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Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses

Eric P Caragata, Chinmay V Tikhe and George Dimopoulos

Mosquitoes naturally harbor a diverse community of microorganisms that play a crucial role in their biology. Mosquito–microbiota interactions are abundant and complex. They can dramatically alter the mosquito immune response, and impede or enhance a mosquito's ability to transmit medically important arboviral pathogens. Yet critically, given the massive public health impact of arboviral disease, few such interactions have been well characterized. In this review, we describe the current state of knowledge of the role of microorganisms in mosquito biology, how microbial-induced changes to mosquito immunity moderate infection with arboviruses, cases of mosquito–microbial–virus interactions with a defined mechanism, and the molecular interactions that underlie the endosymbiotic bacterium *Wolbachia*'s ability to block virus infection in mosquitoes.

Address
W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States

Corresponding author: Dimopoulos, George (gdimopo1@jhu.edu)

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Introduction

Although small in size, mosquitoes are incredibly deadly animals capable of transmitting many disease-causing pathogens to humans. Mosquito-transmitted viral diseases, including dengue (DENV), chikungunya (CHIKV), Japanese encephalitis (JEV), West Nile (WNV), yellow fever (YFV), and Zika (ZIKV), are a serious public health problem, causing millions of infections per year [1–3]. Infection can have severe consequences including chronic symptoms, developmental disorders, and death [4–6]. These arthropod-borne viruses (arboviruses) exploit a mosquito's need to feed on vertebrate blood in order to produce eggs. They are ingested when a mosquito feeds on the blood of an infected person. They invade and replicate within mosquito cells, suppress, and hide from the sophisticated mosquito innate immune system [7,8], and are eventually passed to a new human host during subsequent blood feeding. Key vector species such as *Aedes aegypti* and *Aedes albopictus* have extensive geographic distributions, and can transmit multiple viruses [9,10].

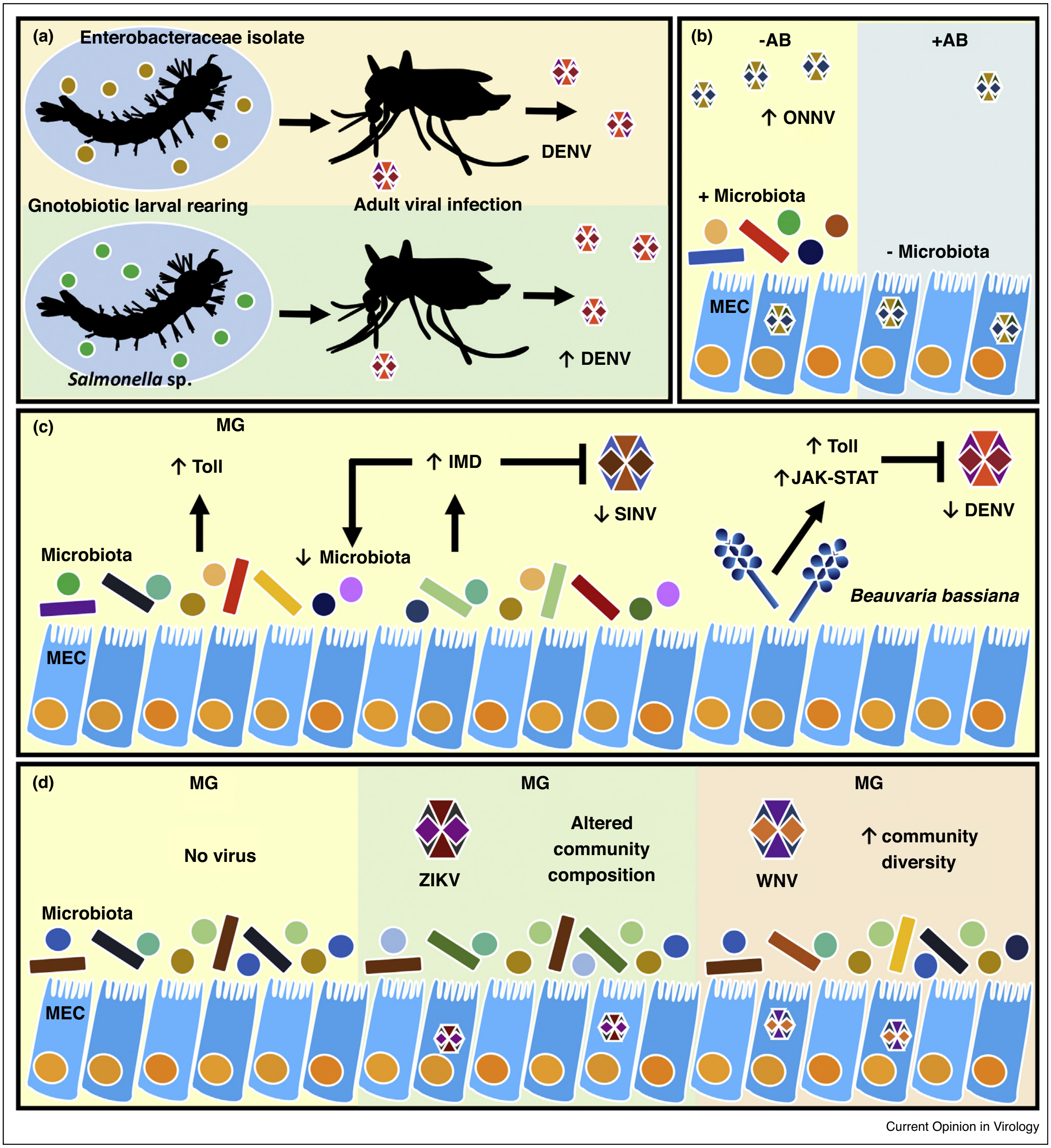
One essential component of mosquito–virus interactions is often overlooked: the microbiota. A mosquito is naturally host to a community of diverse bacteria, fungi, parasites, and viruses with most dwelling within the gut [11]. Community composition is dynamic, highly variable between individuals, and greatly influenced by environmental factors [12,13]. Some interactions occur transiently, some persist for the mosquito's life [14], while others form long-term relationships and are passed to subsequent generations. Some community members are beneficial to the host, while others are opportunistic, pathogenic, or even lethal [15]. Critically, host–microbe interactions can moderate the mosquito immune system, and influence a mosquito's ability to transmit medically important viruses (Figure 1). In this review, we describe the curious entanglement of mosquito–microbiota–virus interactions, and their contribution to arboviral disease.

The role of the microbiota in mosquito biology

Mosquito larvae are aquatic and acquire most of their bacterial community members from their breeding site water [16], although only certain bacterial taxa found in the aquatic environment can colonize the larval gut [16,17]. Other select bacteria, including *Asaia*, *Serratia*, and *Elizabethkingia*, can be passed from mother mosquito to larva by attaching to the egg surface [18–20].

In larvae, the presence of live bacteria, or live eukaryotic cells may be essential for development to adulthood [16,21], potentially because these organisms induce hypoxia in the larval gut [22]. However, a recent study has demonstrated that larvae can develop to adulthood without live bacteria or eukaryotes when their diet meets certain nutritional requirements [23]. In the laboratory, larvae can be developed gnotobiotically, reared in association with a solitary bacterial taxon. Developmental success in these cases depends on the bacterium, suggesting that some bacteria are more useful for mosquito development than others [16,24]. Further elucidating the role of microorganisms in larval development is an interesting question for future research.

Figure 1



Key types of interactions between mosquitoes, their microbiota, and arboviruses.
(a) The larval microbiota can influence adult vector competence. Rearing *Ae. aegypti* larvae gnotobiotically with an Enterobacteraceae isolate or a *Salmonella* sp. differentially affects susceptibility to DENV infection in adults. **(b)** The presence of the gut microbiota can influence viral infection. *An. gambiae* where the microbiota has been cleared by antibiotic treatment (+AB) are less effective hosts for ONNV than those with their natural microbiota (−AB). **(c)** The microbiota stimulates the mosquito immune system and moderates arboviral infection. The gut microbiota induces Toll and IMD pathway activity, the latter hinders SINV infection, and prevents excess microbial proliferation. The fungus *B. bassiana* activates the Toll and JAK-STAT pathways thereby restricting DENV infection. **(d)** Infection with an arbovirus, including ZIKV and WNV, can alter the composition of the microbiota. Abbreviations: −AB: without antibiotics, +AB: with antibiotics, DENV: Dengue virus, MEC: midgut epithelial cells, MG: midgut, ONNV: O'Nyong-nyong virus, SINV: Sindbis virus, WNV: West Nile virus, ZIKV: Zika virus.

