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## Complex interactions in a host-symbiont-microbiome triangle

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# Chapter 2

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## Host, symbionts, and the microbiome: The missing tripartite interaction

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### Abstract

Symbiosis between microbial associates and a host is a ubiquitous feature of life on earth, modulating host phenotypes. In addition to endosymbionts, organisms harbour a collection of host-associated microbes, the microbiome that can impact important host traits. In this opinion we will argue that the mutual influences of the microbiome and endosymbionts, as well as their combined influence on the host are still understudied. Focussing on the endosymbiont *Wolbachia*, we will present growing evidence indicating that host phenotypic effects are exerted in interaction with the remainder microbiome and the host. We thus advocate that only through an integrated approach that considers multiple interacting partners and environmental influences, we will be able to gain a better understanding of host-microbe associations.

## Interacting entities

Arthropods commonly host a wide variety of microbes, some of which live within a host in a close and long-term biological interaction. Such endosymbionts can exert effects on the host ranging from positive (mutualistic, i.e. providing benefits; Brownlie and Johnson, 2009) to negative (parasitic, i.e. imposing substantial costs; Ivanov and Littman, 2011) interactions. Thus, endosymbionts are important modulators of host phenotypes, providing heritable variation upon which natural selection can act (Moran et al., 2008; Corbin et al., 2017).

### Highlights

- Microbial associates, symbionts and the microbial community – the microbiome – are important modulators of host phenotype, providing heritable variation upon which natural selection acts.
- Symbiont, host, microbiome and microbes in the environment interact with each other as part of a complex ecosystem.
- Microbe-microbe interactions between symbionts and the remainder microbiome but also between host-associated microbes and microbes in the environment are increasingly recognized as important driving forces in ecosystems.
- Therefore a more holistic approach, especially in symbiont research, is needed in order to understand how these interactions shape the phenotype of a host.

Historically, symbiosis research has focused on binary interactions between hosts and individual endosymbionts. In recent years this view was broadened to include all microbes that copiously colonize animals, the so-called microbiome, as they are additional important modulators of host traits (Box 1). Due to the historical focus on binary interactions comparatively little is known about interactions between microbes within the microbiome and how these interactions impact the host (Deines and Bosch, 2016). A more holistic approach towards the multitude of interactions is however needed for a better understanding of the varied mechanisms by which microbes drive animal health, development, and evolution (Petersen and Osvatic, 2018). This is especially true as symbionts are part of a complex ecosystem including host, symbiont, microbiome and their environment. Here symbionts, host and the remainder microbiome interact with each other, but are also influenced by free-living microbial communities and environmental conditions, e.g. temperature, diet, as well as other organisms (Box 2). Focusing only on one type of interaction, i.e. between host and symbiont or between host and the microbiome, under artificial conditions that do not reflect the potential influence of the environment (Box 3) will provide only an incomplete picture of host-microbe interactions.

