

Title: Diversity begets diversity: Do parasites promote variation in protective symbionts?

Authors: Nina Hafer¹ & Christoph Vorburger^{1,2}

1: EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133,
8600 Dübendorf, Switzerland

2: Institute of Integrative Biology, ETH Zürich, Universitätsstrasse 16, 8092 Zürich, Switzerland

E-mail:

NH: nina.hafer@eawag.ch

CV: christoph.vorburger@eawag.ch

Corresponding author: Nina Hafer

Abstract

Insects commonly possess heritable microbial symbionts that increase their resistance to particular parasites. A diverse community of defensive symbionts may thus provide hosts with effective and specific protection against multiple parasites, although costs might constrain the accumulation of many symbionts. In parallel to the allelic diversity in the MHC complex of the vertebrate immune system, parasite diversity could be the driving force behind symbiont diversity. There is indeed evidence that parasites have the ability to drive frequencies of defensive symbionts in their hosts, and that these symbionts influence parasite communities, but direct evidence that parasite diversity can promote symbiont diversity is still lacking. We provide suggestions to investigate this potential link.

Highlights:

- Heritable microbes provide insects with effective protection against parasites
- Such protective symbionts are diverse, specific and costly
- These properties are similar to those of the MHC in the vertebrate immune system
- Akin to the MHC, symbiont diversity could be driven by parasite diversity

Introduction

One of the great challenges any living organism faces is how to defend itself against a multitude of parasitic organisms such as parasitoids, macroparasites, fungi, bacteria and viruses. In addition to their own immune system, many organisms rely on microbial symbionts to help them with this challenge. Such defensive symbionts are especially abundant among insects and other arthropods [e.g. 1,2]. Different secondary symbionts, for example, provide aphids with protection against different parasitoid wasps and pathogenic fungi [3–5]. In this paper, we briefly summarize the evidence for the diversity, specificity, and costs of protective symbionts in insects and draw comparisons with the specific protection afforded to vertebrates by their adaptive immune system. We then go on to discuss the potential role of parasites in shaping and maintaining symbiont diversity.

Properties of defensive symbionts in insects

Symbiont-mediated protection has been reported from a wide variety of insects. Flies can be protected against parasitoid wasps or parasitic nematodes by bacteria of the genus *Spiroplasma* [6–8], and against viral pathogens by *Wolbachia* [e.g. 9–11]. Another example are antibiotic-producing bacteria protecting developing beewolves (*Philanthus* sp.) or the eggs of Lagriinae beetles against pathogenic fungi [12,13]. Symbiont diversity has been studied most exhaustively in aphids with at least 9 different species of heritable facultative endosymbionts described to date, 7 of which have been shown to confer protection against entomopathogenic fungi or parasitoids [5]. These different symbionts occur in many aphid species [14], and they can co-occur within the same host species, the same host population, or even the same host individual [1,3–5,15,16]. The frequencies of infection with particular symbionts show extensive variation among aphid populations from different sites or different host plants [1,3–5,15,16] as also observed in other groups of insects [17,18]. This variation is ecologically relevant, because symbiont-conferred resistance is strong, often stronger than any innate resistance [reviewed by 3–5,19,20], and parasite-specific. In *Drosophila*, for example, *Wolbachia* provides protection against different RNA-viruses, but not against DNA-viruses [10,11]. In aphids, the endosymbiont *Hamiltonella defensa* typically provides protection against parasitoids and *Regiella insecticola* protects against fungal pathogens [reviewed e.g. by 3,4], although notable exceptions exist [e.g. 21,22]. This specificity even extends to variation within species. Particular strains of the

symbionts protect well against some parasite genotypes but not against others ($G \times G$ interactions), such that no symbiont offers the best protection against all parasites [22–29].

Symbionts as a modular defence toolbox?

Multiple symbionts might provide a way to overcome the restrictions of this specificity by providing wider or more effective protection. Infections with more than one symbiont are regularly observed in the field [reviewed by 3,5,15,16] and a recent laboratory study confirmed that they can be stable [30]. Indeed, symbionts seem to maintain their protective effect in the presence of another symbiont species or strain [30,31]. Some studies have even found indications that co-infections can enhance protection [26,32,33] or ensure protection even under adverse condition such as heat stress, which normally reduces the protective effect of *Hamiltonella* [34], but see [35]. On the other hand, the possession of multiple defensive symbionts might be constrained by a reduced fidelity of vertical transmission [36,37], or by detrimental effects of symbiont infection. In flies and aphids, protective symbionts have repeatedly been found to be associated with fitness costs such as shorter lifespan or reduced competitive ability [3,4,23,25,38]. Infection with multiple symbiont species or strains can have even more severe fitness costs in aphids [26,30,31,33], although this might depend on host genotype [31], host environment [26], and symbiont-symbiont combination [30].