The larval microbiota can influence immune response and vector competence in adult mosquitoes. For instance, bacteria can 'prime' the immune system, generating an improved immune response upon subsequent exposure to a pathogen [25]. In *Aedes* mosquitoes, sublethal exposure of larvae to *Escherichia coli* stimulates production of key immune factors including nitric oxide and antimicrobial peptides (AMPs), and provides better protection during later infection [26].

There have been several examples of larval microbiota influencing adult response to arboviral infection. *Ae. aegypti* larvae reared gnotobiotically with an Enterobacteriaceae isolate experience reduced DENV load in adults compared to those gnotobiotically reared with *Salmonella* sp. [27]. Sublethal exposure to the pathogenic bacterium *Bacillus thuringiensis* subsp. israelensis (*Bti*) in *Aedes* larvae that were genetically resistant to *Bti* increases adult susceptibility to DENV, but not CHIKV [28]. Interestingly, *Bti* infection can alter the larval community composition [29], and this was hypothesized as a potential factor in the differential susceptibility to DENV [28].

These findings suggest that the larval microbiota play a critical role in mosquito life history, and response to infection. Future studies should focus on better characterizing host–microbiota interactions at this stage, particularly those that influence host immunity.

The larval microbiota and breeding environment have a major influence on the composition of the adult mosquito microbiota. The adult community is generally considered to be less diverse than the larval community [30,31], as only select taxa are transmitted directly from larva to pupa to adult [16,18,19,24,32]. Many microorganisms are lost during metamorphosis, but some are reacquired from the larval breeding site as newly eclosed adults come into contact with, or drink from, the water. Adult community composition and diversity is strongly influenced by geography [11], and breeding site type [33,34]. However, in spite of these differences, there appears to be a 'core' microbiota—a collection of key bacterial taxa that commonly infect different mosquito species [13,35], although the specific roles of these taxa remain unclear.

The adult microbiota is not necessary for mosquito survival, but they do influence many critical life-history traits [24,36–38]. The community is particularly responsive to blood feeding and digestion. Blood intake initiates the proliferation of a select few taxa, leading to an increase in the total number of bacteria in the community, but a decline in overall diversity [39]. Clearing the community with antibiotics impairs blood digestion, and because of reduced availability of nutrients that promote egg development, also reduces fecundity [36,38]. Community composition influences mating preference, as seen in transgenic *Anopheles stephensi* that overexpress key immune genes. These mosquitoes have increased resistance to *Plasmodium* infection, but this increased immune activity alters community composition and reduces the overall number of bacteria, which, serendipitously, makes wild-type females more attractive mates to transgenic males [37]. That such dramatic effects result from changes to the community highlights the critical role it plays in mosquito biology.

Interactions between the microbiota and the mosquito immune system

The mosquito innate immune system primarily utilizes four innate immune pathways: the siRNA, JAK-STAT, Toll, and IMD pathways to restrict infection with bacterial, protozoal, fungal, and viral pathogens [40]. Each pathway is activated in response to pathogen-specific pattern recognition cues, meaning that certain pathways deal with certain pathogens but not others. Critically, the presence of the microbial community induces several of these pathways, and this can indirectly moderate the host response to arbovirus infection.

The Toll, IMD, and JAK-STAT pathways all involve signaling cascades that result in the production of a range of different anti-pathogen effector molecules. The Toll pathway plays an important role in protecting the host against fungal, protozoal, viral, and bacterial pathogens [41–44]. IMD pathway activity has been linked to infection with bacteria, *Plasmodium* parasites and some viruses [45–47]. While the JAK-STAT pathway is a key part of the mosquito antiviral defense, and is induced during DENV and ZIKV infection [48,49].

The gut microbiota can activate host immune pathways, thereby indirectly influencing arboviral infection [44,45]. Toll pathway activity is decreased when the microbiota is removed using antibiotics, and this, in turn, makes antibiotic-treated mosquitoes less resistant to DENV infection [44]. Similarly, blood meal-induced proliferation of the microbiota induces IMD pathway activity, which subsequently suppresses infection with Sindbis virus in *Ae. aegypti* [45]. This microbiota-mediated IMD pathway activity has also been implicated in defense against *Plasmodium* infection in anopheline mosquitoes [50]. JAK-STAT pathway activity is induced during bacterial infection in *Drosophila* and in *Anopheles* mosquitoes [51,52]. Similarly, infection with the entomopathogenic fungus *Beauveria bassiana* induces JAK-STAT and Toll pathway activity, thereby increasing host resistance to DENV [41]. However, there is still no clear link between the JAK-STAT pathway and the bacterial microbiota in *Aedes* mosquitoes, as neither silencing nor overexpression of pathway genes influenced host resistance to bacterial infection [49,53].

The small interfering RNA (or RNAi) pathway is primarily involved in response to viral infection, and results in the recognition and cleavage of viral RNA, thereby restricting viral replication [54]. There is currently no evidence showing that the RNAi pathway interacts with bacteria directly. However, there is evidence of RNAi-JAK-STAT pathway crosstalk, where WNV infection in *Culex pipiens* activates the RNAi pathway including the antiviral gene *Vago*, which in turn activates the JAK-STAT pathway [55]. Although, it is unclear if this interaction affects the microbiota.

The microbiota interacts with other immune processes in insects that are known to indirectly influence arbovirus infection. This includes oxidative stress, a key component of the immune system that occurs in response to excess production of reactive oxygen species (ROS) [56,57]. These particles can damage and kill host cells and microbiota. In fact, ROS production via the Mesh-DUOX pathway restricts excess bacterial proliferation [58]. Interestingly, heme released during the digestion of blood meal proteins reduces ROS and thereby allows bacterial proliferation in the gut [59]. Arbovirus infection in mosquito cells induces ROS [60], and higher ROS levels can induce Toll pathway activity [61]. But arboviruses can overcome ROS-mediated defenses, as seen with ZIKV, where glycosylation of the envelope protein reduces ROS levels, and facilitates invasion of the midgut epithelial cells [62]. Given both virus and the microbiota are ROS-responsive, this is an interesting direction for future research.

Mosquitoes produce a variety of C-type lectins (CTLs), proteins that bind different carbohydrates, which can have a broad range of immune roles. Some CTLs protect mosquitoes against bacterial infection [63], while others facilitate infection with *Plasmodium* and fungal pathogens [64–66]. Other CTLs are induced during infection with DENV, JEV, and WNV, and these promote viral infection [67–69]. Mosquito gut bacteria also induce the production of CTLs, which bind to their cell walls and protect them from host AMPs [70]. It is currently unclear if any individual CTLs interact with both the microbiota and arboviruses.

Further interesting interactions involve the MAPK/ERK pathway, a nutritionally responsive kinase signaling cascade that restricts arboviral entry to cells, and hinders the course of infection within cells [71]. In *Drosophila*, MAPK/ERK is activated by microbiota-induced induction of NF-κB immune signaling [72]. The high degree of MAPK/ERK pathway conservation between *Drosophila* and *Aedes* mosquitoes [71] suggests it may be involved in microbiota–arbovirus–mosquito interactions.

Interactions between microbiota and arboviruses

There is a growing body of evidence suggesting that microbiota–arbovirus interactions are important to virus infection and transmission. Several studies have demonstrated that the presence of a specific microorganism can alter mosquito vector competence [73,74]. These include examples of microorganisms that promote virus infection in mosquitoes. For instance, *An. gambiae* become less susceptible to O'Nyong'nyong virus infection when their microbiota is cleared via antibiotic treatment [75]. While in *Ae. aegypti*, infection with the mosquito-associated fungus *Talaromyces* sp. increases susceptibility to DENV by suppressing the expression of digestive enzymes [76].

