Gut microbiota dictates the metabolic response of *Drosophila* to diet. J Exp Biol 2014;217:1894–901. https://doi.org/10.1242/jeb.101725.

Wong CNA, Ng P, Douglas AE. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. Environ Microbiol 2011;13:1889–900. https://doi.org/10.1111/j.1462-2920.2011.02511.x.

Wrońska AK, Kaczmarek A, Boguś MI, Kuna A. Lipids as a key element of insect defense systems. Front Genet 2023;14:1183659. https://doi.org/10.3389/fgene.2023.1183659.

Wu M, Sun L V, Vamathevan J, Riegler M, Deboy R, Brownlie JC, et al. Phylogenomics of the reproductive parasite *Wolbachia pipientis w*Mel: A streamlined genome overrun by mobile genetic elements. PLoS Biol 2004;2:E69. https://doi.org/10.1371/journal.pbio.0020069.

Wu W, Shan H-W, Li J-M, Zhang C-X, Chen J-P, Mao Q. Roles of bacterial symbionts in transmission of plant virus by hemipteran vectors. Front Microbiol 2022;13:805352. https://doi.org/10.3389/fmicb.2022.805352.

Xie J, Cai Z, Zheng W, Zhang H. Integrated analysis of miRNA and mRNA expression profiles in response to gut microbiota depletion in the abdomens of female *Bactrocera dorsalis*. Insect Sci 2023;30:443–58. https://doi.org/10.1111/1744-7917.13091.

Xu Z, Jiang W, Huang W, Lin Y, Chan FKL, Ng SC. Gut microbiota in patients with obesity and metabolic disorders — a systematic review. Genes Nutr 2022;17:2. https://doi.org/10.1186/s12263-021-00703-6.

Zhang FQ, McMullen JG, Douglas AE, Ankrah NYD. Succinate: A microbial product that modulates *Drosophila* nutritional physiology. Insect Sci 2022;29:315–8. https://doi.org/10.1111/1744-7917.12905.

Zhang H-B, Cao Z, Qiao J-X, Zhong Z-Q, Pan C-C, Liu C, et al. Metabolomics provide new insights into mechanisms of *Wolbachia*-induced paternal defects in *Drosophila melanogaster*. PLoS Pathog 2021;17:e1009859. https://doi.org/10.1371/journal.ppat.1009859.

Zhang T, Feng W, Ye J, Li Z, Zhou G. Metabolomic changes in *Sogatella furcifera* under Southern rice black-streaked dwarf virus infection and temperature stress. Viruses 2018;10:344. https://doi.org/10.3390/v10070344.

Zhang X, Zhang F, Lu X. Diversity and functional roles of the gut microbiota in lepidopteran insects. Microorganisms 2022;10:1234. https://doi.org/10.3390/microorganisms10061234.

1346 Zheng Y, Wang J-L, Liu C, Wang C-P, Walker T, Wang Y-F. Differentially expressed profiles
1347 in the larval testes of *Wolbachia* infected and uninfected *Drosophila*. BMC Genomics
1348 2011;12:595. https://doi.org/10.1186/1471-2164-12-595.

1349 Zhou X, Ling X, Guo H, Zhu-Salzman K, Ge F, Sun Y. *Serratia symbiotica* enhances fatty acid
1350 metabolism of pea aphid to promote host development. Int J Mol Sci 2021;22:5951.
1351 https://doi.org/10.3390/ijms22115951.

1352 Zug R, Hammerstein P. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that
1353 40% of terrestrial arthropod species are infected. PLoS One 2012;7:e38544.
1354 https://doi.org/10.1371/journal.pone.0038544.

1355