Parallels and differences between protective symbionts and the vertebrate MHC

The defensive symbionts of insects can be seen as a second line of defence in addition to their innate immunity. With its remarkable diversity and specificity of action, symbiont-conferred resistance shows some interesting parallels to the vertebrate major histocompatibility complex (MHC) that are worth exploring (Table 1). The MHC is part of the adaptive vertebrate immune system and MHC-encoded proteins (i.e. MHC molecules) are responsible for the specific recognition of parasites and pathogens (Box 1). As for protective symbionts, there are numerous variants of the MHC, which provide effective and specific protection against different parasites and pathogens, but each individual can only possess a limited number of different MHC variants. Their number, however, is usually higher than the number of different protective symbionts within a single insect host and, unlike symbionts, MHC is present in each individual. Being part of the nuclear genome the MHC can change only through recombination during reproduction, albeit immune memory can improve control of familiar parasites and pathogens during an individual's lifetime. Protective symbionts, by contrast, remain organisms of their own that are usually transmitted vertically by the mother only, but can also be lost or acquired horizontally [16,36,39]. Additionally, symbionts can change through horizontal gene transfer, such as the loss or acquisition of bacteriophages, which in *Hamiltonella* are responsible for the protection of their aphid hosts against parasitoids [40–42]. This will also alter their benefits and costs to the host.

Diverse parasite communities should favour high symbiont diversity

Their many shared properties suggest that the MHC and protective symbionts are subject to similar selection pressures. Hence, parasites should be important drivers in maintaining symbiont diversity akin to the maintenance of MHC diversity (see Box 1). This could occur via balancing selection in the face of multiple parasites or via frequency dependent selection [43–48], albeit drift, horizontal transmission and imperfect vertical transmission might also play a role [3,46,49]. Seppälä and Jokela [50] recently argued that parasites could help maintain the diversity of other parasites, if the fitness of one parasite species or genotype depends on the community of co-infecting parasites, which will be highly variable. This should be especially true for co-infections of protective symbionts and parasites, where the consequences of sharing a host will often be a matter of life or death for one of the antagonists. Hence, higher parasite diversity should result in higher symbiont diversity (Figure 1).

Evidence for parasites affecting symbiont communities

While we are not aware of any studies addressing whether parasite diversity enhances symbiont diversity, there is comparative as well as experimental evidence for parasite-mediated selection on communities of protective symbionts. Spatial or temporal correlations between the risk of parasitism and the abundance of specific species or functional groups of symbionts are at least consistent with selection by parasites affecting symbiont frequencies (Table 2A), and there is good evidence that a geographic cline of infection with *Spiroplasma* in *Drosophila neotestacea* reflects a recent spread of this symbiont, driven by its ability to protect flies against parasitic nematodes [8].

Several experimental studies on captive populations of insects have recorded changes in symbiont frequencies depending on the presence or absence of parasitoids (Table 2B). For example, Oliver et al. [51] observed that the frequency of *Hamiltonella* (protective against parasitoids) increased in the presence of parasitoids, but decreased in their absence [51]. Support that the presence of parasitoids drives shifts in symbiont frequencies comes also from *Spiroplasma* infections in *Drosophila hydei* which increase in the presence of the parasitoids against which they confer protection, but remain constant in their absence [7]. Transferring these findings to a natural system with several selectively protective symbionts, a diverse symbiont community should only be maintained given a diverse parasite community and costs to symbiont infection. This remains to be tested experimentally, although there are suggestive results from an experiment that manipulated diversity on a different level. Zytynska et al. [52] found that in field plots with higher plant diversity, which is correlated with the diversity of natural enemies [53], three species of aphids possessed a higher diversity of endosymbionts, even though each aphid species only grew on a single plant species within these communities [52].

Experimental evidence that symbiont communities can alter parasite communities

Because of the specificity in the protection they provide, defensive symbionts may also exert a bottom-up effect on the composition of parasite communities establishing in host populations (Table 2C). In a field experiment using pea aphid populations with two different symbionts that provided protection either against parasitoids (*Hamiltonella*) or fungi (*Regiella*), Hrček et al. [54] observed a shift in the incidence of these parasitic groups in line with the expected protection. The presence of *Hamiltonella* alone has been found to alter the outcome of competition between two parasitoid species in the lab [55,56] and enhance parasitoid diversity in the field by suppressing the otherwise dominant parasitoid species [57]. Simple correlations between symbiont and parasite diversity may thus not be informative about the directionality of a potential effect, and it is indeed plausible that parasites and symbionts drive each other's diversity.

