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1 **Host, symbionts, and the microbiome: the missing tripartite interaction**

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3

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10

11 **Abstract:**

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

23

24 **Keywords:** Insects, Phenotype, *Wolbachia*, Mosquitoes, Metacommunity, *Drosophila*

25

26 **Interacting entities**

27 Arthropods commonly host a wide variety of **microbes** (see Glossary), some of which
28 live within a **host** in a close and long-term biological interaction. Such **endosymbionts**
29 can exert effects on the host ranging from positive (mutualistic, i.e. providing benefits
30 [1]) to negative (parasitic, i.e. imposing substantial costs [2]) interactions. Thus,
31 endosymbionts are important modulators of host **phenotypes**, providing heritable
32 variation upon which natural selection can act [3,4].

33 Historically, symbiosis research has focused on binary interactions between
34 hosts and individual endosymbionts. In recent years this view was broadened to
35 include all microbes that copiously colonize animals, the so-called **microbiome**, as
36 they are additional important modulators of host **traits** (Box 1). Due to the historical
37 focus on binary interactions comparatively little is known about interactions between
38 microbes within the microbiome and how these interactions impact the host [5]. A
39 more holistic approach towards the multitude of interactions is however needed for
40 a better understanding of the varied mechanisms by which microbes drive animal
41 health, development, and evolution [6]. This is especially true as **symbionts** are part
42 of a complex **ecosystem** including host, symbiont, microbiome and their environment.
43 Here symbionts, host and the **remainder microbiome** interact with each other, but
44 are also influenced by free-living microbial communities and environmental
45 conditions, e.g. temperature, diet, as well as other organisms (Box 2). Focusing only
46 on one type of interaction, i.e. between host and symbiont or between host and the
47 microbiome, under artificial conditions that do not reflect the potential influence of

48 the environment (Box 3) will provide only an incomplete picture of **host-microbe**
49 interactions.

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

65

66 **Symbionts, the sole manipulator?**

67 In the last years researchers started to investigate microbe-symbiont interactions in
68 model organisms such as the fruit fly *Drosophila melanogaster* [7], the parasitoid
69 wasps *Nasonia* [8], and in mosquitoes as vectors of important human diseases [9].
70 Additionally, projects like the parasite-microbiome-project [10] started investigating
71 microbiome dynamics within and across parasite-host interactions. Nevertheless,

72 microbe-microbe interactions within a host, their influence on the host and how these
73 interactions are influenced by the environment are still understudied [5].

74 Symbionts and the remainder microbiome can influence each other and by
75 doing so potentially shape their effects on host phenotype. For example the
76 microbiome can be a potential barrier to transmission of heritable symbionts through
77 competitive exclusion of maternally inherited bacteria, as shown for the American dog
78 tick *Dermacentor variabilis* [11] and the fruit fly *D. melanogaster* [12]. On the other
79 hand, symbionts and host can together control and shape the microbiome as shown
80 in Lepidoptera [13], *D. melanogaster* [7,14] and the mosquito *Aedes aegypti* [15].

81 These interactions between the different members of the microbial
82 community within a host can either be direct via competition for resources and space
83 [12], or indirect via the induction of a general immune response [16,17]. A direct
84 competition has been hypothesized for the protective phenotypes induced by the
85 endosymbiont *Wolbachia* against pathogens in *Drosophila* and *Aedes*, resulting in
86 abundance-dependent protection [18,19]. Competition for resources or space
87 between *Wolbachia* and other bacteria is also likely for the terrestrial isopod
88 *Armadillidium vulgare*. In this pill bug, total bacterial loads increase in some, but not
89 all tissues of *Wolbachia*-infected individuals [20] and *Wolbachia* presence decreases
90 the abundance of bacterial phylotypes [21]. Indirect interaction between the different
91 members of the microbial community of a host has also been found. In bumble bees
92 (*Bombus terrestris*), variation in gut microbiome seems to drive the general defence
93 against parasites and the evolution of gut parasites by interactions with the remainder
94 microbiome as well as with host genotypes [17]. Similarly, in ticks (*Ixodes scapularis*),
95 parasites induce the expression of specific glycoproteins, which alter the host

96 microbiome to their advantage, i.e. to promote infection [16]. For the nutritional
97 mutualisms between *Wolbachia* and the bedbug *Cimex lectularius* the exchange of
98 genetic material between *Wolbachia* and other symbionts (likely *Cardinium* or
99 *Rickettsia*) coinfecting the same insect host, have likely enabled *Wolbachia* to become
100 an obligate, needed, symbiont providing B vitamins for the nutrition of *Cimex*
101 *lectularius* [22,23]. These studies highlight the complexity of the tripartite interaction
102 between host species, microbiome and symbionts, across different hosts and foster
103 the development of a framework in which interactions, host phenotype and
104 environment are jointly explored.