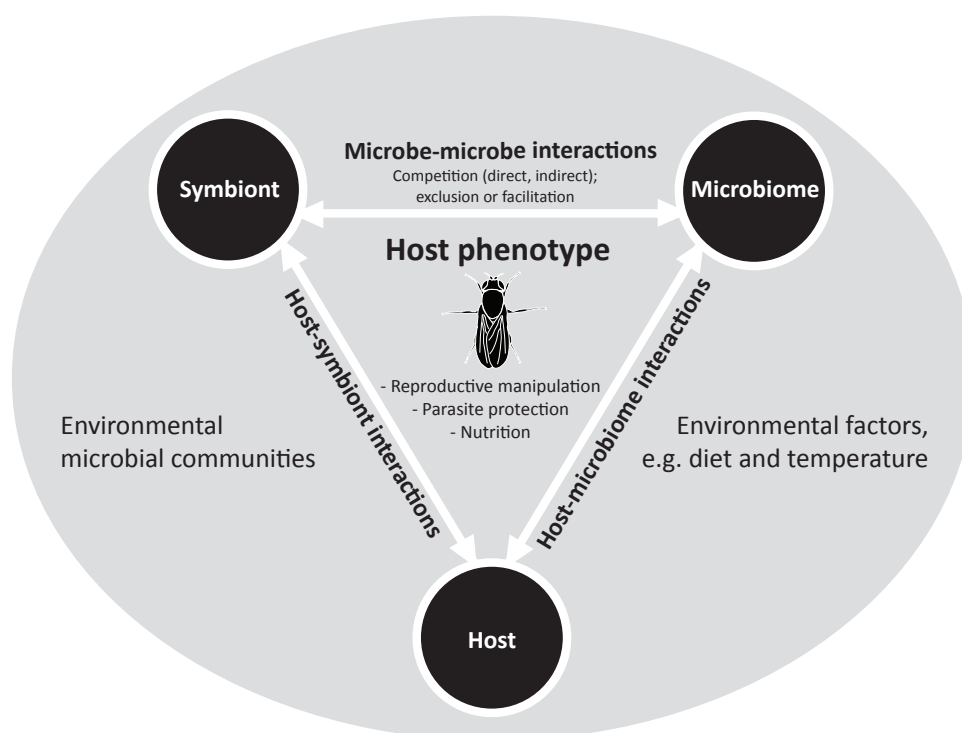
**Box 1: The host as an Ecosystem**

Historically, symbiosis research in insects has focused primarily on binary interactions between hosts and individual endosymbionts (Moran et al., 2008; Werren et al., 2008) and therefore observed phenotypic effects were attributed to the single symbiont. This binary point of view was challenged in recent years. The 'microbiome revolution' (Blaser, 2014) of the past 10 years revealed that all animals are copiously colonized by microorganisms, collectively called the microbiome, of which the symbionts are part. During this revolution it was realized that similar to single symbionts, the microbiome can also impact important host traits (Koch and Schmid-Hempel, 2012; Wong et al., 2014; Shropshire and Bordenstein, 2016) and thus influence the ecology and evolution of their hosts (McFall-Ngai et al., 2013), acting as an extended genome of the host, the hologenome (Zilber-Rosenberg and Rosenberg, 2008). Therefore, it has been proposed that the host itself should be viewed as a complex ecosystem, in which not only single symbionts interact with the host, but also the microbiome interacts with symbionts and hosts (McFall-Ngai et al., 2013; Sicard et al., 2014). An additional scale of complexity has recently gained attention, namely that host-microbe associations are also part of a wider microbial community maintained by transmission between individual hosts and dispersal between host-associated and free-living microbial communities (Dittmer et al., 2016; Adair and Douglas, 2017). In 2017, Carrier & Reitzel (Carrier and Reitzel, 2017) introduced the idea of a "host-associated microbial repertoire", which is the sum of microbial species a host may associate with over the course of its life under all encountered environmental circumstances. Due to this plasticity in the microbiome, the microbiome genome could allow hosts to adapt and survive under changing environmental conditions thus providing the time necessary for the host genome to adapt and evolve (Rosenberg and Zilber-Rosenberg, 2018). From this it becomes clear that one host can harbour a diverse microbial community, the entities of which interact with each other, potentially competing for space, energy, and resources, and ultimately influencing the condition of the host by conferring multiple detrimental, neutral, or beneficial effects (Bonnet et al., 2017). Therefore, a more holistic approach in studying the interaction between the different partners is needed.

In this opinion article, we argue that an important area for future research relies on disentangling how endosymbionts, the remainder microbiome and the host interact with each other and how their environment is shaping these interactions (Fig. 1). Note that we consider symbionts here as a separate microbial entity due to historical focus on binary interactions of symbiont and host. With this separation we can highlight the differences between both, but in fact the microbial symbionts are part of the microbiome and as such, should be studied together. We postulate that phenotypic effects of symbionts are modulated by other microbes, the host and the tripartite interaction between them. Drawing on the wealth of information on the endosymbiont *Wolbachia*, we will discuss studies that embraced this holistic view. We will argue for re-investigating well-known symbioses with respect to the interactions with other microbes, reviewing studies that indicate that symbioses are more influenced by other partners than the host itself. Throughout this opinion we will advocate the importance of this broader approach to gain a better understanding of symbionts and the role of the microbiome and the host in shaping host fitness.