Outlook

How can we test whether symbiont diversity promotes parasite diversity, and *vice versa*? Experimental evolution and coevolution have proven crucial for an in-depth understanding of host-parasite interactions [58]. The same tools are suited, in principle, to address the question posed here. Symbiont-polymorphic host populations could be exposed to parasite communities of high and low diversity over multiple generations, parasite communities could be selected on hosts with high vs. low symbiont diversity, and it would also be possible to monitor symbiont and parasite diversity in a setup where host and parasite populations are free to evolve.

For vertebrates there is overwhelming evidence that hosts go to great lengths to ensure that their offspring is equipped with the best possible MHC alleles via mate choice [e.g. 44,47]. Unlike the biparentally inherited MHC, however, inheritance of defensive symbionts is predominantly maternal. The benefits of symbiont-based mate choice would thus mostly accrue to males, but males have generally more to gain from just maximizing mating opportunities [e.g. 59]. Nevertheless, this might

play a role in species where males have evolved to be choosy [60]. Symbionts can be lost during vertical transfer or obtained horizontally during an individual's lifetime through a number of different routes, albeit these events are relatively rare [16,36,39]. Are hosts able to influence these processes to facilitate a better set of symbionts in a given environment? While there is no evidence of this to date, at least theoretically it would give hosts a different means to influence their symbiont composition not available to vertebrates with their genetically encoded MHC.

While we are the first to concede that the parallels between the vertebrate MHC and defensive symbionts of insects are tentative at best, we believe that taking this perspective and thus benefitting from an extensive body of research can aid in understanding the role of symbionts in insect defence.

Acknowledgements

We thank K.M. Oliver for eliciting this article. Our research is supported by the Deutsche Forschungsgemeinschaft (DFG fellowship nr. HA 8471/1-1 to NH) and the Swiss National Science Foundation (Sinergia grant nr. CRSII3_154396 to CV).

References

- Flórez L V., Biedermann PHW, Engl T, Kaltenpoth M: **Defensive symbioses of animals with prokaryotic and eukaryotic microorganisms.** *Nat Prod Rep* 2015, **32**:904–936.
- Brownlie JC, Johnson KN: **Symbiont-mediated protection in insect hosts.** *Trends Microbiol* 2009, **17**:348–354.
- Oliver KM, Smith AH, Russell JA: **Defensive symbiosis in the real world - advancing ecological studies of heritable, protective bacteria in aphids and beyond.** *Funct Ecol* 2014, **28**:341–355.
- Vorburger C: **The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids.** *Insect Sci* 2014, **21**:251–264.
- Guo J, Hatt S, He K, Chen J, Francis F, Wang Z: **Nine facultative endosymbionts in aphids. A review.** *J Asia Pac Entomol* 2017, **20**:794–801.
- Xie J, Vilchez I, Mateos M: ***Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*.** *PLoS One* 2010, **5**:e12149.
- Xie J, Winter C, Winter L, Mateos M: **Rapid spread of the defensive endosymbiont *Spiroplasma* in *Drosophila hydei* under high parasitoid wasp pressure.** *FEMS Microbiol Ecol* 2015, **91**:1–11.
- Jaenike J, Unckless R, Cockburn SN, Boelio LM, Perlman SJ: **Adaptation via symbiosis: Recent spread of a *Drosophila* defensive symbiont.** *Science* 2010, **329**:212–215.
- Hedges LM, Brownlie JC, O'Neill SL, Johnson KN: ***Wolbachia* and virus protection in insects.** *Science* 2008, **322**:702.
- Osborne SE, Leong YS, O'Neill SL, Johnson KN: **Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*.** *PLoS Pathog* 2009, **5**:e1000656.
- Teixeira L, Ferreira Á, Ashburner M: **The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*.** *PLoS Biol* 2008, **6**:2753–2763.
- Kaltenpoth M, Göttinger W, Herzner G, Strohm E: **Symbiotic bacteria protect wasp larvae from fungal infestation.** *Curr Biol* 2005, **15**:475–479.