105

106

107 **The environmental component**

108 The environment influences microbes within a host and thus potentially their
109 interactions as well as their effect on host phenotype. It is for example known that the
110 abiotic environment, i.e. temperature, affects symbiont density [4,24]. As example a
111 reduction, or elimination of *Wolbachia* due to high temperature was found for *D.*
112 *melanogaster*, mites, and other species [4,24–26]. In line with this, seasonal changes
113 of *Wolbachia* density were observed in Lepidoptera [26], mosquitoes [9] and other
114 blood sucking arthropods [27]. In mosquitoes, high temperature caused a reduction
115 of *Wolbachia* density and a concomitant greater host susceptibility to viruses [9]. Also
116 the biotic environment could potentially influence within-host microbe-microbe
117 interactions through **horizontal exchange** of microbes from free-living microbial
118 populations [28] (Box 2). This could lead to microbial community shifts and therefore
119 changes in microbe-microbe interactions potentially influencing host fitness. These

120 interactions are defined by **metacommunities**, local communities linked by dispersal,
121 but also extinction and recolonization of potentially interacting species [29]. Thus,
122 local communities are influenced by processes operating at the level of the entire
123 metacommunity [26]. The few studies that investigated the influence of
124 metacommunities discovered that the microbial communities associated with
125 different interaction partners, e.g. plants and insects, shared microbes [30,31], such
126 as **vertically transmitted** symbionts from the genus *Wolbachia*, *Rickettsia*, and
127 *Spiroplasma* [30].

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

139

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

141 *Wolbachia* in interaction: known facts

142 The endosymbiont *Wolbachia* – one of the most widely distributed symbionts
143 worldwide, infecting an estimated 40% of terrestrial arthropods [32] – is a strong

144 manipulator of a wide range of host traits [33]. It gained specific interest due to its
145 protection against various viruses in naturally infected fruit flies [34] and its capacity
146 to reduce the density and transmission of pathogens in mosquito species [35,36].

147 In contrast to long-held beliefs, *Wolbachia* is not restricted to host germ line
148 cells and reproductive organs, but is present in cells throughout somatic tissues and
149 even in the gut lumen of some insects and their faeces [37,38]. Thus, direct
150 interactions with other microbes of the host or indirect interactions via the hosts'
151 immune system are likely. Direct interactions between *Wolbachia* and other microbes
152 have been observed in fruit flies. A co-infection with the endobacterium *Spiroplasma*
153 reduced *Wolbachia* density while *Spiroplasma* numbers remained unaffected by the
154 presence of *Wolbachia* [12]. The investigation of the effect of *Wolbachia* infection on
155 the composition of the gut microbiome in *D. melanogaster* showed an even more
156 complex picture. Here the presence of *Wolbachia* is a significant determinant of the
157 overall composition of the gut microbiome. Interestingly this was neither caused by a
158 direct interaction between *Wolbachia* and the gut microbiome, as *Wolbachia* is absent
159 from the gut lumen in the fly, nor was it indirectly modulated through the activation
160 of the fly's immune system through *Wolbachia* [7]. This highlights the importance of
161 considering a multitude of possible interactions between microbes and between
162 microbes and the host in studies of the dynamics and effects of *Wolbachia*-infections.

163

164 *The quest for disease eradication*

165 An integrated approach, considering multiple interactions between microbes and
166 between microbes and hosts, is especially important when developing microbe-based
167 disease vector control strategies. *Wolbachia* is currently being developed as a novel

168 arthropod-borne disease control agent ([http:// www.eliminatedengue.com](http://www.eliminatedengue.com)); hereby
169 the mode of a successful transmission is an important factor. Under laboratory
170 conditions the native microbiome of *Anopheles* mosquitoes was found to impede
171 vertical transmission of *Wolbachia* through antagonistic microbe-microbe interactions
172 between the bacterium *Asaia* and *Wolbachia* [39]. Similar antagonistic microbe
173 interactions were found in a survey of various mosquito species in Canada, with the
174 presence and abundance of *Wolbachia* fluctuating over season, as well as with the
175 presence of the bacteria *Asaia* and *Pseudomonas* [9]. This suggests that, in addition to
176 environmental effects, interaction of *Wolbachia* with other microbes may explain
177 some of the variation in vector competence of mosquitoes. In contrast to these
178 results, a stable infection with *Wolbachia* in lab-reared mosquitoes *A. aegypti* had only
179 few effects on the microbiome. Moreover, significant changes in the microbiome
180 composition did not affect the dengue virus blocking phenotype caused by *Wolbachia*
181 infection in this mosquito [40]. However, analyses of *A. aegypti* transinfected with
182 *Wolbachia*, released in the field in Brazil and Vietnam to inhibit the dengue virus,
183 revealed that *Wolbachia* increases susceptibility of mosquitoes to dengue infection.
184 This contradicting result was due to the wide variability in exposure doses of
185 *Wolbachia* naturally experienced by mosquitoes [41]. The authors concluded that
186 reliable predictions of **vectorial capacity** of transinfected mosquitoes require an
187 informed account of mosquito pathogens and their interplay with *Wolbachia*.
188 Additionally recent interaction networks, looking at co-occurrence and co-exclusion
189 of microbes, established for several mosquito species (laboratory vs. field) revealed
190 that *Wolbachia* is a highly interconnected taxon, mostly co-exclusionary with other
191 bacteria [42].