### Symbionts, the sole manipulator?

In the last years researchers started to investigate microbe-symbiont interactions in model organisms such as the fruit fly *Drosophila melanogaster* (Simhadri et al., 2017), the parasitoid wasps *Nasonia* (Dittmer et al., 2016), and in mosquitoes as vectors of important human diseases (Novakova et al., 2017). Additionally, projects like the parasite-microbiome-project (Dheilly et al., 2017) started investigating microbiome dynamics within and across parasite-host interactions. Nevertheless, microbe-microbe interactions within a host, their influence on the host and how these interactions are influenced by the environment are still understudied (Deines and Bosch, 2016).



**Figure 1: Multipartite interactions affect the host phenotype.** Symbiosis between microbial associates and a host is a ubiquitous feature of life on earth, modulating host phenotypes (host-symbiont interactions). In addition to endosymbionts, organisms harbour a collection of host-associated microbes, the microbiome that can impact important host traits (host-microbiome interactions). These microbes interact with each other either direct via competition for resources and space, or indirect via the induction of a general immune response, potentially leading in changes of microbial community diversity, or abundance (microbe-microbe interactions). Therefore, a symbiont induced host phenotype, such as reproductive manipulation, parasite protection, or nutrition, is not only modulated by binary interactions, but also by a multitude of interaction between host, symbiont and the remainder microbiome, continuously influencing each other. Additionally, these interactions are influenced by their environment (grey circle), such as temperature, or diet, but by direct interaction with free living microbial communities. We thus advocate that only through an integrated approach that considers multiple interacting partners and environmental influences will we be able to gain a better understanding of host-microbe associations.

Symbionts and the remainder microbiome can influence each other and by doing so potentially shape their effects on host phenotype. For example the microbiome can be a

potential barrier to transmission of heritable symbionts through competitive exclusion of maternally inherited bacteria, as shown for the American dog tick *Dermacentor variabilis* (Macaluso et al., 2002) and the fruit fly *D. melanogaster* (Goto et al., 2006). On the other hand, symbionts and host can together control and shape the microbiome as shown in Lepidoptera (Johnston and Rolff, 2015), *D. melanogaster* (Simhadri et al., 2017; Ye et al., 2017) and the mosquito *Aedes aegypti* (Audsley et al., 2018).

**Box 2: Factors influencing host microbiome specificity**

Host-microbe interactions are shaped by a multitude of factors, i.e. factors associated with the host such as immunity (Chandler et al., 2011), phylogeny (Brooks et al., 2016), host population background (Chaplinska et al., 2016), physicochemical conditions in the insect habitat (e.g., gut pH, oxygen tensions), and structural filters in the gut (Engel and Moran, 2013) but also environmental factors such as diet (Colman et al., 2012) and temperature (Russell and Moran, 2006). Abiotic factors do have a crucial effect on microbes and the host, and therefore on their interactions. External environmental conditions significantly affect the infective states of hosts including the density of the endosymbionts inhabiting the host body, e.g. high temperature (van Opijnen and Breeuwer, 1999; Corbin et al., 2017; Sumi et al., 2017) with occurring seasonal changes of symbiont density such as *Wolbachia* (Cohen et al., 2015; Novakova et al., 2017; Sumi et al., 2017).

In addition, these associations are also part of a wider microbial community maintained by transmission between individual hosts and dispersal between host-associated and free-living microbial communities (Dittmer et al., 2016; Adair and Douglas, 2017; Carrier and Reitzel, 2017). While our understanding of the factors that affect the composition and abundance of the microbiome is expanding, there are still many unanswered questions regarding microbiome assembly and maintenance. Exposure to environmental microbes has undoubtedly a major influence on the microbial communities of an organism (Hegde et al., 2018), as metacommunity studies revealed that microbial communities associated with different interaction partners (species) differ in terms of composition and abundance, but shared microbes among the macro-partners (Bili et al., 2016; Pringle and Moreau, 2017). Unravelling the role of environment in shaping the host-associated microbiome (including symbionts) is crucial to place the specificity of interactions in an evolutionary context, for instance, by understanding whether deterministic processes lead to the selection of the horizontally transmitted microbes.