- 193 13.** Flórez L V., Scherlach K, Gaube P, Ross C, Sitte E, Hermes C, Rodrigues A, Hertweck C,
194 Kaltenpoth M: **Antibiotic-producing symbionts dynamically transition between plant**
195 **pathogenicity and insect-defensive mutualism.** *Nat Commun* 2017, **8**:15172.
- 196 A comprehensive study providing evidence for the evolutionary transition of a plant pathogen towards an
197 antibiotic-producing defensive symbiont of beetles that protects their eggs against infection.
- 198 14. Henry LM, Maiden MCJ, Ferrari J, Godfray HCJ: **Insect life history and the evolution of**
199 **bacterial mutualism.** *Ecol Lett* 2015, **18**:516–525.
- 200 15. McLean AHC, Parker BJ, Hrčák J, Henry LM, Godfray HCJ: **Insect symbionts in food webs.**
201 *Philos Trans R Soc B* 2016, **371**:20150325.
- 202 16. Zytynska SE, Weisser WW: **The natural occurrence of secondary bacterial symbionts in**
203 **aphids.** *Ecol Entomol* 2016, **41**:13–26.
- 204 17. Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M: **Biotpe-**
205 **dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*.**
206 *Bull Entomol Res* 2007, **97**:407–413.
- 207 18. Zhu Y-X, Song Y-L, Zhang Y-K, Hoffmann AA, Zhou J-C, Sun J-T, Hong X-Y: **Incidence of**
208 **facultative bacterial endosymbionts in spider mites associated with local environment and**
209 **host plant.** *Appl Environ Microbiol* 2018, **84**:e02546-17.
- 210 19. Oliver KM, Degnan PH, Burke GR, Moran NA: **Facultative Symbionts in Aphids and the**
211 **Horizontal Transfer of Ecologically Important Traits.** *Annu Rev Entomol* 2010, **55**:247–
212 266.
- 213 20. Vorburger C, Perlman SJ: **The role of defensive symbionts in host-parasite coevolution.** *Biol*
214 *Rev* 2018, doi:10.1111/brv.12417.
- 215 21. Lenhart PA, White JA: **A defensive endosymbiont fails to protect aphids against the**
216 **parasitoid community present in the field.** *Ecol Entomol* 2017, **42**:680–684.
- 217 22. Vorburger C, Gehrer L, Rodriguez P: **A strain of the bacterial symbiont *Regiella insecticola***
218 **protects aphids against parasitoids.** *Biol Lett* 2010, **6**:109–111.
- 219 23. Cayetano L, Vorburger C: **Symbiont-conferred protection against Hymenopteran**
220 **parasitoids in aphids: How general is it?** *Ecol Entomol* 2015, **40**:85–93.
- 221 24. Vorburger C, Rouchet R: **Are aphid parasitoids locally adapted to the prevalence of**
222 **defensive symbionts in their hosts?** *BMC Evol Biol* 2016, **16**:271.
- 223 25. Cayetano L, Rothacher L, Simon J, Vorburger C: **Cheaper is not always worse: strongly**
224 **protective isolates of a defensive symbiont are less costly to the aphid host.** *Proc R Soc B*
225 2015, **282**:1–10.
- 226 26. Leclair M, Pons I, Mahéo F, Simon J-C, Outreman Y: **Diversity in symbiont consortia in the**
227 **pea aphid complex is associated with large phenotypic variation in the insect host.** *Evol*
228 *Ecol* 2016, **30**:925–941.
- 229 27. Rouchet R, Vorburger C: **Strong specificity in the interaction between parasitoids and**
230 **symbiont-protected hosts.** *J Evol Biol* 2012, **25**:2369–2375.
- 231 28. Parker BJ, Hrčák J, McLean AHC, Godfray HCJ: **Genotype specificity among hosts,**
232 **pathogens, and beneficial microbes influences the strength of symbiont-mediated**
233 **protection.** *Evolution* 2017, **71**:1222–1231.
- 234 29. Schmid M, Sieber R, Zimmermann YS, Vorburger C: **Development, specificity and sublethal**
235 **effects of symbiont-conferred resistance to parasitoids in aphids.** *Funct Ecol* 2012, **26**:207–
236 215.
- 237 30.* McLean AHC, Parker BJ, Hrčák J, Kavanagh JC, Wellham PAD, Godfray HCJ:

- 238 **Consequences of symbiont co-infections for insect host phenotypes.** *J Anim Ecol* 2018,
239 **87**:478–488.
- 240 An elegant laboratory study demonstrating that co-infections of two different heritable endosymbionts in pea
241 aphids can be very stable and have little effect on the protection afforded by the individual symbionts, and that
242 some co-infections were more costly to the host than single infections.
- 243 31. Leclair M, Polin S, Jousseau T, Simon JC, Sugio A, Morlière S, Fukatsu T, Tsuchida T,
244 Outreman Y: **Consequences of coinfection with protective symbionts on the host**
245 **phenotype and symbiont titres in the pea aphid system.** *Insect Sci* 2017, **24**:798–808.
- 246 32. Nyabuga FN, Outreman Y, Simon JC, Heckel DG, Weisser WW: **Effects of pea aphid**
247 **secondary endosymbionts on aphid resistance and development of the aphid parasitoid**
248 ***Aphidius ervi*: A correlative study.** *Entomol Exp Appl* 2010, **136**:243–253.
- 249 33. Oliver KM, Moran NA, Hunter MS: **Costs and benefits of a superinfection of facultative**
250 **symbionts in aphids.** *Proc R Soc B Biol Sci* 2006, **273**:1273–1280.
- 251 34. Guay JF, Boudreault S, Michaud D, Cloutier C: **Impact of environmental stress on aphid**
252 **clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in**
253 **association with a new facultative symbiont of the pea aphid.** *J Insect Physiol* 2009,
254 **55**:919–926.
- 255 35. Doremus MR, Smith AH, Kim KL, Holder AJ, Russell JA, Oliver KM: **Breakdown of a**
256 **defensive symbiosis, but not endogenous defences, at elevated temperatures.** *Mol Ecol*
257 2018, **27**:2138–2151.
- 258 36. * Rock DI, Smith AH, Joffe J, Albertus A, Wong N, O'Connor M, Oliver KM, Russell JA:
259 **Context-dependent vertical transmission shapes strong endosymbiont community**
260 **structure in the pea aphid, *Acyrtosiphon pisum*.** *Mol Ecol* 2018, **27**:2039–2056.
- 261 A very comprehensive study combining field surveys and field experiments to show that even though
262 endosymbiont communities in pea aphids are rather dynamic, certain associations are consistently
263 overrepresented and thus likely maintained by selection via their combined effects on the host.
- 264 37. Moran NA, Dunbar HE: **Sexual acquisition of beneficial symbionts in aphids.** *Proc Natl*
265 *Acad Sci* 2006, **103**:12803–12806.
- 266 38. Heyworth ER, Ferrari J: **A facultative endosymbiont in aphids can provide diverse**
267 **ecological benefits.** *J Evol Biol* 2015, **28**:1753–1760.
- 268 39. Martinez AJ, Ritter SG, Doremus MR, Russell JA, Oliver KM: **Aphid-encoded variability in**
269 **susceptibility to a parasitoid.** *BMC Evol Biol* 2014, **14**:1–10.
- 270 40. Brandt JW, Chevignon G, Oliver KM, Strand MR: **Culture of an aphid heritable symbiont**
271 **demonstrates its direct role in defence against parasitoids.** *Proc R Soc B Biol Sci* 2017,
272 doi:10.1098/rspb.2017.1925.
- 273 41. Weldon SR, Strand MR, Oliver KM: **Phage loss and the breakdown of a defensive**
274 **symbiosis in aphids.** *Proc R Soc B Biol Sci* 2012, **280**:20122103–20122103.
- 275 42. Oliver KM, Degnan PH, Hunter MS, Moran NA: **Bacteriophages encode factors required**
276 **for protection in a symbiotic mutualism.** *Science* 2009, **325**:992–994.
- 277 43. Apanius V, Penn D, Slev PR, Ruff LR, Potts WK: **The Nature of Selection on the Major**
278 **Histocompatibility Complex.** *Crit Rev Immunol* 1997, **17**:179–224.
- 279 44. Piertney SB, Oliver MK: **The evolutionary ecology of the major histocompatibility**
280 **complex.** *Heredity* 2006, **96**:7–21.
- 281 45. Wegner KM, Kalbe M, Schaschl H, Reusch TBH: **Parasites and individual major**
282 **histocompatibility complex diversity - An optimal choice?** *Microbes Infect* 2004, **6**:1110–