Arbovirus infection can dramatically impact the microbiota. Community composition differs between DENV-susceptible and DENV-resistant *Ae. aegypti* populations [77,78], with this variation linked to population-specific differences in branched chain amino acid degradation [78]. Likewise, community composition varies depending on whether or not mosquitoes are from areas with endemic DENV transmission [79]. Infection with WNV in *Culex* mosquitoes increases bacterial diversity [80]. Infection with ZIKV alters the community profile in *Ae. aegypti* [81], while infection with CHIKV in *Ae. albopictus* increases the abundance of Enterobacteriaceae, but reduces the abundance of *Wolbachia* [82].

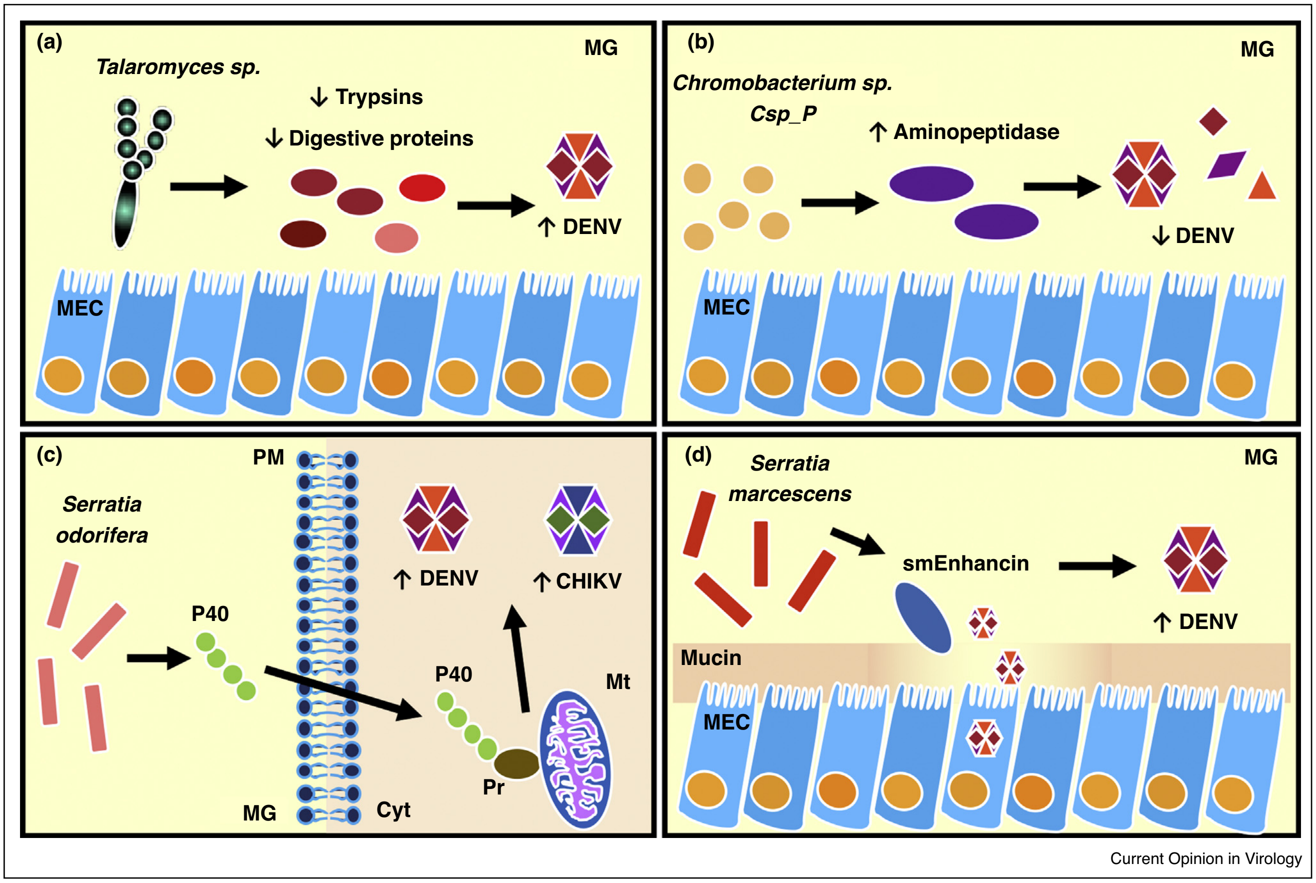
There are few characterized microbiota–arbovirus interactions with a defined mechanism (Figure 2). One example is *Chromobacterium* species Panama (*Csp_P*), a mosquito gut commensal bacterium that reduces DENV infection in *Ae. aegypti* [83] by producing an aminopeptidase that degrades the DENV envelope protein [84]. Interestingly, *Csp_P* also restricts infection with *Plasmodium falciparum* in *An. gambiae* by producing the antiparasitic protein Rhomidepsin [85].

The mosquito gut-associated bacterium, *Serratia odorifera*, enhances infection with either DENV or CHIKV in *Ae. aegypti* [86,87], with the effect attributed to a bacterial-encoded polypeptide, P40, that interacts with prohibitin [86], a protein linked to arboviral infection in mosquito cells [88]. Similarly, a *Serratia marcescens* isolate enhances DENV infection in *Ae. aegypti* by secreting a protein, smEnhancin, that digests the mucin layer of the mosquito midgut epithelium, rendering them more permissive to infection [89]. Interestingly, smEnhancin did not produce a similar effect with Sindbis virus in *Cx. pipiens* [89].

Mosquitoes, arboviruses and *Wolbachia*

There has been a great deal of research seeking to understand tripartite interactions between mosquitoes, arboviruses and the endosymbiotic bacterium *Wolbachia pipientis*. *Wolbachia* is a highly abundant, maternally transmitted symbiont found in at least 40% of all terrestrial insect species [90]. It naturally infects many important mosquito vectors including *Ae. albopictus*, *Cx. pipiens*, and *Culex quinquefasciatus*. Native infections have also been found at low prevalence in some populations of major

Figure 2



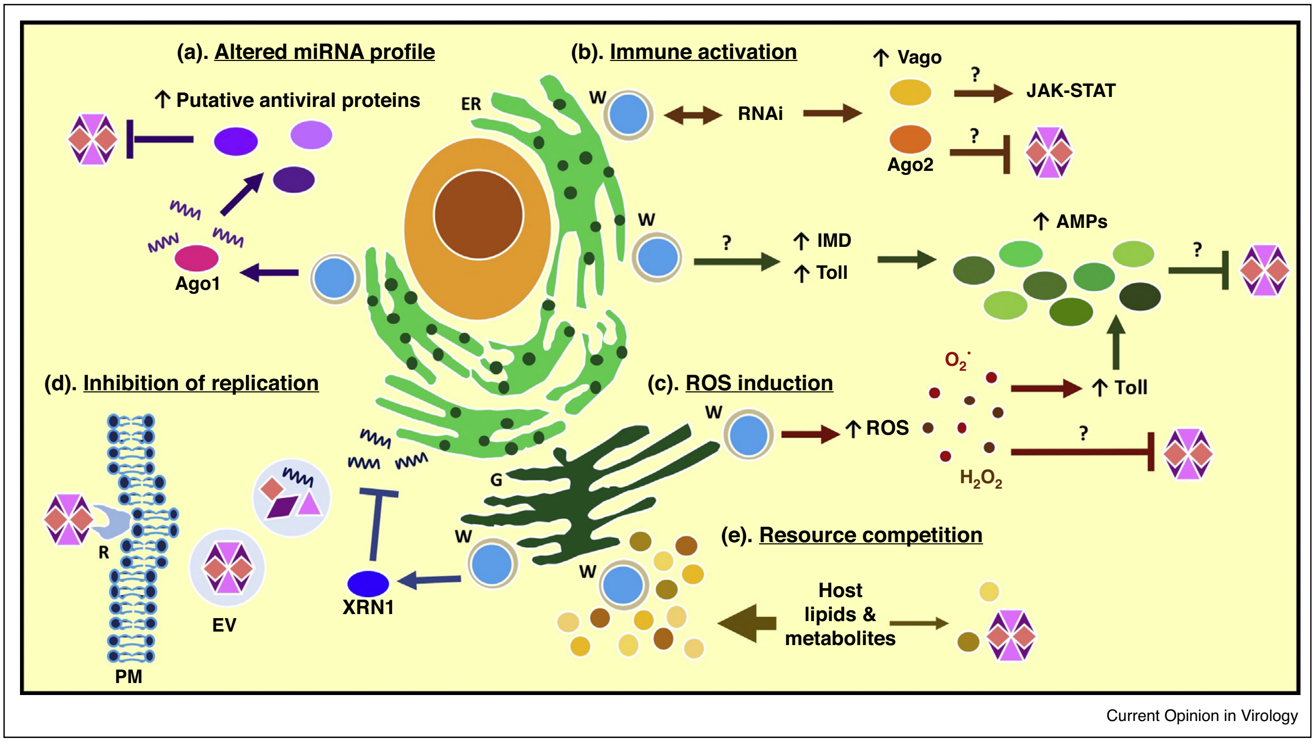
Mosquito–microbiota–virus interactions have diverse mechanisms.
There are few instances where the mechanisms used by microbiota to alter viral infection in mosquitoes have been identified. **(a)** In *Ae. aegypti*, the fungus *Talaromyces* sp. decreases host production of trypsin and other digestive proteins, which enhances DENV infection. **(b)** In *Ae. aegypti*, the bacterium *Chromobacterium* sp. Panama (*Csp_P*) restricts DENV infection by producing an aminopeptidase that digests the DENV envelope protein. **(c)** In *Ae. aegypti*, the bacterium *Serratia odorifera* produces the P40 polypeptide, which associates with the protein Prohibitin and enhances infection with DENV and CHIKV. **(d)** In *Ae. aegypti*, the bacterium *S. marcescens* produces the protein smEnhancin, which digests the mucin layer in the mosquito midgut, making invasion of the midgut epithelial cells easier for DENV. Abbreviations: CHIKV: Chikungunya virus, Cyt: Cytoplasm, DENV: Dengue virus, MEC: midgut epithelial cells, MG: midgut, Mt: Mitochondria, P40: Polypeptide P40, PM: Plasma membrane, Pr: Prohibitin.