These interactions between the different members of the microbial community within a host can either be direct via competition for resources and space (Goto et al., 2006), or indirect via the induction of a general immune response (Koch and Schmid-Hempel, 2012; Abraham et al., 2017). A direct competition has been hypothesized for the protective phenotypes induced by the endosymbiont *Wolbachia* against pathogens in *Drosophila* and *Aedes*, resulting in abundance-dependent protection (Lu et al., 2012; Osborne et al., 2012). Competition for resources or space between *Wolbachia* and other bacteria is also likely for the terrestrial isopod *Armadillidium vulgare*. In this pill bug, total bacterial loads increase in

some, but not all tissues of *Wolbachia*-infected individuals (Dittmer et al., 2014) and *Wolbachia* presence decreases the abundance of bacterial phylotypes (Dittmer and Bouchon, 2018). Indirect interaction between the different members of the microbial community of a host has also been found. In bumble bees (*Bombus terrestris*), variation in gut microbiome seems to drive the general defence against parasites and the evolution of gut parasites by interactions with the remainder microbiome as well as with host genotypes (Koch and Schmid-Hempel, 2012). Similarly, in ticks (*Ixodes scapularis*), parasites induce the expression of specific glycoproteins, which alter the host microbiome to their advantage, i.e. to promote infection (Abraham et al., 2017). The exchange of genetic material between *Wolbachia* and other symbionts (likely *Cardinium* or *Rickettsia*) coinfecting the bedbug *Cimex lectularius*, have likely enabled *Wolbachia* to become an obligate, needed, symbiont providing B vitamins leading to a nutritional mutualism (Hosokawa et al., 2010; Nikoh et al., 2014). These studies highlight the complexity of the tripartite interaction between host species, microbiome and symbionts, across different hosts and foster the development of a framework in which interactions, host phenotype and environment are jointly explored.

### **The environmental component**

The environment influences microbes within a host and thus potentially their interactions as well as their effect on host phenotype. It is for example known that the abiotic environment, i.e. temperature, affects symbiont density (Hurst et al., 2000; Corbin et al., 2017). As example a reduction, or elimination of *Wolbachia* due to high temperature was found for *D. melanogaster*, mites, and other species (van Opijnen and Breeuwer, 1999; Hurst et al., 2000; Corbin et al., 2017; Sumi et al., 2017). In line with this, seasonal changes of *Wolbachia* density were observed in Lepidoptera (Sumi et al., 2017), mosquitoes (Novakova et al., 2017) and other blood sucking arthropods (Cohen et al., 2015). In mosquitoes, high temperature caused a reduction of *Wolbachia* density and a concomitant greater host susceptibility to viruses (Novakova et al., 2017). Also the biotic environment could potentially influence within-host microbe-microbe interactions through horizontal exchange of microbes from free-living microbial populations (Adair and Douglas, 2017) (Box 2). This could lead to microbial community shifts and therefore changes in microbe-microbe interactions potentially influencing host fitness. These interactions are defined by metacommunities, local communities linked by dispersal, but also extinction and recolonization of potentially interacting species (Leibold et al., 2004). Thus, local communities are influenced by processes operating at the level of the entire metacommunity (Sumi et al., 2017). The few studies that investigated the influence of metacommunities discovered that the microbial communities associated with different interaction partners, e.g. plants and insects, shared microbes (Bili et al., 2016; Pringle and Moreau, 2017), such as vertically transmitted symbionts from the genus *Wolbachia*, *Rickettsia*, and *Spiroplasma* (Bili et al., 2016).

As phenotypic host effects of a symbiosis are closely tied to interactions with the

remainder microbiome and environmental factors, biotic and abiotic, the absence of an integrative approach might mask the mechanistic interpretation of the data, leading to inconclusive results. Taking *Wolbachia* as model, in the next section we will provide a brief introduction of what is known for *Wolbachia*-microbe interactions and two examples in which a deeper understanding of the complex interactions between hosts, symbionts and the microbiome could explain discrepancies. The first one is of great relevance for human health and refers to investigations on the vector-competence of *Wolbachia* infected mosquitoes and their role in the transmission of human pathogens. The second example refers the reproductive manipulation by *Wolbachia* in several arthropod species and takes a more evolutionary perspective.