- 283 1116.
- 284 46. Jaenike J: **Population genetics of beneficial heritable symbionts.** *Trends Ecol Evol* 2012,
285 **27**:226–232.
- 286 47. Milinski M: **The Major Histocompatibility Complex, Sexual Selection, and Mate Choice.**
287 *Annu Rev Ecol Evol Syst* 2006, **37**:159–186.
- 288 48. Smith AH, Łukasik P, O'Connor MP, Lee A, Mayo G, Drott MT, Doll S, Tuttle R, Disciullo
289 RA, Messina A, et al.: **Patterns, causes and consequences of defensive microbiome**
290 **dynamics across multiple scales.** *Mol Ecol* 2015, **24**:1135–1149.
- 291 49. Kwiatkowski M, Engelstädter J, Vorburger C: **On Genetic Specificity in Symbiont-Mediated**
292 **Host-Parasite Coevolution.** *PLoS Comput Biol* 2012, **8**:e1002633.
- 293 50.** Seppälä O, Jokela J: **Do Coinfections Maintain Genetic Variation in Parasites?** *Trends*
294 *Parasitol* 2016, **32**:930–938.
- 295 An interesting conceptual review article arguing that interactions with co-infecting parasites contribute to the
296 maintenance of genetic diversity in parasites.
- 297 51. Oliver KM, Campos J, Moran NA, Hunter MS: **Population dynamics of defensive symbionts**
298 **in aphids.** *Proc R Soc B Biol Sci* 2008, **275**:293–299.
- 299 52. Zytynska SE, Meyer ST, Sturm S, Ullmann W, Mehrparvar M, Weisser WW: **Secondary**
300 **bacterial symbiont community in aphids responds to plant diversity.** *Oecologia* 2016,
301 **180**:735–747.
- 302 53. Petermann JS, Müller CB, Weigelt A, Weisser WW, Schmid B: **Effect of plant species loss on**
303 **aphid-parasitoid communities.** *J Anim Ecol* 2010, **79**:709–720.
- 304 54. Hrčák J, McLean AHC, Godfray HCJ: **Symbionts modify interactions between insects and**
305 **natural enemies in the field.** *J Anim Ecol* 2016, **85**:1605–1612.
- 306 55.* Kraft LJ, Kopco J, Harmon JP, Oliver KM: **Aphid symbionts and endogenous resistance**
307 **traits mediate competition between rival parasitoids.** *PLoS One* 2017, **12**:1–16.
- 308 56.* McLean AHC, Godfray HCJ: **The outcome of competition between two parasitoid species is**
309 **influenced by a facultative symbiont of their aphid host.** *Funct Ecol* 2017, **31**:927–933.
- 310 Two laboratory studies on pea aphids showing that the specificity of symbiont-conferred protection has the
311 potential to affect parasitoid communities by mediating competition among different parasitoid species.
- 312 57.** Rothacher L, Ferrer-Suay M, Vorburger C: **Bacterial endosymbionts protect aphids in the**
313 **field and alter parasitoid community composition.** *Ecology* 2016, **97**:1712–1723.
- 314 A study demonstrating the significance and selectivity of symbiont-conferred resistance against natural
315 enemies under field conditions, corroborating laboratory-based evidence.
- 316 58. Brockhurst MA, Koskella B: **Experimental coevolution of species interactions.** *Trends Ecol*
317 *Evol* 2013, **28**:367–375.
- 318 59. Glutton-Brock TH, Vincent ACJ: **Sexual selection and the potential reproductive rates of**
319 **males and females.** *Nature* 1991, **351**:58–60.
- 320 60. Bonduriansky R: **The evolution of male mate choice in insects: A synthesis of ideas and**
321 **evidence.** *Biol Rev Camb Philos Soc* 2001, **76**:305–339.
- 322 61. Sommer S: **The importance of immune gene variability (MHC) in evolutionary ecology**
323 **and conservation.** *Front Zool* 2005, **2**:1–18.
- 324 62. Matzaraki V, Kumar V, Wijmenga C, Zhernakova A: **The MHC locus and genetic**
325 **susceptibility to autoimmune and infectious diseases.** *Genome Biol* 2017, **18**:76.

