Nine facultative endosymbionts in aphids. A review

Jianqing Guo, Séverin Hatt, Kanglai He, Julian Chen, Frédéric Francis, Zhenying Wang

PII: S1226-8615(17)30026-2
DOI: doi: 10.1016/j.aspen.2017.03.025
Reference: ASPEN 967

To appear in: *Journal of Asia-Pacific Entomology*

Received date: 14 January 2017
Revised date: 28 February 2017

Please cite this article as: Jianqing Guo, Séverin Hatt, Kanglai He, Julian Chen, Frédéric Francis, Zhenying Wang , Nine facultative endosymbionts in aphids. A review. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Aspen(2017), doi: 10.1016/j.aspen.2017.03.025

Nine facultative endosymbionts in aphids. A review

Jianqing Guo[1,2,3], Séverin Hatt[1,2,3], Kanglai He[1], Julian Chen[1], Frédéric Francis[2, *], Zhenying Wang[1, *]

[1] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

[2] *Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium*

[3] *TERRA - AgricultureIsLife, Gembloux Agro–Bio Tech, University of Liège, Passage des Déportés 2, B–5030 Gembloux, Belgium*

[*] *Corresponding authors, Frédéric Francis: Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, B-5030 Gembloux, Belgium. E-mail: frederic.francis@ulg.ac.be. Tel: +32 81622283; Zhenying Wang: Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Beijing 100193, China. E-mail: zywang@ippcaas.cn. Tel: +86 1062815945*

Abstract

Aphids are frequently engaged in mutualistic associations with endosymbionts. Symbionts are bacterial or fungal microorganisms that can be obligate or facultative to aphids. Research showed facultative (or secondary) symbionts have numerous effects on their host aphids such as resistance to heat shock, parasitoids and fungus etc., which may consequently promote a co-evolution between symbionts and hosts. However, this symbiotic relation may be affected by several factors, such as the ability of symbionts to spread from aphids to others within and across populations, and the cost of infections for hosts. Moreover, aphid-symbionts interactions may be affected by aphid living environment such as its host plant, the presence of natural enemies or the temperature. Here we firstly described the functions of nine facultative symbionts (*Serratia symbiotica*, *Hamiltonella defensa*, *Regiella insecticola*, *Rickettsia*, *Rickettsiella*, PAXS (pea aphid X-type symbiont), *Spiroplasma*, *Wolbachia* and *Arsenophonus*) studied in aphids one by one, and discussed the associations between these symbionts and aphids, plants and environment. We aim to have a better knowledge of the roles the facultative symbionts play in aphid biology, ecology and evolution, which we believe can provide new inspirations for aphid control.

Key words: Facultative symbionts; Aphididae; Function; Interaction; Evolution

INTRODUCTION

Symbionts are symbiotic microorganisms known to have a close and ongoing mutualistic relationship (i.e., win-win interactions, for instance when one partner species provides shelter or nutrition while the other offers protection against natural enemies (Janzen, 1966)) with a wide diversity of insects. Aphid (Hemiptera: Aphididae) -symbiont interactions are estimated to have started 160 to 280 million years ago (Fukatsu, 1994). Since then, co-evolution of symbionts with their hosts allowed mutualism on a long-term.

Some symbionts are obligates (also called primary) while others are facultative (also called secondary or S-symbionts). In aphids, obligate symbiont *Buchnera aphidicola* is indispensable for that it can provide them essential amino acids they cannot obtain from the phloem of plants (Douglas, 1998; Shigenobu et al., 2000; Baumann, 2005). Facultative symbionts are numerous and exhibit diverse effects on hosts through various functions that can be beneficial or detrimental to aphids (Oliver et al., 2010). Frequency of facultative symbionts in nature is maintained intermediately (Oliver, 2014) as it exists because of the possible cost for the infection such as longevity reduction (Vorburger, 2011).

The functions conferred by facultative symbionts may depend on various factors (Chen et al., 2000; Asplen et al., 2014; Peccoud et al., 2015). Indeed, the ability of symbionts to spread within and across aphid populations will determine the infection frequency and finally whether symbiont functions can remain in some environments.