## **Interactions between the endosymbiont *Wolbachia* and other microbes**

### *Wolbachia in interaction: known facts*

The endosymbiont *Wolbachia* – one of the most widely distributed symbionts worldwide, infecting an estimated 40% of terrestrial arthropods (Zug and Hammerstein, 2012) – is a strong manipulator of a wide range of host traits (Werren et al., 2008). It gained specific interest due to its protection against various viruses in naturally infected fruit flies (Teixeira et al., 2008) and its capacity to reduce the density and transmission of pathogens in mosquito species (Moreira et al., 2009; Pereira et al., 2018).

In contrast to long-held beliefs, *Wolbachia* is not restricted to host germ line cells and reproductive organs, but is present in cells throughout somatic tissues and even in the gut lumen of some insects and their faeces (Sicard et al., 2014; Pietri et al., 2016). Thus, direct interactions with other microbes of the host or indirect interactions via the hosts' immune system are likely. Direct interactions between *Wolbachia* and other microbes have been observed in fruit flies. A co-infection with the endobacterium *Spiroplasma* reduced *Wolbachia* density while *Spiroplasma* numbers remained unaffected by the presence of *Wolbachia* (Goto et al., 2006). The investigation of the effect of *Wolbachia* infection on the composition of the gut microbiome in *D. melanogaster* showed an even more complex picture. Here the presence of *Wolbachia* is a significant determinant of the overall composition of the gut microbiome. Interestingly this was neither caused by a direct interaction between *Wolbachia* and the gut microbiome, as *Wolbachia* is absent from the gut lumen in the fly, nor was it indirectly modulated through the activation of the fly's immune system through *Wolbachia* (Simhadri et al., 2017). This highlights the importance of considering a multitude of possible interactions between microbes and between microbes and the host in studies of the dynamics and effects of *Wolbachia*-infections.



*The quest for disease eradication*

An integrated approach, considering multiple interactions between microbes and between microbes and hosts, is especially important when developing microbe-based disease vector control strategies. *Wolbachia* is currently being developed as a novel arthropod-borne disease control agent ([http:// www.eliminatedengue.com](http://www.eliminatedengue.com)); hereby the mode of a successful transmission is an important factor. Under laboratory conditions the native microbiome of *Anopheles* mosquitoes was found to impede vertical transmission of *Wolbachia* through antagonistic microbe-microbe interactions between the bacterium *Asaia* and *Wolbachia* (Hughes et al., 2014). Similar antagonistic microbe interactions were found in a survey of various mosquito species in Canada, with the presence and abundance of *Wolbachia* fluctuating over season, as well as with the presence of the bacteria *Asaia* and *Pseudomonas* (Novakova et al., 2017). This suggests that, in addition to environmental effects, interaction of *Wolbachia* with other microbes may explain some of the variation in vector competence of mosquitoes. In contrast to these results, a stable infection with *Wolbachia* in lab-reared mosquitoes *A. aegypti* had only few effects on the microbiome. Moreover, significant changes in the microbiome composition did not affect the dengue virus blocking phenotype caused by *Wolbachia* infection in this mosquito (Audsley et al., 2017). However, analyses of *A. aegypti* transinfected with *Wolbachia*, released in the field in Brazil and Vietnam to inhibit the dengue virus, revealed that *Wolbachia* increases susceptibility of mosquitoes to dengue infection. This contradicting result was due to the wide variability in exposure doses of *Wolbachia* naturally experienced by mosquitoes (King et al., 2018). The authors concluded that reliable predictions of vectorial capacity of transinfected mosquitoes require an informed account of mosquito pathogens and their interplay with *Wolbachia*. Additionally recent interaction networks, looking at co-occurrence and co-exclusion of microbes, established for several mosquito species (laboratory vs. field) revealed that *Wolbachia* is a highly interconnected taxon, mostly co-exclusionary with other bacteria (Hegde et al., 2018).