malaria vectors [91–95]. Native *Wolbachia* infections typically occur at low bacterial density, and either have no effect on arbovirus infection [96,97], or a minor inhibitory effect [98].

Artificial *Wolbachia* infections in *Ae. aegypti* have been generated by embryonic microinjection [99–101]. These *Wolbachia* transinfections strongly inhibit infection, including by DENV [100,102], CHIKV [103], WNV [104], YFV [105], and ZIKV [106,107], and completely, or nearly completely, restrict viral transmission. *Wolbachia* also induces a reproductive manipulation known as cytoplasmic incompatibility that facilitates spread of the bacterium through wild populations [108–110]. Because of these properties, *Wolbachia*-infected mosquitoes are widely used as a mosquito biocontrol strategy, to generate mosquito populations with reduced transmission potential [111–114]. But critically, the exact nature of the mechanism of *Wolbachia*-mediated virus blocking remains unclear (Figure 3).

Studies implicate *Wolbachia* density, or bacterial load, as a major contributory factor in virus blocking at the organismal level. As *Wolbachia* strains that grow to higher overall density are more likely to induce blocking [100,115]. Density varies greatly between tissues, with the highest bacterial load occurring in reproductive tissues. However, blocking at the tissue level does not correlate with *Wolbachia* load [116], which suggests that the mechanism is somewhat independent of density.

Figure 3



Interactions putatively linked to *Wolbachia*-mediated arbovirus blocking.

Wolbachia are intracellular bacteria that dwell within membrane-bound vacuoles that associate with the ER and Golgi. Multiple *Wolbachia*–mosquito interactions have been implicated in *Wolbachia*'s ability to block arboviral infection: **(a)** *Wolbachia* interact with the miRNA pathway gene AGO1, altering the mosquito miRNA profile, which leads to the expression of antiviral genes. **(b)** *Wolbachia* stimulate different mosquito immune pathways, leading to the production of AMPs that target the virus. **(c)** *Wolbachia* cause the excess production of ROS, which stimulate the Toll pathway, and/or target the virus directly. **(d)** Viruses enter *Wolbcahia*-infected cells as normal via receptor mediated endocytosis, but *Wolbachia* restricts viral replication by promoting mosquito XRN1-mediated degradation of viral RNA. **(e)** *Wolbachia* preferentially uses mosquito resources, particularly lipids, that are required by the virus to infect the mosquito. Some or all of these processes, or other as-yet-unidentified processes may contribute to virus blocking. Abbreviations/Key: '?': It is unclear how or whether this interaction occurs, AMPs: Antimicrobial peptides, ER: Endoplasmic reticulum, EV: Endocytotic vesicle, G: Golgi apparatus, Nu: Nucleus, PM: Plasma membrane, R: Receptor mediating viral entry into cell, ROS: Reactive oxygen species, W: *Wolbachia*.

Like other bacteria, *Wolbachia* stimulates the mosquito immune system, including the Toll and IMD pathways [61,117], potentially priming the host against viral infection. Production of AMPs, including cecropins and defensins, is strongly induced, and blocking efficacy is reduced when the expression of these genes is silenced [61,117,118]. *Wolbachia* also induces Vago expression, suggesting potential interactions with both the RNAi and JAK/STAT pathways [119]. Furthermore, silencing of the RNAi pathway gene AGO2 also increases DENV load in *Wolbachia*-infected cells [120]. *Wolbachia* strongly induces production of ROS, and dysregulates both the prooxidant and antioxidant responses in mosquitoes [61,117]. Critically, neither immune activation nor ROS induction occur in all *Wolbachia*-infected insects where virus blocking is observed [121,122].

Wolbachia infection affects the expression of AGO1, a gene involved in producing micro RNAs (miRNAs) [123], and affects the expression of miRNAs that regulate the expression of DNA methyltransferase AaDmnt2, which appears to regulate both *Wolbachia* density and DENV infection [124]. Other *Wolbachia*-induced changes to miRNAs could feasibly influence host–virus interactions, and these should be investigated further. At the cellular level, studies have indicated that *Wolbachia* does not affect viral entry into mosquito cells, but inhibits viral replication in the cell, potentially by promoting XRN1 exoribonuclease-mediated degradation of viral RNA [125,126].

Blocking may involve *Wolbachia*-induced changes to mosquito metabolism, as both *Wolbachia* and viruses rely on mosquito resources. *Wolbachia* infection alters the expression of many metabolism genes [61,117], and alters host lipid profiles [127,128]. Likewise, DENV infection in *Ae. aegypti*, alters lipid profiles, increasing levels of fatty acyls, glycerophospholipids and sphingolipids [129]. There may also be a role for cholesterol, which is an important factor in arboviral infection [130,131]. *Wolbachia*-infected *Ae. aegypti* cells have altered cholesterol transport and accumulation, and blocking efficacy is decreased after treatment with cholesterol-depleting agents [132]. While in *Wolbachia*-infected *Drosophila*, increased dietary cholesterol intake also produced less effective blocking [133].

These studies suggest that the mechanism underlying *Wolbachia*–mosquito–arbovirus interactions is complex, and potentially involves multiple components, or even other unidentified factors. Resolving the mechanism may require a change of experimental approach: utilizing molecular genetics techniques to assess gene function, using multiple *Wolbachia* strain/mosquito/cell line combinations to search for commonalities, or experimental evolution to assess the response of viral genomes to the selection pressure induced by *Wolbachia*.

Conclusions

There is a clear need to improve our understanding of mosquito–microbiota interactions that moderate virus infection in mosquitoes, as these may have a substantial impact on disease transmission in the field. Many aspects of this area of research remain a mystery. We have far more knowledge about the role of the community as a whole, than we do about the roles of individual taxa. Likewise, there is little information on the role of mosquito-associated fungi on vector competence, as most studies have focused on bacteria. Few studies have looked at mosquito–microbiota–virus interaction in the field, which is critical as the microbiota of field and laboratory-reared mosquitoes can differ greatly. One of the most crucial future research directions will be to identify the mechanisms underlying these interactions. Expanding our knowledge of these subjects could lead to the development of new antiviral drugs, transmission blocking vaccines, or virus-resistant mosquito strains, and provide us with new or improved disease control strategies.

Conflict of interest statement

Nothing declared.