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

200

201 **The joint reproductive manipulation of *Wolbachia* and other microbes**

202 The endosymbiont *Wolbachia* is especially well known for its four distinct
203 reproductive phenotypes, that promote its own vertical transmission from mother to
204 offspring [43]. There is growing evidence that the reproductive manipulation by
205 *Wolbachia* is not only exerted by the endosymbiont alone but in interaction with other
206 microbes, i.e. the microbiome of the host, other symbionts, or the host itself.
207 *Wolbachia* has repeatedly been reported to cause different phenotypes, either in
208 experimental settings when transferred between hosts, for example in Lepidoptera
209 [44] or *Drosophila* sibling species [45], or naturally over evolutionary timescales, e.g.
210 in moths and fruit flies [46]. Additionally, many species show geographical variation in
211 symbiont prevalence, including *Wolbachia* with a lower presence in warmer regions
212 [4], as for example reported for many species infected with **parthenogenesis**-inducing
213 *Wolbachia* [47]. The causes for this distributional pattern in prevalence remain

214 speculative, but a possible explanation is that it is driven by variation in microbial
215 communities of host populations in interaction with their abiotic environment (Box 2).

216 Another line of evidence indicating a modulating role of the microbiome in
217 reproductive manipulation by *Wolbachia* comes from studies investigating *Wolbachia*
218 abundance (titre) in a host. The efficiency and phenotype of reproductive
219 manipulation can depend on a threshold of *Wolbachia* titre, i.e. a minimum number
220 of bacteria are required for exerting the manipulative action. A low titre can lead to a
221 switch of the *Wolbachia*-induced phenotype in *Drosophila bifasciata* [24], or to
222 changes in the efficiency of parthenogenesis induction in the parasitic wasp *Asobara*
223 *japonica* [48]. In both studies, variation in the *Wolbachia* titre were manifested under
224 identical rearing conditions, for the latter even in a clonal host reproduction system,
225 suggesting a strong influence on *Wolbachia* titre by other partners, such as the
226 microbiome.

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

234

235 **Concluding remarks**

236 Throughout this manuscript we have pointed out growing evidence that host
237 phenotypic effects such as reproductive manipulation by the endosymbiont

238 *Wolbachia* are not only exerted by an endosymbiont alone but in interaction with
239 others microbes. This and other examples, call for an integrative approach in studying
240 host-microbe associations including host gene expression and interactions between
241 microbes and environmental factors, on these interacting partners (see Outstanding
242 Questions). The latter is especially important in the light of the upcoming challenges
243 of our world, e.g. global warming and disease control. For instance, the protective
244 effect of *Wolbachia* against important human diseases in insect vectors [49] is highly
245 dependent upon temperature. Therefore global warming might cause a decrease in
246 protective *Wolbachia*, undermining on-going long-term biological control
247 programmes of mosquitoes. In this respect, a broader and more natural approach in
248 studying host associated microbes is needed, as laboratory studies might often not be
249 directly translatable to the field [41] (Box 3). Although in this manuscript our focus is
250 on the traits vector-competence and reproductive manipulation conferred by
251 *Wolbachia* in arthropod associations, we would like to point out that an involvement
252 of other host associated microbes is also likely for other traits conferred by *Wolbachia*.
253 As an example, the nutritional symbiosis between *Wolbachia* and bedbugs showed
254 that *Wolbachia*-microbe interactions, i.e. the complementation of functions by gene
255 exchange between different components of the microbiome, can strongly influence
256 the host phenotype through genetic changes in the symbiont [22,23]. As similar
257 microbe-microbe interactions are not restricted to *Wolbachia* but also other
258 symbionts, a holistic approach should be extended to all symbioses [50].

259 Finally, the interpretation of data on host-microbe associations has to be done
260 carefully, keeping in mind that small changes in composition and/or abundance of the
261 microbial community might have great phenotypic consequences for the host, as low-

262 abundance or rare microbial taxa can represent **hub species** [51] that are crucial for
263 the host functioning, as shown for plants and soil ecosystems [52]. Network analyses
264 of the host-associated microbial communities might represent an important tool [53]
265 for basic insights into interaction dynamics within microbial communities. For
266 instance, this approach has recently revealed that for several mosquito species (in the
267 laboratory and field), *Wolbachia* is a highly interconnected taxon, being mostly
268 negatively correlated with other bacteria (i.e. its abundance leads to a reduction in
269 the abundance of other species)[42]. The integration of microbial network analyses
270 with host gene expression networks could provide valuable insights into the
271 complexity of the tripartite interactions.