The mosquito studies indeed show that the abundance and effect of *Wolbachia* is closely tied to the remaining microbiome. This highlights the importance of considering the composition of the microbiome and host genetic background in studies investigating phenotypes induced by *Wolbachia* and when formulating microbe-based disease vector control strategies. In line with that, assessing the involvement of microbe-microbe interactions within a host and how they are influenced in the field, due to biotic or abiotic factors, is critical as it may affect the efficiency of *Wolbachia*-mediated manipulations (Box 2, Box 3).

**Box 3: Importance of laboratory versus field studies**

Given the strong influence of environmental factors (Box1, Box2) on host-microbe interactions, the transitions from laboratory studies to the field might bear some difficulty (Carrier and Reitzel, 2017). Laboratory settings potentially restrict the full spectrum of host-microbe-associations compared to the natural setting where these associations have evolved (Chandler et al., 2011). Thus, it may limit the interpretation of the functional roles microbes play in host biology (Carrier and Reitzel, 2017; Box2).

A good example for this is the *Wolbachia*-mediated inhibition of dengue virus. Under laboratory condition it was indicated that the microbiome composition of the mosquito *Aedes aegypti* is not critical for inhibition (Audsley et al., 2017). However, when released into the wild the picture became more complex. *Wolbachia*, when introgressed into different genetic backgrounds increased the mean and the variance in mosquito susceptibility to dengue infection (King et al., 2018). While the respective impacts of these factors are not easily disentangled, similarly complex multifactorial patterns likely underlie many host-microbe associations under ecologically realistic conditions. Given that *Wolbachia* appears to modify host susceptibility to a broad spectrum of pathogens (Teixeira et al., 2008; Martinez et al., 2014), reliable predictions of invasiveness and vectorial capacity of transinfected mosquitoes require an informed account of natural mosquito pathogens and their interplay with *Wolbachia* (King et al., 2018). A first step to get a more complete picture of the symbiotic effects of *Wolbachia* is to investigate these complex interactions simultaneously in the lab and field. In addition, a beneficial approach would be the mimicry of environmental factors, biotic and abiotic, in the lab. Microbiome and symbiont manipulation is often achieved through the use of antibiotics. However such approaches may have several overlooked side-effects. First, antibiotics may affect different components of the microbiome differently and hence alter the interaction networks. Second, results obtained in the absence of (parts of) the microbiome tell only one side of the story, as microbe-microbe interactions might modulate host response. Hence, in order to unravel the interactions and impact of host phenotype, host recolonization by a variety of well characterized microbes, or whole microbial communities, is required. Also the enrichment of the microbial diversity, mimicking possible biotic environmental influences such as transmission of free-living microbial communities, might be worthwhile. Although these manipulations can only provide a mechanistic understanding of the tripartite interaction and may not be translatable to field settings, they nevertheless are an important first step in gaining a better understanding of host-microbe interactions.

**The joint reproductive manipulation of *Wolbachia***

The endosymbiont *Wolbachia* is especially well known for its four distinct reproductive phenotypes, that promote its own vertical transmission from mother to offspring (Hurst and Frost, 2015). There is growing evidence that the reproductive manipulation by *Wolbachia* is not only exerted by the endosymbiont alone but in interaction with other microbes, i.e. the microbiome of the host, other symbionts, or the host itself. *Wolbachia* has repeatedly been

reported to cause different phenotypes, either in experimental settings when transferred between hosts, for example in Lepidoptera (Sasaki et al., 2002) or *Drosophila* sibling species (Jaenike, 2009), or naturally over evolutionary timescales, e.g. in moths and fruit flies (Ma et al., 2014). Additionally, many species show geographical variation in symbiont prevalence, including *Wolbachia* with a lower presence in warmer regions (Corbin et al., 2017), as for example reported for many species infected with parthenogenesis-inducing *Wolbachia* (Haag and Ebert, 2004). The causes for this distributional pattern in prevalence remain speculative, but a possible explanation is that it is driven by variation in microbial communities of host populations in interaction with their abiotic environment (Box 2).