- 326 63. Bernatchez L, Landry C: **MHC studies in nonmodel vertebrates: What have we learned**
327 **about natural selection in 15 years?** *J Evol Biol* 2003, **16**:363–377.
- 328 64. Eizaguirre C, Lenz TL: **Major histocompatibility complex polymorphism: Dynamics and**
329 **consequences of parasite-mediated local adaptation in fishes.** *J Fish Biol* 2010, **77**:2023–
330 2047.
- 331 65. Eizaguirre C, Lenz TL, Sommerfeld RD, Harrod C, Kalbe M, Milinski M: **Parasite diversity,**
332 **patterns of MHC II variation and olfactory based mate choice in diverging three-spined**
333 **stickleback ecotypes.** *Evol Ecol* 2011, **25**:605–622.
- 334 66. Wegner KM, Reusch TBH, Kalbe M: **Multiple parasites are driving major**
335 **histocompatibility complex polymorphism in the wild.** *J Evol Biol* 2003, **16**:224–232.
- 336 67. Göüy De Bellocq J, Charbonnel N, Morand S: **Coevolutionary relationship between**
337 **helminth diversity and MHC class II polymorphism in rodents.** *J Evol Biol* 2008, **21**:1144–
338 1150.
- 339 68. Šimková A, Ottová E, Morand S: **MHC variability, life-traits and parasite diversity of**
340 **European cyprinid fish.** *Evol Ecol* 2006, **20**:465–477.
- 341 69. Bordes F, Morand S: **The impact of multiple infections on wild animal hosts: a review.**
342 *Infect Ecol Epidemiol* 2011, **1**:7346.
- 343 70. Hansen AK, Jeong G, Paine TD, Stouthamer R: **Frequency of secondary symbiont infection**
344 **in an invasive psyllid relates to parasitism pressure on a geographic scale in California.**
345 *Appl Environ Microbiol* 2007, **73**:7531–7535.
- 346 71. Herzog J, Muller CB, Vorburger C: **Strong parasitoid-mediated selection in experimental**
347 **populations of aphids.** *Biol Lett* 2007, **3**:667–669.
- 348 72.* Käch H, Mathé-Hubert H, Dennis AB, Vorburger C: **Rapid evolution of symbiont-mediated**
349 **resistance compromises biological control of aphids by parasitoids.** *Evol Appl* 2018,
350 **11**:220–230.
- 351 An experimental study using caged laboratory populations to demonstrate that selection by parasitic wasps
352 results in a rapid increase of symbiont-protected aphids, to the point that the increasingly resistant pest
353 populations escape control by their natural enemies.
- 354 73.** Sanders D, Kehoe R, van Veen FF, McLean A, Godfray HCJ, Dicke M, Gols R, Frago E:
355 **Defensive insect symbiont leads to cascading extinctions and community collapse.** *Ecol*
356 *Lett* 2016, **19**:789–799.
- 357 A convincing demonstration of the strength of protection provided by a defensive symbiont and its
358 consequences for insect communities. When protected by a symbiont, one aphid species can escape control by
359 parasitoids and outcompete other aphid species on the same host plants.
- 360 74. Rouchet R, Vorburger C: **Experimental evolution of parasitoid infectivity on symbiont-**
361 **protected hosts leads to the emergence of genotype specificity.** *Evolution* 2014, **68**:1607–
362 1616.
- 363 75. Dennis AB, Patel V, Oliver KM, Vorburger C: **Parasitoid gene expression changes after**
364 **adaptation to symbiont-protected hosts.** *Evolution* 2017, **71**:2599–2617.
- 365 76. Dion E, Zélé F, Simon JC, Outreman Y: **Rapid evolution of parasitoids when faced with the**
366 **symbiont-mediated resistance of their hosts.** *J Evol Biol* 2011, **24**:741–750.

Box 1: The MHC of vertebrates and parasite driven selection

The major histocompatibility complex (MHC) is a gene region that consists of multiple loci encoding parts of the adaptive immune system of all jawed vertebrates. For each locus there are numerous alleles making the MHC extremely diverse. The MHC molecules, i.e. antigen binding transmembrane proteins encoded by this region, contain a highly variable antigen-binding site capable of the specific recognition of pathogens. In each MHC molecule, this site binds to specific parasite-derived antigenic peptides and presents them on the cell surface where they are recognised by specific T-cells, which then activate a specific immune response. In order to prevent self-reactivity, all T-cells that bind to self-peptides are eliminated during T-cell production (negative T-cell selection). Each individual possesses only a limited number of different MHC alleles and hence is not equally effective in fighting off all parasites. Certain MHC molecules have frequently been found to be associated with resistance to particular parasites and the evidence for some relationship between protection against parasites and individual MHC diversity is convincing, but the exact shape of this relationship is less clear and selection might favour optimal rather than maximal MHC diversity [reviewed by 43,45,47,61]. Such an optimum in MHC diversity would ensue if there were a cost to high MHC diversity. Indeed, individual MHC diversity might be restricted by negative T-cell selection limiting the capability of increasing the number of parasites that can be recognised through increasing MHC diversity and the risk of autoimmune diseases that increases with increasing MHC diversity [43,45,47]. Additionally, certain MHC alleles, in addition to their protective effect, are associated with susceptibility to autoimmune diseases or parasites [43,45,62]. The MHC repertoire and its diversity differs between populations and species [47,63,64]. Frequently, populations or species co-occurring with a more diverse parasite community have a more diverse MHC repertoire [65–69]. It has been the subject of an ongoing debate whether MHC diversity is driven by heterozygote advantage and/ or frequency dependent selection, but in either case it seems clear that parasites are the driving force behind the diversity [43–45,47,61,63].