Moreover, co-infection (i.e., infection of multiple symbiont species in one host simultaneously) as well as aphid living environment (e.g., aphid host plant, presence of natural enemies, temperature) may modify the way symbionts affect their hosts.

In the present paper, we systematically described the effects that have been reported of nine most studied facultative symbionts on aphids. We first described the occurrence of facultative symbionts, then reviewed the nine most studied aphid symbionts and their functions, and finally analyzed the interactions with environmental factors that may affect symbionts presence and functions.

1 Coexistence with host aphid

1.1 Transmission and co-infection

There are two transmission ways for symbionts to spread their distribution. Vertical transfer (i.e., from mother to offspring) is symbiont's main pathway to spread within a population, while horizontal transfer (i.e., from one individual to another within one population or between species) occasionally happens, allowing them to spread more widely helping to shape their distributions (Russell et al., 2003; Russell and Moran, 2005; Jaenike, 2012). Horizontal transfer is notably possible by the use of an intermediary individual. For instance, parasitoids, that sequentially stab infected or uninfected aphids, can act as vectors to transfer symbionts (Gehrer and Vorburger, 2012). However, horizontal transmission rate is considered low (Russell and Moran, 2005). Study indicated that through aphid sexual reproduction, maternal transmission can fail while paternal transmission is very rare (Peccoud et al., 2014). Also, selection against symbiont-positive aphids may occur (Darby et al., 2001).

Co-infection (i.e., infection of multiple symbiont species in one host simultaneously) are frequently observed (Gómez-Valero et al., 2004; Tsuchida et al., 2004; Oliver et al., 2006; Frantz et al., 2009; Nyabuga et al., 2010; Ferrari et al., 2012; Russell et al., 2013; Oliver et al., 2014; Vorburger, 2014). However, the frequency of co-infection and combination preference among different symbionts are rarely studied. Pea aphid *Acyrthosiphon pisum* commonly harbor one facultative symbiont rather than two (Tsuchida et al., 2002) since single infection is stable while symbiont loss is observed with multiple infection (Smith et al., 2015).

Coexistent symbionts may affect each other which play a significant role in determining the distributions and functions of symbionts in their host. All negative, neutral and positive interactions are possible. For instance, it was reported that *B. aphidicola* population can be significantly suppressed by the presence of *Rickettsia*, particularly at the young stage of aphid adults (Sakurai et al., 2005), while the pathogen-protective phenotype conferred by *Rickettsia* and *Spiroplasma* was found not to be influenced in the presence of *H. defensa* (Łukasik et al., 2013b). Finally, co-infection of *H. defensa*-*Spiroplasma*, *R. insecticola*-*Spiroplasma* (Nyabuga et al., 2010) or *H. defensa*-*S. symbiotica* (Oliver et al., 2006) may provide greater protection, compared to aphids infected by *H. defensa* or *R. insecticola* alone. Moreover, it can also be complicated: the protective phenotype of *H. defensa* is not affected by the coexistence of *Rickettsiella*, but the abundance of *H. defensa* is suppressed; whereas *H. defensa* is negative to *Rickettsiella*-mediated phenotype, but no influence on *Rickettsiella* abundance (Leclair et al., 2016a). However, the interactions among different symbionts can vary according to the genotype of the hosts, the species, the number of symbionts and even their density in the hosts (Sakurai et al., 2005; Łukasik et al., 2013b).

1.2 Frequency and cost of infection

Although insects benefit from facultative symbionts substantially, the frequency of facultative symbionts remains at intermediate level (Castañeda et al., 2010; Oliver et al., 2014). It can vary according to temporal and spatial gradients, food plant associations (Oliver et al., 2014) or the presence of natural enemies (Oliver et al., 2008). One reason for the moderate frequency of infection is that it may represent a cost for aphids (Scarborough et al., 2005; Oliver et al., 2003; 2008). Evidences for costs are variable between studies, with some documenting no cost under benign conditions (Castañeda et al., 2010), while others reporting aphid fitness reduction (Sakurai et al., 2005; Simon et al., 2007; Laughton et al., 2013). For example, aphids affected with *S. symbiotica* or *H. defensa* could suffer reduced longevity (Chen et al., 2000; Vorburger and Gouskov, 2011), shorter lifetime reproduction when the parasitoid is absent (Vorburger et al., 2013), delayed development time and reduced mass (Dykstra et al., 2014). Interestingly, for *H. defensa*, the stronger protection one strain confers, the less reduced longevity and lifetime reproduction unparasitized aphid suffers (Cayetano et al., 2015).