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References

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O *et al.*: **The global distribution and burden of dengue**. *Nature* 2013, **496**:504-507.
2. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E *et al.*: **Dengue: a continuing global threat**. *Nat Rev Microbiol* 2010, **8**:S7-S16.
3. WHO: **Vector-borne diseases**. *Fact Sheets*. 2017. October 31st.
4. Calvet G, Aguiar RS, Melo ASO, Sampaio SA, de Filippis I, Fabri A, Araujo ESM, de Sequeira PC, de Mendonca MCL, de Oliveira L *et al.*: **Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study**. *Lancet Infect Dis* 2016, **16**:653-660.
5. Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, Dub T, Baudouin L, Teissier A, Larre P *et al.*: **Guillain-Barre syndrome outbreak associated with Zika virus infection in French polynesia: a case-control study**. *Lancet* 2016, **387**:1531-1539.
6. Manimunda SP, Vijayachari P, Uppoor R, Sugunan AP, Singh SS, Rai SK, Sudeep AB, Muruganandam N, Chaitanya IK, Guruprasad DR: **Clinical progression of Chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging**. *Trans R Soc Trop Med Hyg* 2010, **104**:392-399.
7. Cheng G, Liu Y, Wang PH, Xiao XP: **Mosquito defense strategies against viral infection**. *Trends Parasitol* 2016, **32**:177-186.
8. Simões ML, Caragata EP, Dimopoulos G: **Diverse host and restriction factors regulate mosquito-pathogen interactions**. *Trends Parasitol* 2018, **34**:603-616.
9. Campbell LP, Luther C, Moo-Llanes D, Ramsey JM, Danis-Lozano R, Peterson AT: **Climate change influences on global distributions of dengue and Chikungunya virus vectors**. *Philos Trans R Soc Lond B Biol Sci* 2015, **370**.
10. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W *et al.*: **The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus***. *eLife* 2015, **4**:e08347.
11. Guégan M, Zouache K, Démichel C, Minard G, Van VT, Potier P, Mavingui P, Moro CV: **The mosquito holobiont: fresh insight into mosquito-microbiota interactions**. *Microbiome* 2018, **6**.
12. Akorli J, Gendrin M, Pels NAP, Yeboah-Manu D, Christophides GK, Wilson MD: **Seasonality and locality affect the diversity of *Anopheles gambiae* and *Anopheles coluzzii* midgut microbiota from Ghana**. *PLoS One* 2016, **11**.
13. Segata N, Baldini F, Pompon J, Garrett WS, Truong DT, Dabire RK, Diabate A, Levashina EA, Catteruccia F: **The reproductive tracts of two malaria vectors are populated by a core microbiome and by gender-and swarm-enriched microbial biomarkers**. *Sci Rep* 2016, **6**.
14. Gimonneau G, Tchioffo MT, Abate L, Boissiere A, Awono-Ambene PH, Nsango SE, Christen R, Morlais I: **Composition of *Anopheles coluzzii* and *Anopheles gambiae* microbiota from larval to adult stages**. *Infect Genet Evol* 2014, **28**:715-724.
15. Minard G, Mavingui P, Moro CV: **Diversity and function of bacterial microbiota in the mosquito holobiont**. *Parasites Vectors* 2013, **6**.
16. Coon KL, Vogel KJ, Brown MR, Strand MR: **Mosquitoes rely on their gut microbiota for development**. *Mol Ecol* 2014, **23**:2727-2739.
17. Coon KL, Brown MR, Strand MR: **Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats**. *Mol Ecol* 2016, **25**:5806-5826.
18. Chen SC, Bagdasarian M, Walker ED: ***Elizabethkingia anophelis*: molecular manipulation and interactions with mosquito hosts**. *Appl Environ Microbiol* 2015, **81**:2233-2243.
19. Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, Rizzi A, Urso R, Brusetti L, Borin S *et al.*: **Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector**. *Proc Natl Acad Scii U S A* 2007, **104**:9047-9051.
20. Wang SB, Dos-Santos ALA, Huang W, Liu KC, Oshaghi MA, Wei G, Agre P, Jacobs-Lorena M: **Driving mosquito refractoriness to *Plasmodium falciparum* with engineered symbiotic bacteria**. *Science* 2017, **357**:1399.
21. Valzania L, Martinson VG, Harrison RE, Boyd BM, Cooncurrency KL, Brown MR, Strand MR: **Both living bacteria**

and eukaryotes in the mosquito gut promote growth of larvae. *PLoS Negl Trop Dis* 2018, **12**.

22. Coon KL, Valzania L, McKinney DA, Vogel KJ, Brown MR, Strand MR: **Bacteria-mediated hypoxia functions as a signal for mosquito development**. *Proc Natl Acad Sci U S A* 2017, **114**: E5362-E5369.

23. Correa MA, Matusovsky B, Brackney DE, Steven B: **Generation of axenic *Aedes aegypti* demonstrate live bacteria are not required for mosquito development**. *Nat Commun* 2018, **9**.

24. Coon KL, Brown MR, Strand MR: **Gut bacteria differentially affect egg production in the anautogenous mosquito *Aedes aegypti* and facultatively autogenous mosquito *Aedes atropalpus* (Diptera: Culicidae)**. *Parasites Vectors* 2016, **9**.

25. Ramirez JL, Oliveira GD, Calvo E, Dalli J, Colas RA, Serhan CN, Ribeiro JM, Barillas-Mury C: **A mosquito lipoxin/lipocalin complex mediates innate immune priming in *Anopheles gambiae***. *Nat Commun* 2015, **6**.

26. Moreno-Garcia M, Vargas V, Ramirez-Bello I, Hernandez-Martinez G, Lanz-Mendoza H: **Bacterial exposure at the larval stage induced sexual immune dimorphism and priming in adult *Aedes aegypti* mosquitoes**. *PLoS One* 2015, **10**.

27. Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, Ghozlane A, Bouchier C, Ayala D, Paupy C, Moro CV *et al.*: **Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector**. *Sci Adv* 2017, **3**.

28. Moltini-Conclois I, Stalinski R, Tetreau G, Despres L, Lambrechts L: **Larval exposure to the bacterial insecticide *Bti* enhances dengue virus susceptibility of adult *Aedes aegypti* mosquitoes**. *Insects* 2018, **9**.

29. Tetreau G, Grizard S, Patil CD, Tran FH, Van VT, Stalinski R, Laporte F, Mavingui P, Despres L, Moro CV: **Bacterial microbiota of *Aedes aegypti* mosquito larvae is altered by intoxication with *Bacillus thuringiensis israelensis***. *Parasites Vectors* 2018, **11**.

30. Muturi EJ, Ramirez JL, Rooney AP, Kim CH: **Comparative analysis of gut microbiota of mosquito communities in central Illinois**. *PLoS Negle Trop Dis* 2017, **11**.

31. Wang XM, Liu T, Wu Y, Zhong DB, Zhou GF, Su XH, Xu JB, Sotero CF, Sadruddin AA, Wu K *et al.*: **Bacterial microbiota assemblage in *Aedes albopictus* mosquitoes and its impacts on larval development**. *Mol Ecol* 2018, **27**:2972-2985.

32. Chavshin AR, Oshaghi MA, Vatandoost H, Yakhchali B, Zarenejad F, Terenius O: **Malpighian tubules are important determinants of *Pseudomonas* transstadial transmission and longtime persistence in *Anopheles stephensi***. *Parasite Vectors* 2015, **8**.

33. Buck M, Nilsson LKJ, Brunius C, Dabire RK, Hopkins R, Terenius O: **Bacterial associations reveal spatial population dynamics in *Anopheles gambiae* mosquitoes**. *Sci Rep* 2016, **6**.

34. Yee DA, Allgood D, Kneitel JM, Kuehn KA: **Constitutive differences between natural and artificial container mosquito habitats: vector communities, resources, microorganisms, and habitat parameters**. *J Med Entomol* 2012, **49**:482-491.

35. Villegas LM, Pimenta PFP: **Metagenomics, paratransgenesis and the *Anopheles* microbiome: a portrait of the geographical distribution of the anopheline microbiota based on a meta-analysis of reported taxa**. *Memorias Do Instituto Oswaldo Cruz* 2014, **109**:672-U295.