272

273

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280

281 **References:**

282

- 283 1 Brownlie, J.C. and Johnson, K.N. (2009) Symbiont-mediated protection in
284 insect hosts. *Trends Microbiol.* 17, 348–354
- 285 2 Ivanov, I.I. and Littman, D.R. (2011) Modulation of immune homeostasis by
286 commensal bacteria. *Curr. Opin. Microbiol.* 14, 106–114
- 287 3 Moran, N.A. *et al.* (2008) Genomics and evolution of heritable bacterial
288 symbionts. *Annu. Rev. Genet.* 42, 165–190
- 289 4 Corbin, C. *et al.* (2017) Heritable symbionts in a world of varying temperature.
290 *Heredity (Edinb).* 118, 10–20
- 291 5 Deines, P. and Bosch, T.C.G. (2016) Transitioning from microbiome
292 composition to microbial community interactions: The potential of the
293 metaorganism *Hydra* as an experimental model. *Front. Microbiol.* 7, 1610
- 294 6 Petersen, J.M. and Osvatic, J. (2018) Microbiomes *in natura*: Importance of
295 invertebrates in understanding the natural variety of animal-microbe
296 Interactions. *mSystems* 3, e00179-17
- 297 7 Simhadri, R.K. *et al.* (2017) The gut commensal microbiome of *Drosophila*
298 *melanogaster* is modified by the endosymbiont *Wolbachia*. *mSphere* 2,
299 e00287-17
- 300 8 Dittmer, J. *et al.* (2016) Disentangling a holobiont – Recent advances and
301 perspectives in *Nasonia* wasps. *Front. Microbiol.* 7, 1478
- 302 9 Novakova, E. *et al.* (2017) Mosquito microbiome dynamics, a background for
303 prevalence and seasonality of West Nile virus. *Front. Microbiol.* 8, 526
- 304 10 Dheilly, N.M. *et al.* (2017) Parasite Microbiome Project: Systematic

305 investigation of microbiome dynamics within and across parasite-host
306 interactions. *mSystems* 2, e00050-17

307 11 Macaluso, K.R. *et al.* (2002) Rickettsial infection in *Dermacentor variabilis*
308 (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. *J.*
309 *Med. Entomol.* 39, 809–813

310 12 Goto, S. *et al.* (2006) Asymmetrical interactions between *Wolbachia* and
311 *Spiroplasma* endosymbionts coexisting in the same insect host. *Appl. Environ.*
312 *Microbiol.* 72, 4805–4810

313 13 Johnston, P.R. and Rolff, J. (2015) Host and symbiont jointly control gut
314 microbiota during complete metamorphosis. *PLoS Pathog.* 11, e1005246

315 14 Ye, Y.H. *et al.* (2017) Gut microbiota in *Drosophila melanogaster* interacts with
316 *Wolbachia* but does not contribute to *Wolbachia*-mediated antiviral
317 protection. *J. Invertebr. Pathol.* 143, 18–25

318 15 Audsley, M.D. *et al.* (2018) *Wolbachia* infection alters the relative abundance
319 of resident bacteria in adult *Aedes aegypti* mosquitoes, but not larvae. *Mol.*
320 *Ecol.* 27, 297–309

321 16 Abraham, N.M. *et al.* (2017) Pathogen-mediated manipulation of arthropod
322 microbiota to promote infection. *Proc. Natl. Acad. Sci. U. S. A.* 12, E781–E790

323 17 Koch, H. and Schmid-Hempel, P. (2012) Gut microbiota instead of host
324 genotype drive the specificity in the interaction of a natural host-parasite
325 system. *Ecol. Lett.* 15, 1095–1103

326 18 Lu, P. *et al.* (2012) *Wolbachia* induces density-dependent inhibition to dengue
327 virus in mosquito cells. *PLoS Negl. Trop. Dis.* 6, e1754

328 19 Osborne, S.E. *et al.* (2012) Antiviral protection and the importance of

- 329 Wolbachia density and tissue tropism in *Drosophila simulans*. *Appl. Environ.*
330 *Microbiol.* 78, 6922–6929
- 331 20 Dittmer, J. *et al.* (2014) Host tissues as microhabitats for *Wolbachia* and
332 quantitative insights into the bacterial community in terrestrial isopods. *Mol.*
333 *Ecol.* 23, 2619–2635
- 334 21 Dittmer, J. and Bouchon, D. (2018) Feminizing *Wolbachia* influence microbiota
335 composition in the terrestrial isopod *Armadillidium vulgare*. *Sci. Rep.* 8, 6998
- 336 22 Hosokawa, T. *et al.* (2010) *Wolbachia* as a bacteriocyte-associated nutritional
337 mutualist. *Proc. Natl. Acad. Sci. U. S. A.* 107, 769–774
- 338 23 Nikoh, N. *et al.* (2014) Evolutionary origin of insect-*Wolbachia* nutritional
339 mutualism. *Proc. Natl. Acad. Sci. U. S. A.* 111, 10257–10262
- 340 24 Hurst, G.D.D. *et al.* (2000) Male-killing *Wolbachia* in *Drosophila*: A
341 temperature-sensitive trait with a threshold bacterial density. *Genetics* 156,
342 699–709
- 343 25 Van Opijnen, T. and Breeuwer, J.A. (1999) High temperatures eliminate
344 *Wolbachia*, a cytoplasmic incompatibility inducing endosymbiont, from the
345 two-spotted spider mite. *Exp. Appl. Acarol.* 23, 871–881
- 346 26 Sumi, T. *et al.* (2017) *Wolbachia* density changes seasonally amongst
347 populations of the pale grass blue butterfly, *Zizeeria maha* (Lepidoptera:
348 Lycaenidae). *PLoS One* 12, e0175373
- 349 27 Cohen, C. *et al.* (2015) Similarities and seasonal variations in bacterial
350 communities from the blood of rodents and from their flea vectors. *ISME J.* 9,
351 1662–1676
- 352 28 Adair, K.L. and Douglas, A.E. (2017) Making a microbiome: The many