Moreover, facultative symbionts may negatively affect the interactions between their hosts and other organisms. For instance, *A. pisum* infected by parasitoid-protective symbionts may exert less defensive behaviors against predators such as ladybeetles, thus suffering from a higher predation than symbiont-free aphids (Polin et al., 2014). Similar observations were reported for aphids co-infected with *Rickettsiella viridis* and *H. defensa*, compared with aphids that were single infected with *R. viridis* (Polin et al., 2015).

2 Nine facultative symbionts: descriptions and functions

Facultative symbionts are pleomorphic (i.e., tubular, globular and short rods) under different endosymbiotic conditions (Fukatsu et al., 2000). They can live in hemolymph within sheath cells that are related to the bacteriome, which also host primary symbiont *B. aphidicola*, or can be located within bacteriocytes, in this case excluding *B. aphidicola* (Oliver et al., 2010). So far, nine facultative symbiont species have been repeatedly studied in aphids (Table 1).

Facultative symbiotic bacteria can influence several aspects of aphid ecology (Table 2; Figure 1). Oliver et al. (2003) first proposed the "symbiont-mediated resistance" to describe that symbionts can endow aphids with defense against parasitoid attacks. Moreover, symbionts are associated with aphid fitness, competitiveness, their capacity of exploiting their host plants and stress resistance

(Montllor et al., 2002; Leonardo and Muiru, 2003; Oliver et al., 2005; Haine, 2008). Aphids, like any other animals, carry their own immune response to harmful environmental factors such as the presence of pathogens, parasitism and high temperature. However, when infected by symbionts, some species (e.g., the pea aphid) may miss critical genes involved in recognition, signaling and killing of microbes (Elsik, 2010; Gerardo et al., 2010). An explanation may be that their association with mutualistic symbionts could benefit their vitality without a strong immune protection. For instance, the expression levels of Macrophage Migration Inhibitory Factors genes (*ApMIFs*) in *A. pisum*, that increase upon challenge with a parasitoid or a Gram-negative bacteria, is reduced in the presence of facultative symbionts (Dubreuil et al., 2014). As for physiological responses, the presence of facultative symbiont in *A. pisum* can significantly influence the encapsulation response and immune cell counts, whereas the host genotype has less effect on determining immunity outcomes (Laughton et al., 2016). Finally, the facultative symbionts play important roles in shaping their host immunity. Most of the studies focused on one single effect of a facultative symbiont on one aphid species (Scarborough et al., 2005; Guay et al., 2009; Oliver et al., 2009; Vorburger et al., 2009a; Simon et al., 2011). However, a single endosymbiont can also simultaneously confer multiple ecological benefits to its host (Heyworth and Ferrari, 2015).

2.1 *Serratia symbiotica*

Chen and Purcell (1997) designated pea aphid secondary symbiont as PASS and the symbiont was later referred to as R-type (Sandström et al., 2001). Moran et al. (2005) then designated R-type symbionts as "*Candidatus* Serratia symbiotica" by phylogenetic analysis through gyrB, recA and 16S rRNA genes.

Serratia symbiotica has been detected in different aphid species and was diverged into two phylogenetic clades (cluster A and B) based on the 16 rRNA gene (Lamelas et al., 2008). Furthermore, it may undergo the transition from facultative symbiont to obligate symbiont (Pérez-Brocal et al., 2006; Lamelas et al., 2008; Manzano-Marín and Latorre, 2014). For instance, one of its cluster (cluster B) has become a co-obligate endosymbiont of *B. aphidicola* as the primary symbiont in the aphid *Cinara cedri* Mimeur (Lamelas et al., 2008; 2011). Nevertheless, in other aphid species such as *A. pisum*, such a change has not been observed (Manzano-Marín and Latorre, 2014).