396 **Tables**

397

398 **Table 1: Comparison between properties of MHC and symbiont-conferred protection**

	Symbionts	MHC
Prevalence	Intermediate, highly variable (encoded by facultative symbionts)	100% (encoded by the host genome)
Diversity between individuals	High	Very high
Differences between Populations/ environments	Yes	Yes
Effective protection against pathogens/ parasites	Yes	Yes
Specificity	Yes	Yes
Costs	Yes	Yes (depend on individual MHC diversity)

Table 2: Evidence for associations between symbiont and parasite diversity

Approach	Host	Symbiont	Parasite	Finding	Reference
A: Correlation between symbiont and parasite communities in natural populations					
Field survey	Lerp psyllid, <i>Glycaspis brimblecombei</i>	<i>Psyllaphaegus bliteus</i>	Parasitoid, <i>Psyllaphaegus bliteus</i>	Positive correlation between symbiont and parasitoid frequency	[70]
	Pea aphid, <i>Acyrtosiphon pisum</i>	Various, split up by parasitoid and fungal defender	Natural parasitoid and fungus communities	Some correlations between symbiont and parasite prevalence, but not all positive, large variation between species and sites	[48]
B: Parasites affecting symbiont frequencies and communities					
Experimental evolution	Peach-potato aphid, <i>Myzus persicae</i>	<i>Regiella</i> (natural infection)	Parasitoids, <i>Diaeretiella rapae</i> and <i>Aphidius colemani</i>	<i>Regiella</i> -infected aphid clone increases in the presence of either parasitoid	[71]
	<i>A. pisum</i>	<i>Hamiltonella</i> , <i>Serratia</i>	Parasitoid, <i>Aphidius ervi</i>	<i>Hamiltonella</i> increases in the presence of parasitoids and decreases in its absence; <i>Serratia</i> always decreases	[51]
	Black bean aphid, <i>Aphis fabae</i>	<i>Hamiltonella</i>	Parasitoid, <i>Lysiphlebus fabarum</i>	<i>Hamiltonella</i> increases (up to complete fixation in some replicates) in the presence of parasitoids	[72]
	<i>Drosophila hydei</i>	<i>Spiroplasma</i>	Parasitoid, <i>Leptopilina heterotoma</i>	<i>Spiroplasma</i> increases in the presence of parasitoids, but remains constant in their absence	[7]
C: Symbionts affecting parasite frequencies and communities					
Field experiment	<i>A. pisum</i>	<i>Regiella</i> and <i>Hamiltonella</i>	Natural parasite community (parasitoids, fungi)	Causes of mortality altered by symbionts; reduced incidence of fungi in the presence of <i>Regiella</i> , reduced incidence of parasitoids in the presence of <i>Hamiltonella</i>	[54]
Lab experiment		Defensive and non-defensive <i>Hamiltonella</i>	<i>A. ervi</i>	Protection can drive parasitoids to extinction	[73]
		<i>Hamiltonella</i>	Parasitoids, <i>Praon pequodorum</i> and <i>A. ervi</i>	Altered competition between the parasitoids; <i>Hamiltonella</i> reverses competitive abilities and reduces the differences in competitive outcomes	[55]
Experimental infections				Parasitoids, <i>Aphelinus abdominalis</i> and <i>A. ervi</i>	<i>Hamiltonella</i> excludes the parasitoid that in its absence can exclude the other parasite
Field experiment	<i>A. fabae</i>		Natural parasitoid community	Altered parasitoid community and increased parasitoid diversity in the presence of <i>Hamiltonella</i>	[57]
Experimental Evolution		<i>Hamiltonella</i> (different strains)	Parasitoid, <i>Lysiphlebus fabarum</i>	Parasitoids evolve to overcome symbiont conferred resistance; specific to the co-evolving strain	[74,75]
	<i>A. pisum</i>	<i>Hamiltonella</i>	<i>A. ervi</i>	Parasitoids evolve to overcome symbiont conferred resistance	[76]

Figure legends

Figure 1: Expected effect of parasite diversity on symbiont diversity. Two types of parasites and two types of protective symbionts are represented by different coloured wasps and different coloured circles within aphid hosts, respectively. If symbiont protection is specific (i.e. protection by the blue symbiont only works against the blue parasite and the red symbiont only protects against the red parasite) and only one type of parasite occurs, only the corresponding symbiont that transfers protection against that particular parasite will be maintained over evolutionary time (A, B). If, however, both types of parasites co-occur, both symbionts confer some protection (and hence fitness advantage) and should hence be maintained (C).