353 determinants of host-associated microbial community composition. *Curr.*
354 *Opin. Microbiol.* 35, 23–29

355 29 Leibold, M.A. *et al.* (2004) The metacommunity concept: A framework for
356 multi-scale community ecology. *Ecol. Lett.* 7, 601–613

357 30 Bili, M. *et al.* (2016) Bacterial community diversity harboured by interacting
358 species. *PLoS One* 11, e0155392

359 31 Pringle, E.G. and Moreau, C.S. (2017) Community analysis reveals microbial
360 sharing and specialization in a Costa Rican ant-plant-hemipteran symbiosis.
361 *Proc. R. Soc. London B Biol. Sci.* 284, 20162770

362 32 Zug, R. and Hammerstein, P. (2012) Still a host of hosts for *Wolbachia*:
363 Analysis of recent data suggests that 40% of terrestrial arthropod species are
364 infected. *PLoS One* 7, e38544

365 33 Werren, J.H. *et al.* (2008) *Wolbachia*: Master manipulators of invertebrate
366 biology. *Nat. Rev. Microbiol.* 6, 741–751

367 34 Teixeira, L. *et al.* (2008) The bacterial symbiont *Wolbachia* induces resistance
368 to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.* 6, e1000002

369 35 Moreira, L.A. *et al.* (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits
370 infection with dengue, chikungunya, and plasmodium. *Cell* 139, 1268–1278

371 36 Pereira, T.N. *et al.* (2018) *Wolbachia* significantly impacts the vector
372 competence of *Aedes aegypti* for Mayaro virus. *Sci. Rep.* 8, 6889

373 37 Sicard, M. *et al.* (2014) A host as an ecosystem: *Wolbachia* coping with
374 environmental constraints. *Environ. Microbiol.* 16, 3583–3607

375 38 Pietri, J.E. *et al.* (2016) The rich somatic life of *Wolbachia*. *Microbiologyopen* 5,
376 923–936

- 377 39 Hughes, G.L. *et al.* (2014) Native microbiome impedes vertical transmission of
378 *Wolbachia* in *Anopheles* mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 111, 12498–
379 12503
- 380 40 Audsley, M.D. *et al.* (2017) The microbiome composition of *Aedes aegypti* is
381 not critical for *Wolbachia*-mediated inhibition of dengue virus. *PLoS Negl.*
382 *Trop. Dis.* 11, e0005426
- 383 41 King, J.G. *et al.* (2018) Variation in *Wolbachia* effects on *Aedes* mosquitoes as
384 a determinant of invasiveness and vectorial capacity. *Nat. Commun.* 9, 1483
- 385 42 Hegde, S. *et al.* (2018) Microbiome interaction networks and community
386 structure from lab reared and field-collected *Aedes aegypti*, *Aedes albopictus*,
387 and *Culex quinquefasciatus* mosquito vectors. *bioRxiv* at
388 <<http://dx.doi.org/10.1101/337311>>
- 389 43 Hurst, G.D.D. and Frost, C.L. (2015) Reproductive parasitism: Maternally
390 inherited symbionts in a biparental world. *Cold Spring Harb. Perspect. Biol.* 7,
391 a017699
- 392 44 Sasaki, T. *et al.* (2002) Interspecific transfer of *Wolbachia* between two
393 lepidopteran insects expressing cytoplasmic incompatibility: A *Wolbachia*
394 variant naturally infecting *Cadra cautella* causes male killing in *Ephesia*
395 *kuehniella*. *Genetics* 162, 1313–1319
- 396 45 Jaenike, J. (2009) Coupled population dynamics of endosymbionts within and
397 between hosts. *Oikos* 118, 353–362
- 398 46 Ma, W.J. *et al.* (2014) Manipulation of arthropod sex determination by
399 endosymbionts: Diversity and molecular mechanisms. *Sex. Dev.* 8, 59–73
- 400 47 Haag, C.R. and Ebert, D. (2004) A new hypothesis to explain geographic

401 parthenogenesis. *Ann. Zool. Fennici* 41, 539–544

402 48 Ma, W.-J. *et al.* (2015) Diploid males support a two-step mechanism of
403 endosymbiont-induced thelytoky in a parasitoid wasp. *BMC Evol. Biol.* 15, 84

404 49 Flores, H.A. and O’Neill, S.L. (2018) Controlling vector-borne diseases by
405 releasing modified mosquitoes. *Nat. Rev. Microbiol.* 16, 508–518