Another line of evidence indicating a modulating role of the microbiome in reproductive manipulation by *Wolbachia* comes from studies investigating *Wolbachia* abundance (titre) in a host. The efficiency and phenotype of reproductive manipulation can depend on a threshold of *Wolbachia* titre, i.e. a minimum number of bacteria are required for exerting the manipulative action. A low titre can lead to a switch of the *Wolbachia*-induced phenotype in *Drosophila bifasciata* (Hurst et al., 2000), or to changes in the efficiency of parthenogenesis induction in the parasitic wasp *Asobara japonica* (Ma et al., 2015). In both studies, variation in the *Wolbachia* titre were manifested under identical rearing conditions, for the latter even in a clonal host reproduction system, suggesting a strong influence on *Wolbachia* titre by other partners, such as the microbiome.

Together, these examples illustrate that *Wolbachia* may be a potent manipulator of host reproduction, but not in isolation but rather in interaction with the host genome and the remainder of the microbiome and in addition influenced by interaction with the environment. By shifting the focus away from *Wolbachia* as the only manipulator it becomes clear that manipulation of a host phenotype is likely not only caused by a single microbe (*Wolbachia*), but also strongly influenced by interaction with other microbes, and by the host genotype itself.

### **Concluding remarks**

Throughout this manuscript we have pointed out growing evidence that host phenotypic effects such as reproductive manipulation by the endosymbiont *Wolbachia* are not only exerted by an endosymbiont alone but in interaction with others microbes. This and other examples, call for an integrative approach in studying host-microbe associations including host gene expression and interactions between microbes and environmental factors, on these interacting partners (see Outstanding Questions). The latter is especially important in the light of the upcoming challenges of our world, e.g. global warming and disease control. For instance, the protective effect of *Wolbachia* against important human diseases in insect vectors (Flores and O'Neill, 2018) is highly dependent upon temperature. Therefore global warming might cause a decrease in protective *Wolbachia*, undermining on-going long-term biological control programmes of mosquitoes. In this respect, a broader and more natural

approach in studying host associated microbes is needed, as laboratory studies might often not be directly translatable to the field (King et al., 2018) (Box 3). Although in this manuscript our focus is on the traits vector-competence and reproductive manipulation conferred by *Wolbachia* in arthropod associations, we would like to point out that an involvement of other host associated microbes is also likely for other traits conferred by *Wolbachia*. As an example, the nutritional symbiosis between *Wolbachia* and bedbugs showed that *Wolbachia*-microbe interactions, i.e. the complementation of functions by gene exchange between different components of the microbiome, can strongly influence the host phenotype through genetic changes in the symbiont (Hosokawa et al., 2010; Nikoh et al., 2014). As similar microbe-microbe interactions are not restricted to *Wolbachia* but also other symbionts, a holistic approach should be extended to all symbioses (Santos-Garcia et al., 2018).

**Outstanding Question:**

- Are interactions between single symbionts and the remainder of the microbiome or microbes in the environment the exception or the rule?
- Is the effect of microbe-microbe interactions, e.g. between the symbiont and members of the microbiome or between symbionts and the environmental microbial community on host phenotypes phylogenetically conserved?
- How stable is the host phenotype induced by a symbiont over time and season? Or does it fluctuate potentially indicating the influence of unknown environmental drivers?
- Are the observations between lab-based and field-based studies congruent? If not, which unknown factors influencing are driving the differences?

Finally, the interpretation of data on host-microbe associations has to be done carefully, keeping in mind that small changes in composition and/or abundance of the microbial community might have great phenotypic consequences for the host, as low-abundance or rare microbial taxa can represent hub species (Agler et al., 2016) that are crucial for the host functioning, as shown for plants and soil ecosystems (Jousset et al., 2017). Network analyses of the host-associated microbial communities might represent an important tool (Layeghifard et al., 2017) for basic insights into interaction dynamics within microbial communities. For instance, this approach has recently revealed that for several mosquito species (in the laboratory and field), *Wolbachia* is a highly interconnected taxon, being mostly negatively correlated with other bacteria (i.e. its abundance leads to a reduction in the abundance of other species)(Hegde et al., 2018). The integration of microbial network analyses with host gene expression networks could provide valuable insights into the complexity of the tripartite interactions.

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