36. Gaio AD, Gusmao DS, Santos AV, Berbert-Molina MA, Pimenta PFP, Lemos FJA: **Contribution of midgut bacteria to blood digestion and egg production in *Aedes aegypti* (Diptera: Culicidae) (L.)**. *Parasites Vectors* 2011, **4**.

37. Pike A, Dong YM, Dizaji NB, Gacita A, Mongodin EF, Dimopoulos G: **Changes in the microbiota cause genetically modified *Anopheles* to spread in a population**. *Science* 2017, **357**:1396-1399.

38. Sharma A, Dhayal D, Singh OP, Adak T, Bhatnagar RK: **Gut microbes influence fitness and malaria transmission potential of Asian malaria vector *Anopheles stephensi***. *Acta Trop* 2013, **128**:41-47.

39. Wang Y, Gilbreath TM, Kukutla P, Yan GY, Xu JN: **Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya**. *PLoS One* 2011, **6**.

40. Kumar A, Srivastava P, Sirisena PDNN, Dubey SK, Kumar R, Shrinet J, Sunil S: **Mosquito Innate Immunity**. *Insects* 2018, **9**.

41. Dong Y, Morton JC Jr, Ramirez JL, Souza-Neto JA, Dimopoulos G: **The entomopathogenic fungus *Beauveria bassiana* activate toll and JAK-STAT pathway-controlled effector genes and anti-dengue activity in *Aedes aegypti***. *Insect Biochem Mol Biol* 2012, **42**:126-132.

42. Garver LS, Dong Y, Dimopoulos G: **Caspar controls resistance to *Plasmodium falciparum* in diverse anopheline species**. *PLoS Pathog* 2009, **5**:e1000335.

43. Meister S, Kanzok SM, Zheng XL, Luna C, Li TR, Hoa NT, Clayton JR, White KP, Kafatos FC, Christophides GK *et al.*: **Immune signaling pathways regulating bacterial and malaria parasite infection of the mosquito *Anopheles gambiae***. *Proc Natl Acad Sci U S A* 2005, **102**:11420-11425.

44. Xi Z, Ramirez JL, Dimopoulos G: **The *Aedes aegypti* toll pathway controls dengue virus infection**. *PLoS Pathog* 2008, **4**: e1000098.

45. Barletta ABF, Nascimento-Silva MCL, Talyuli OAC, Oliveira JHM, Pereira LOR, Oliveira PL, Sorgine MHF: **Microbiota activates IMD pathway and limits Sindbis infection in *Aedes aegypti***. *Parasites Vectors* 2017, **10**.

46. Cooper DM, Chamberlain CM, Lowenberger C: ***Aedes* FADD: a novel death domain-containing protein required for antibacterial immunity in the yellow fever mosquito, *Aedes aegypti***. *Insect Biochem Mol Biol* 2009, **39**:47-54.

47. Garver LS, Bahia AC, Das S, Souza-Neto JA, Shiao J, Dong Y, Dimopoulos G: ***Anopheles* IMD pathway factors and effectors in infection intensity-dependent anti-*plasmodium* action**. *PLoS Pathog* 2012, **8**:e1002737.

48. 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.

49. Souza-Neto JA, Sim S, Dimopoulos G: **An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense**. *Proc Natl Acad Sci U S A* 2009, **106**:17841-17846.

50. Blumberg BJ, Trop S, Das S, Dimopoulos G: **Bacteria- and IMD pathway-independent immune defenses against *Plasmodium falciparum* in *Anopheles gambiae***. *PLoS One* 2013, **8**.

51. Barillas-Mury C, Han YS, Seeley D, Kafatos FC: ***Anopheles gambiae* Ag-STAT, a new insect member of the STAT family, is activated in response to bacterial infection**. *EMBO J* 1999, **18**:959-967.

52. Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B: ***Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation**. *Cell Host Microbe* 2009, **5**:200-211.

53. Jupatanakul N, Sim S, Anglero-Rodriguez YI, Souza-Neto J, Das S, Poti KE, Rossi SL, Bergren N, Vasilakis N, Dimopoulos G: **Engineered *Aedes aegypti* JAK/STAT pathway-mediated immunity to dengue virus**. *PLoS Negl Trop Dis* 2017, **11**: e0005187.

54. Adelman ZN, Blair CD, Carlson JO, Beaty BJ, Olson KE: **Sindbis virus-induced silencing of dengue viruses in mosquitoes**. *Insect Mol Biol* 2001, **10**:265-273.

55. Paradkar PN, Trinidad L, Voysey R, Duchemin JB, Walker PJ: **Secreted vago restricts West Nile virus infection in *Culex* mosquito cells by activating the Jak-STAT pathway**. *Proc Natl Acad Sci U S A* 2012, **109**:18915-18920.

56. Champion CJ, Xu JN: **The impact of metagenomic interplay on the mosquito redox homeostasis**. *Free Radic Biol Med* 2017, **105**:79-85.

57. Molina-Cruz A, DeJong RJ, Charles B, Gupta L, Kumar S, Jaramillo-Gutierrez G, Barillas-Mury C: **Reactive oxygen species modulate *Anopheles gambiae* immunity against bacteria and *Plasmodium***. *J Biol Chem* 2008, **283**:3217-3223.

58. Xiao XP, Yang LJ, Pang XJ, Zhang RD, Zhu YB, Wang PH, Gao GJ, Cheng G: **A Mesh-Duox pathway regulates homeostasis in the insect gut**. *Nat Microbiol* 2017, **2**.

59. Oliveira JH, Goncalves RL, Lara FA, Dias FA, Gandara AC, Menna-Barreto RF, Edwards MC, Laurindo FR, Silva-Neto MA, Sorgine MH *et al.*: **Blood meal-derived heme decreases ROS levels in the midgut of *Aedes aegypti* and allows proliferation of intestinal microbiota**. *PLoS Pathog* 2011, **7**:e1001320.

60. Chen TH, Chiang YH, Hou JN, Cheng CC, Sofiyatun E, Chiu CH, Chen WJ: **XBP1-mediated BiP/GRP78 upregulation copes with oxidative stress in mosquito cells during dengue 2 virus infection**. *Biomed Res Int* 2017, **17**:3519158 p.14 *https://www.hindawi.com/journals/bmri/2017/3519158/*.

61. Pan X, Zhou G, Wu J, Bian G, Lu P, Raikhel AS, Xi Z: ***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 U S A* 2012, **109**:E23-E31.

62. Wen D, Li SH, Dong FF, Zhang YA, Lin YF, Wang JM, Zou Z, Zheng AH: **N-glycosylation of viral E protein is the determinant for vector midgut invasion by flaviviruses**. *mBio* 2018, **9**.

63. Schnitger AKD, Yassine H, Kafatos FC, Osta MA: **Two C-type lectins cooperate to defend *Anopheles gambiae* against Gram-negative bacteria**. *J Biol Chem* 2009, **284**:17616-17624.

64. Christophides GK, Zdobnov E, Barillas-Mury C, Birney E, Blandin S, Blass C, Brey PT, Collins FH, Danielli A, Dimopoulos G *et al.*: **Immunity-related genes and gene families in *Anopheles gambiae***. *Science* 2002, **298**:159-165.

65. Osta MA, Christophides GK, Kafatos FC: **Effects of mosquito genes on *Plasmodium* development**. *Science* 2004, **303**:2030-2032.

66. Wang YH, Hu Y, Xing LS, Jiang H, Hu SN, Raikhel AS, Zou Z: **A critical role for CLSP2 in the modulation of antifungal immune response in mosquitoes**. *PLoS Pathog* 2015, **11**.

67. Cheng G, Cox J, Wang PH, Krishnan MN, Dai JF, Qian F, Anderson JF, Fikrig E: **A C-type lectin collaborates with a CD45 phosphatase homolog to facilitate West Nile virus infection of mosquitoes**. *Cell* 2010, **142**:714-725.

68. Liu K, Qian YJ, Jung YS, Zhou B, Cao RB, Shen T, Shao DH, Wei JC, Ma ZY, Chen PY *et al.*: **mosGCTL-7, a C-type lectin protein, mediates Japanese Encephalitis virus infection in mosquitoes**. *J Virol* 2017, **91**.

69. Liu Y, Zhang FC, Liu JY, Xiao XP, Zhang SY, Qin CF, Xiang Y, Wang PH, Cheng G: **Transmission-blocking antibodies against mosquito C-type lectins for dengue prevention**. *PLoS Pathog* 2014, **10**.