406 50 Santos-Garcia, D. *et al.* (2018) To B or not to B : Comparative genomics
407 suggests *Arsenophonus* as a source of B vitamins in whiteflies. *Front.*
408 *Microbiol.* 9, 2254

409 51 Agler, M.T. *et al.* (2016) Microbial hub taxa link host and abiotic factors to
410 plant microbiome variation. *PLoS Biol.* 14, e1002352

411 52 Jousset, A. *et al.* (2017) Where less may be more: How the rare biosphere
412 pulls ecosystems strings. *ISME J.* 11, 853–862

413 53 Layeghifard, M. *et al.* (2017) Disentangling interactions in the microbiome: A
414 network perspective. *Trends Microbiol.* 25, 217–228

415 54 Blaser, M.J. (2014) The microbiome revolution. *J. Clin. Invest.* 124, 4162–4165

416 55 Wong, A.C.-N. *et al.* (2014) Gut microbiota dictates the metabolic response of
417 *Drosophila* to diet. *J. Exp. Biol.* 217, 1894–1901

418 56 Shropshire, J.D. and Bordenstein, S.R. (2016) Speciation by symbiosis: The
419 microbiome and behavior. *MBio* 7, e01785

420 57 McFall-Ngai, M. *et al.* (2013) Animals in a bacterial world, a new imperative
421 for the life sciences. *Proc. Natl. Acad. Sci. U. S. A.* 110, 3229–3236

422 58 Zilber-Rosenberg, I. and Rosenberg, E. (2008) Role of microorganisms in the
423 evolution of animals and plants: The hologenome theory of evolution. *FEMS*
424 *Microbiol. Rev.* 32, 723–735

425 59 Carrier, T.J. and Reitzel, A.M. (2017) The hologenome across environments
426 and the implications of a host-associated microbial repertoire. *Front.*
427 *Microbiol.* 8, 802

428 60 Rosenberg, E. and Zilber-Rosenberg, I. (2018) The hologenome concept of
429 evolution after 10 years. *Microbiome* 6, 78

430 61 Bonnet, S.I. *et al.* (2017) The tick microbiome: Why non-pathogenic
431 microorganisms matter in tick biology and pathogen transmission. *Front. Cell.*
432 *Infect. Microbiol.* 7, 236

433 62 Chandler, J.A. *et al.* (2011) Bacterial communities of diverse *Drosophila*
434 species: Ecological context of a host–microbe model system. *PLoS Genet.* 7,
435 e1002272

436 63 Brooks, A.W. *et al.* (2016) Phylosymbiosis : Relationships and functional
437 effects of microbial communities across host evolutionary history. *PLoS Biol.*
438 14, e2000225

439 64 Chaplinska, M. *et al.* (2016) Bacterial communities differ among *Drosophila*
440 *melanogaster* populations and affect host resistance against parasitoids. *PLoS*
441 *One* 11, e0167726

442 65 Engel, P. and Moran, N.A. (2013) The gut microbiota of insects - Diversity in
443 structure and function. *FEMS Microbiol. Rev.* 37, 699–735

444 66 Colman, D.R. *et al.* (2012) Do diet and taxonomy influence insect gut bacterial
445 communities? *Mol. Ecol.* 21, 5124–5137

446 67 Russell, J.A. and Moran, N.A. (2006) Costs and benefits of symbiont infection
447 in aphids: Variation among symbionts and across temperatures. *Proc. R. Soc.*
448 *London B Biol. Sci.* 273, 603–610

449 68 Martinez, J. *et al.* (2014) Symbionts commonly provide broad spectrum
450 resistance to viruses in insects: A comparative analysis of *Wolbachia* strains.
451 *PLoS Pathog.* 10, e1004369
452
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454 **Glossary:**

455 **Ecosystem:** the complex network of living organisms, their physical environment, and
456 their interactions in a particular unit of space. In our context a host, its associated
457 microbiome and all potential interactions among living organisms and with
458 environmental conditions.

459 **Endosymbionts:** microbial associates living within the body or cells of another
460 organism (host).

461 **Hologenome:** the sum of the genetic information of the host and its microbiome.

462 **Horizontal transmission:** transmission of microbes between individuals, species or
463 with free-living microbes.

464 **Host:** organism in which an endosymbiont or microbiome live.

465 **Host-microbe interactions:** interactions between any microbial species or microbial
466 communities (either a symbiont or part of the remainder microbial community) and a
467 host.

468 **Hub species:** microbial species that are strongly interconnected by several links within
469 a network and play an important role in community functioning and/or stability.
470 Abiotic factors and host genotype can directly act on hub species, thus spreading the
471 effects to the whole microbial community.

472 **Metacommunity:** a set of interacting communities that are regulated by processes
473 such as dispersal, extinction and recolonization.

474 **Microbes:** microscopic organisms, including bacteria, fungi, protozoa, and viruses.

475 **Microbiome:** a community of microbes that inhabit a particular environment.

476 **Parthenogenesis:** clonal reproduction, in which an unfertilized egg develops into a
477 new individual.

478 **Phenotype:** the set of observable characteristics of an individual resulting from the
479 interaction of its genotype with the environment.

480 **Phenotypic effects:** changes in a phenotype caused by an external influencing factor,
481 here symbionts.

482 **Remainder microbiome:** the microbiome excluding the symbiont under investigation.

483 Note that we consider symbionts here as a separate microbial entity due to historical
484 reasons and to highlight the differences between both, but in fact they are a part of
485 the microbiome and as such, should be studied together.

486 **Symbiont:** here microbial associate of any type in a close and long-term biological
487 interaction (mutualism, commensalism or parasitism) with biological organisms, of
488 the same or of different species.

489 **Traits:** characteristics or attributes of an organism that are expressed by genes and/or
490 influenced by the environment.

491 **Vectorial capacity:** the capability for disease transmission by a vector to a host, as
492 influenced by behavioral, ecological and environmental factors, such as population
493 density, host preference, feeding habits or frequency, duration of latent period, or
494 longevity.

495 **Vertical transmission:** maternal transmission of microbes to offspring.

496

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

498 Historically, symbiosis research in insects has focused primarily on binary interactions
499 between hosts and individual endosymbionts [3,33] and therefore observed
500 phenotypic effects were attributed to the single symbiont. This binary point of view
501 was challenged in recent years. The ‘microbiome revolution’ [54] of the past 10 years
502 revealed that all animals are copiously colonized by microorganisms, collectively
503 called the microbiome, of which the symbionts are part. During this revolution it was
504 realized that similar to single symbionts, the microbiome can also impact important
505 host traits [17,55,56] and thus influence the ecology and evolution of their hosts [57],
506 acting as an extended genome of the host, the **hologenome** [58]. Therefore, it has
507 been proposed that the host itself should be viewed as a complex ecosystem, in which
508 not only single symbionts interact with the host, but also the microbiome interacts
509 with symbionts and hosts [37,57]. An additional scale of complexity has recently
510 gained attention, namely that host-microbe associations are also part of a wider
511 microbial community maintained by transmission between individual hosts and
512 dispersal between host-associated and free-living microbial communities [8,28]. In
513 2017, Carrier & Reitzel [59] introduced the idea of a “host-associated microbial
514 repertoire,” which is the sum of microbial species a host may associate with over the
515 course of its life under all encountered environmental circumstances. Due to this
516 plasticity in the microbiome, the microbiome genome could allow hosts to adapt and
517 survive under changing environmental conditions thus providing the time necessary
518 for the host genome to adapt and evolve [60]. From this it becomes clear that one
519 host can harbour a diverse microbial community, the entities of which interact with
520 each other, potentially competing for space, energy, and resources, and ultimately

521 influencing the condition of the host by conferring multiple detrimental, neutral, or
522 beneficial effects [61]. Therefore, a more holistic approach in studying the interaction
523 between the different partners is needed.

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

525 Host-microbe interactions are shaped by a multitude of factors, i.e. factors associated
526 with the host such as immunity [62], phylogeny [63], host population background [64],
527 physicochemical conditions in the insect habitat (e.g., gut pH, oxygen tensions), and
528 structural filters in the gut [65] but also environmental factors such as diet [66] and
529 temperature [67]. Abiotic factors do have a crucial effect on microbes and the host,
530 and therefore on their interactions. External environmental conditions significantly
531 affect the infective states of hosts including the density of the endosymbionts
532 inhabiting the host body, e.g. high temperature [4,25,26] with occurring seasonal
533 changes of symbiont density such as *Wolbachia* [9,26,27].

534 In addition, these associations are also part of a wider microbial community
535 maintained by transmission between individual hosts and dispersal between host-
536 associated and free-living microbial communities [8,28,59]. While our understanding
537 of the factors that affect the composition and abundance of the microbiome is
538 expanding, there are still many unanswered questions regarding microbiome
539 assembly and maintenance. Exposure to environmental microbes has undoubtedly a
540 major influence on the microbial communities of an organism [42], as metacommunity
541 studies revealed that microbial communities associated with different interaction
542 partners (species) differ in terms of composition and abundance, but shared microbes
543 among the macro-partners [30,31]. Unravelling the role of environment in shaping the
544 host-associated microbiome (including symbionts) is crucial to place the specificity of

545 interactions in an evolutionary context, for instance, by understanding whether
546 deterministic processes lead to the selection of the horizontally transmitted microbes.