70. Pang XJ, Xiao XP, Liu Y, Zhang RD, Liu JY, Liu QY, Wang PH, Cheng G: **Mosquito C-type lectins maintain gut microbiome homeostasis**. *Nat Microbiol* 2016, **1**.

71. Xu J, Hopkins K, Sabin L, Yasunaga A, Subramanian H, Lamborn I, Gordesky-Gold B, Cherry S: **ERK signaling couples nutrient status to antiviral defense in the insect gut**. *Proc Natl Acad Sci U S A* 2013, **110**:15025-15030.

72. Sansone CL, Cohen J, Yasunaga A, Xu J, Osbom G, Subramanian H, Gold B, Buchon N, Cherry S: **Microbiota-dependent priming of antiviral intestinal immunity in *Drosophila***. *Cell Host Microbe* 2015, **18**:571-581.

73. Joyce JD, Nogueira JR, Bales AA, Pittman KE, Anderson JR: **Interactions between La Crosse virus and bacteria isolated from the digestive tract of *Aedes albopictus* (Diptera: Culicidae)**. *J Med Entomol* 2011, **48**:389-394.

74. Ramirez JL, Souza-Neto J, Torres Cosme R, Rovira J, Ortiz A, Pascale JM, Dimopoulos G: **Reciprocal tripartite interactions between the *Aedes aegypti* midgut microbiota, innate immune system and dengue virus influences vector competence**. *PLoS Negl Trop Dis* 2012, **6**:e1561.

75. Carissimo G, Pondeville E, McFarlane M, Dietrich I, Mitri C, Bischoff E, Antoniewski C, Bourgouin C, Failloux AB, Kohl A *et al.*: **Antiviral immunity of *Anopheles gambiae* is highly compartmentalized, with distinct roles for RNA interference and gut microbiota**. *Proc Natl Acad Sci U S A* 2015, **112**: E176-E185.

76. Angleró-Rodríguez YI, Talyuli OA, Blumberg BJ, Kang S, Demby C, Shields A, Carlson J, Jupatanakul N, Dimopoulos G: **An *Aedes aegypti*-associated fungus increases susceptibility to Dengue virus by modulating gut trypsin activity**. *eLife* 2017, **6**.

77. Charan SS, Pawar KD, Severson DW, Patole MS, Shouche YS: **Comparative analysis of midgut bacterial communities of *Aedes aegypti* mosquito strains varying in vector competence to dengue virus**. *Parasitol Res* 2013, **112**:2627-2637.

78. Short SM, Mongodin EF, MacLeod HJ, Talyuli OAC, Dimopoulos G: **Amino acid metabolic signaling influences *Aedes aegypti* midgut microbiome variability**. *PLoS Negl Trop Dis* 2017, **11**.

79. Charan SS, Pawar KD, Gavhale SD, Tikhe CV, Charan NS, Angel B, Joshi V, Patole MS, Shouche YS: **Comparative analysis of midgut bacterial communities in three aedine mosquito species from dengue-endemic and non-endemic areas of Rajasthan, India**. *Med Vet Entomol* 2016, **30**:264-277.

80. Zink SD, Van Slyke GA, Palumbo MJ, Kramer LD, Ciota AT: **Exposure to West Nile virus increases bacterial diversity and immune gene expression in *Culex pipiens***. *Viruses–Basel* 2015, **7**:5619-5631.

81. Villegas LEM, Campolina TB, Barnabe NR, Orfano AS, Chaves BA, Norris DE, Pimenta PFP, Secundino NFC: **Zika virus infection modulates the bacterial diversity associated with *Aedes aegypti* as revealed by metagenomic analysis**. *PLoS One* 2018, **13**.

82. Zouache K, Michelland RJ, Failloux AB, Grundmann GL, Mavingui P: **Chikungunya virus impacts the diversity of symbiotic bacteria in mosquito vector**. *Mol Ecol* 2012, **21**:2297-2309.

83. Ramirez JL, Short SM, Bahia AC, Saraiva RG, Dong Y, Kang S, Tripathi A, Mlambo G, Dimopoulos G: ***Chromobacterium Csp_P* reduces malaria and dengue infection in vector mosquitoes and has entomopathogenic and in vitro anti-pathogen activities**. *PLoS Pathog* 2014, **10**:e1004398.

84. Saraiva RG, Fang JR, Kang S, Anglero-Rodriguez YI, Dong YM, Dimopoulos G: **Aminopeptidase secreted by *Chromobacterium sp.* panama inhibits Dengue virus infection by degrading the E protein**. *PLoS Negl Trop Dis* 2018, **12**.

85. Saraiva RG, Huitt-Roehl CR, Tripathi A, Cheng YQ, Bosch J, Townsend CA, Dimopoulos G: ***Chromobacterium spp.* mediate their anti-*Plasmodium* activity through secretion of the histone deacetylase inhibitor romidepsin**. *Sci Rep* 2018, **8**.

86. Apte-Deshpande A, Paingankar M, Gokhale MD, Deobagkar DN: ***Serratia odorifera* a midgut inhabitant of *Aedes aegypti* mosquito enhances its susceptibility to dengue-2 virus**. *PLoS One* 2012, **7**.

87. Apte-Deshpande AD, Paingankar MS, Gokhale MD, Deobagkar DN: ***Serratia odorifera* mediated enhancement in susceptibility of *Aedes aegypti* for Chikungunya virus**. *Indian J Med Res* 2014, **139**:762-768.

88. Londono-Renteria B, Troupin A, Conway MJ, Vesely D, Ledizet M, Roundy CM, Cloherty E, Jameson S, Vanlandingham D, Higgs S *et al.*: **Dengue virus infection of *Aedes aegypti* requires a putative cysteine rich venom protein**. *PLoS Pathog* 2015, **11**.

89. Wu P, Sun P, Nie KX, Zhu YB, Shi MY, Xiao CG, Liu H, Liu QY, Zhao TY, Chen XG *et al.*: **A gut commensal bacterium promotes mosquito permissiveness to arboviruses**. *Cell Host Microbe* 2019, **25**:101.

90. Zug R, Hammerstein P: **Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected**. *PLoS One* 2012, **7**:e38544.

91. Baldini F, Rouge J, Kreppel K, Mkandawile G, Mapua SA, Sikulu-Lord M, Ferguson HM, Govella N, Okumu FO: **First report of**

natural *Wolbachia* infection in the malaria mosquito *Anopheles* arabiensis in Tanzania. *Parasites Vectors* 2018, **11**.

92. Gomes FM, Hixson BL, Tyner MDW, Ramirez JL, Canepa GE, Alves ESTL, Molina-Cruz A, Keita M, Kane F, Traore B *et al.*: **Effect of naturally occurring *Wolbachia* in *Anopheles gambiae s.l.* mosquitoes from Mali on *Plasmodium falciparum* malaria transmission**. *Proc Natl Acad Sci U S A* 2017, **114**:12566-12571.

93. Jeffries CL, Lawrence GG, Golovko G, Kristan M, Orsborne J, Spence K, Hurn E, Bandibabone J, Tantely LM, Raharimalala FN *et al.*: **Novel *Wolbachia* strains in *Anopheles* malaria vectors from Sub-Saharan Africa**. *Wellcome Open Res* 2018, **3**:113.

94. Niang EA, Bassene H, Makoundou P, Fenollar F, Weill M, Mediannikov O: **First report of natural *Wolbachia* infection in wild *Anopheles funestus* population in Senegal**. *Malaria J* 2018, **17**.

95. Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, Sawadogo SP, Dabire RK, Diabate A, Catteruccia F: ***Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development**. *Nat Commun* 2016, **7**.

96. Silva JBL, Alves DM, Bottino-Rojas V, Pereira TN, Sorgine MHF, Caragata EP, Moreira LA: ***Wolbachia* and dengue virus infection in the mosquito *Aedes fluviatilis* (Diptera: Culicidae)**. *PLoS One* 2017, **12**.

97. Skelton E, Rances E, Frentiu FD, Kusmintarsih ES, Iturbe-Ormaetxe I, Caragata EP, Woolfit M, O'Neill SL: **A native *Wolbachia* endosymbiont does not limit dengue virus infection in the mosquito *Aedes notoscriptus* (Diptera: Culicidae)**. *J Med Entomol* 2016, **53**:401-408.

98. Mousson L, Zouache K, Arias-Goeta C, Raquin V, Mavingui P, Failloux AB: **The native *Wolbachia* symbionts limit transmission of dengue virus in *Aedes albopictus***. *PLoS Negl Trop Dis* 2012, **6**:e1989.

99. McMeniman CJ, Lane RV, Cass BN, Fong AWC, Sidhu M, Wang YF, O'Neill SL: **Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti***. *Science* 2009, **323**:141-144.

100. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P *et al.*: **A non-virulent *Wolbachia* infection blocks dengue transmission and rapidly invades *Aedes aegypti* populations**. *Nature* 2011, **476**:450-455.

101. Xi Z, Khoo CC, Dobson SL: ***Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population**. *Science* 2005, **310**:326-328.

102. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu GJ, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M *et al.*: **A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium***. *Cell* 2009, **139**:1268-1278.

103. Aliota MT, Walker EC, Uribe Yepes A, Velez ID, Christensen BM, Osorio JE: **The *w*Mel Strain of *Wolbachia* reduces transmission of chikungunya virus in *Aedes aegypti***. *PLoS Negl Trop Dis* 2016, **10**:e0004677.

104. Hussain M, Lu G, Torres S, Edmonds JH, Kay BH, Khromykh AA, Asgari S: **Effect of *Wolbachia* on replication of West Nile virus in a mosquito cell line and adult mosquitoes**. *J Virol* 2013, **87**:851-858.

105. van den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, Day A, Higgs S, O'Neill SL: **Impact of *Wolbachia* on infection with chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti***. *PLoS Negl Trop Dis* 2012, **6**:e1892.

106. Aliota MT, Peinado SA, Velez ID, Osorio JE: **The *w*Mel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti***. *Sci Rep* 2016, **6**:28792.

107. Dutra HL, Rocha MN, Dias FB, Mansur SB, Caragata EP, Moreira LA: ***Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes**. *Cell Host Microbe* 2016, **19**:771-774.

108. Beckmann JF, Ronau JA, Hochstrasser M: **A *Wolbachia* deubiquitylating enzyme induces cytoplasmic Incompatibility**. *Nat Microbiol* 2017, **2**.

109. Hoffmann AA, Turelli M: **Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects**. *Genetics* 1988, **119**:435-444.

110. LePage DP, Metcalf JA, Bordenstein SR, On J, Perlmutter JI, Shropshire JD, Layton EM, Funkhouser-Jones LJ, Beckmann JF, Bordenstein SR: **Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility**. *Nature* 2017, **543**:243-247.

111. Garcia GD, Sylvestre G, Aguiar R, da Costa GB, Martins AJ, Lima JBP, Petersen MT, Lourenco-de-Oliveira R, Shadbolt MF, Rasic G *et al.*: **Matching the genetics of released and local *Aedes aegypti* populations is critical to assure *Wolbachia* invasion**. *PLoS Negl Trop Dis* 2019, **13**.

112. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y *et al.*: **Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission**. *Nature* 2011, **476**:454-U107.

113. O'Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, Dong Y, Kenny N, Paton CJ, Ritchie SA *et al.*: **Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses**. *Gates Open Res* 2018, **2**:36.

114. Schmidt TL, Barton NH, Rasic G, Turley AP, Montgomery BL, Iturbe-Ormaetxe I, Cook PE, Ryan PA, Ritchie SA, Hoffmann AA *et al.*: **Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti***. *PLoS Biol* 2017, **15**:e2001894.

115. Joubert DA, Walker T, Carrington LB, De Bruyne JT, Kien DH, Hoang Nle T, Chau NV, Iturbe-Ormaetxe I, Simmons CP, O'Neill SL: **Establishment of a *Wolbachia* superinfection in *Aedes aegypti* mosquitoes as a potential approach for future resistance management**. *PLoS Pathog* 2016, **12**:e1005434.

116. Amuzu HE, McGraw EA: ***Wolbachia*-based dengue virus inhibition is not tissue-specific in *Aedes aegypti***. *PLoS Negl Trop Dis* 2016, **10**.

117. 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.

118. Kambris Z, Cook PE, Phuc HK, Sinkins SP: **Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes**. *Science* 2009, **326**:134-136.

119. Asad S, Parry R, Asgari S: **Upregulation of *Aedes aegypti* Vago1 by *Wolbachia* and its effect on Dengue virus replication**. *Insect Biochem Mol Biol* 2018, **92**:45-52.

120. Terradas G, Joubert DA, McGraw EA: **The RNAi pathway plays a small part in *Wolbachia*-mediated blocking of dengue virus in mosquito cells**. *Sci Rep* 2017, **7**:43847.

121. Molloy JC, Sinkins SP: ***Wolbachia* do not induce reactive oxygen species-dependent immune pathway activation in *Aedes albopictus***. *Viruses* 2015, **7**:4624-4639.

122. Rancès E, Johnson TK, Popovici J, Iturbe-Ormaetxe I, Zakir T, Warr CG, O'Neill SL: **The toll and IMD pathways are not required for *Wolbachia*-mediated dengue virus interference**. *J Virol* 2013, **87**:11945-11949.

123. Hussain M, O'Neill SL, Asgari S: ***Wolbachia* interferes with the intracellular distribution of Argonaute 1 in the dengue vector *Aedes aegypti* by manipulating the host microRNAs**. *RNA Biol* 2013, **10**:1868-1875.

124. Zhang GM, Hussain M, O'Neill SL, Asgari S: ***Wolbachia* uses a host microRNA to regulate transcripts of a methyltransferase, contributing to dengue virus inhibition in *Aedes aegypti***. *Proc Natl Acad Sci U S A* 2013, **110**:10276-10281.

125. Rainey SM, Martinez J, McFarlane M, Juneja P, Sarkies P, Lulla A, Schnettler E, Varjak M, Merits A, Miska EA *et al.*: ***Wolbachia* blocks viral genome replication early in infection without a**

transcriptional response by the endosymbiont or host small RNA pathways. *PLoS Pathog* 2016, **12**.

126. Thomas S, Verma J, Woolfit M, O'Neilll SL: ***Wolbachia*-mediated virus blocking in mosquito cells is dependent on XRN1-mediated viral RNA degradation and influenced by viral replication rate**. *PLoS Pathogens* 2018, **14**.

127. Caragata EP, Rancès E, O'Neill SL, McGraw EA: **Competition for amino acids between *Wolbachia* and the mosquito host, *Aedes aegypti***. *Microb Ecol* 2014, **67**:205-218.

128. Molloy JC, Sommer U, Viant MR, Sinkins SP: ***Wolbachia* modulates lipid metabolism in *Aedes albopictus* mosquito cells**. *Appl Environ Microbiol* 2016, **82**:3109-3120.

129. Chotiwan N, Andre BG, Sanchez-Vargas I, Islam MN, Grabowskia JM, Hopf-Jannasch A, Gough E, Nakayasu E, Blair CD, Belisle JT *et al.*: **Dynamic remodeling of lipids coincides with dengue virus replication in the midgut of *Aedes aegypti* mosquitoes**. *PLoS Pathog* 2018, **14**.

130. Lee CJ, Lin HR, Liao CL, Lin YL: **Cholesterol effectively blocks entry of flavivirus**. *J Virol* 2008, **82**:6470-6480.

131. Mackenzie JM, Khromykh AA, Parton RG: **Cholesterol manipulation by West Nile virus perturbs the cellular immune response**. *Cell Host Microbe* 2007, **2**:229-239.

132. Geoghegan V, Stainton K, Rainey SM, Ant TH, Dowle AA, Larson T, Hester S, Charles PD, Thomas B, Sinkins SP: **Perturbed cholesterol and vesicular trafficking associated with dengue blocking in *Wolbachia*-infected *Aedes aegypti* cells**. *Nat Commun* 2017, **8**:526.

133. Caragata EP, Rancès E, Hedges LM, Gofton AW, Johnson KN, O'Neill SL, McGraw EA: **Dietary cholesterol modulates pathogen blocking by *Wolbachia***. *PLoS Pathog* 2013, **9**: e1003459.