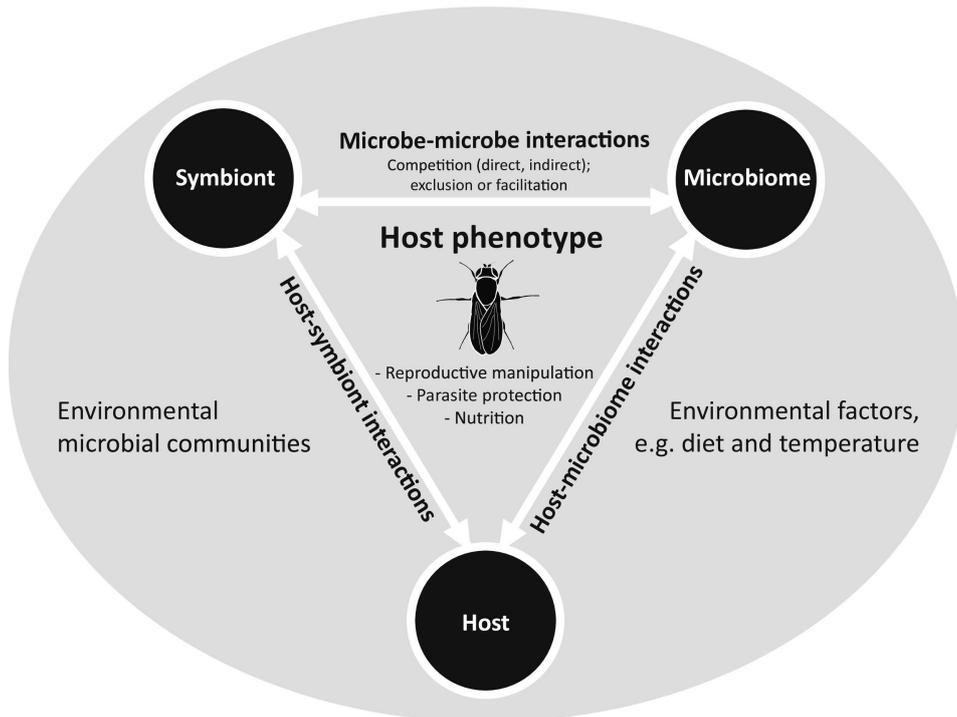
547 **Box 3: Importance of laboratory versus field studies**

548 Given the strong influence of environmental factors (Box1, Box2) on host-microbe
549 interactions, the transitions from laboratory studies to the field might bear some
550 difficulty [59]. Laboratory settings potentially restrict the full spectrum of host-
551 microbe-associations compared to the natural setting where these associations have
552 evolved [62]. Thus, it may limit the interpretation of the functional roles microbes play
553 in host biology ([59]; Box2).

554 A good example for this is the *Wolbachia*-mediated inhibition of dengue virus. Under
555 laboratory condition it was indicated that the microbiome composition of the
556 mosquito *Aedes aegypti* is not critical for inhibition [40]. However, when released into
557 the wild the picture became more complex. *Wolbachia*, when introgressed into
558 different genetic backgrounds increased the mean and the variance in mosquito
559 susceptibility to dengue infection [41]. While the respective impacts of these factors
560 are not easily disentangled, similarly complex multifactorial patterns likely underlie
561 many host-microbe associations under ecologically realistic conditions. Given that
562 *Wolbachia* appears to modify host susceptibility to a broad spectrum of pathogens
563 [34,68], reliable predictions of invasiveness and vectorial capacity of transinfected
564 mosquitoes require an informed account of natural mosquito pathogens and their
565 interplay with *Wolbachia* [41]. A first step to get a more complete picture of the
566 symbiotic effects of *Wolbachia* is to investigate these complex interactions
567 simultaneously in the lab and field. In addition, a beneficial approach would be the
568 mimicry of environmental factors, biotic and abiotic, in the lab. Microbiome and

569 symbiont manipulation is often achieved through the use of antibiotics. However such
570 approaches may have several overlooked side-effects. First, antibiotics may affect
571 different components of the microbiome differently and hence alter the interaction
572 networks. Second, results obtained in the absence of (parts of) the microbiome tell
573 only one side of the story, as microbe-microbe interactions might modulate host
574 response. Hence, in order to unravel the interactions and impact of host phenotype,
575 host recolonization by a variety of well characterized microbes, or whole microbial
576 communities, is required. Also the enrichment of the microbial diversity, mimicking
577 possible biotic environmental influences such as transmission of free-living microbial
578 communities, might be worthwhile. Although these manipulations can only provide a
579 mechanistic understanding of the tripartite interaction and may not be translatable
580 to field settings, they nevertheless are an important first step in gaining a better
581 understanding of host-microbe interactions.
582

583 **Figure 1. Multipartite interactions affect the host phenotype.** Symbiosis between
584 microbial associates and a host is a ubiquitous feature of life on earth, modulating
585 host phenotypes (host-symbiont interactions). In addition to endosymbionts,
586 organisms harbour a collection of host-associated microbes, the microbiome that can
587 impact important host traits (host-microbe interactions). These microbes interact with
588 each other either direct via competition for resources and space, or indirect via the
589 induction of a general immune response, potentially leading in changes of microbial
590 community diversity, or abundance (microbe-microbe interactions). Therefore, a
591 symbiont induced host phenotype, such as reproductive manipulation, parasite
592 protection, or nutrition, is not only modulated by binary interactions, but also by a
593 multitude of interaction between host, symbiont and the remainder microbiome,
594 continuously influencing each other. Additionally, these interactions are influenced by
595 their environment (grey circle), such as temperature, or diet, but by direct interaction
596 with free living microbial communities. We thus advocate that only through an
597 integrated approach that considers multiple interacting partners and environmental
598 influences will we be able to gain a better understanding of host-microbe associations.
599



600
601

Figure 1.