S. symbiotica is involved in defense against various adverse conditions for the host aphids. First is heat resistance (Chen et al., 2000; Montllor et al., 2002; Gómez-Valero et al., 2004). For instance the frequency of *S. symbiotica* in *A. pisum* in California can increase in two-third with increasing seasonal temperature (Montllor et al., 2002) Additionally, *S. symbiotica* plays important role in resistance to parasitoid wasps in inducing the mortality of developing parasitoid larvae four to five days after oviposition (Oliver et al., 2003; 2005). *S. symbiotica* with other symbionts (e.g., *Hamiltonella defensa*, see below) also provide protection against predators, by reducing the fitness and reproduction of ladybeetles (Coleoptera: Coccinellidae) (Costopoulos et al., 2014). Moreover, evidences also showed potential nutrition supply capacity of *S. symbiotica* (Koga et al., 2003; Pérez-Brocal et al., 2006; Koga et al., 2007).

2.2 *Hamiltonella defensa*

The symbiont found in the gut and ovaries of aphids was designated as PABS (pea aphid Bemisia-like symbiont) because of the high 16S rDNA sequence similarity to a facultative symbiont in the silverleaf whiteflies *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Darby et al., 2001). It was also referred to as T-type symbiont (Sandström et al., 2001) and then named as "*Candidatus* Hamiltonella defensa" to honor the evolutionary biologist William D. Hamilton (Moran et al., 2005).

Aphids with *H. defensa* are resistant to heat shock even though not as tolerant as *S. symbiotica* (Russell and Moran, 2006). Castañeda et al. (2010) reported *H. defensa* can increase aphid body mass and fecundity. However, the most famous effect of *H. defensa* is that this bacteria protect aphid against parasitoid wasps by disturbing their development inside aphid host (Oliver et al., 2003; 2005; 2008) and the level of protection depends on various factors. First, it depends on symbiont isolates. Indeed, from various ones of *H. defensa*, the conferred resistance can vary considerably (Leclair et al., 2016b), whereas one *H. defensa* isolate confers similar levels of resistance in different pea aphid clones (Oliver et al., 2005). Besides, protection level also rely on symbiont consortium (Leclair et al., 2016b) and aphid species, because no parasitoid resistance was conferred to *Sitobion avenae* by *H. defensa* (Łukasik et al., 2015). Also, *H. defensa*-conferred resistance varies according to parasitoid species.

Cayetano and Vorburger (2015) notably reported that it provides strong protection against *Lysiphlebus fabarum* Marshall (Hymenoptera: Braconidae) and *Aphidius colemani* Viereck (Hymenoptera: Braconidae), but not so against other parasitoid species. In addition, three asexual lines of *L. fabarum* performed variously when facing different symbiont strains, in the end resulting in a significant genotype-by-genotype interaction (Cayetano and Vorburger, 2015). Moreover, *H. defensa* in *A. pisum* from different host plant provide protection to different parasitoids indicating the specific protection against parasitoids of the symbiont (Mclean and Godfray, 2015). Finally, environmental factors such as heat may reduce the parasitoid resistance conferred by *H. defensa* (Guay et al., 2009).

Sometimes, *H. defensa* may not directly increase aphid resistance to some parasitoid species, but can still decrease their attraction: experienced parasitoids may prefer to oviposit into uninfected aphid compared with infected ones (Łukasik et al., 2013a). However, parasitoids can adapt to aphid resistance after having been exposed to *H. defensa* for a certain period of time (Dion et al., 2011), although they still showed lower emergence, longer development time and smaller size than other parasitoids developing in aphids without *H. defensa* (Schmid et al., 2012). *H. defensa*-based protection depend on a kind of bacteriophages called *Acyrthosiphon pisum* Secondary Endosymbionts (APSEs) (van der Wilk et al., 1999; Sandstrom et al., 2001; Degnan and Moran, 2008a; Oliver et al., 2009; Duron, 2014; Martinez et al., 2014; Vorburger, 2014). To date, seven different APSE strains have been partially sequenced and identified for toxin variation (Degnan and Moran, 2008b).

2.3 *Regiella insecticola*

The bacterium detected from strains belonging to the γ-*Proteobacteria* was firstly designated as U-type (Sandström et al., 2001) and referred to as PAUS (pea aphid U-type symbiont) (Tsuchida et al., 2002). Later, Moran *et al.* (2005) named it "*Candidatus* Regiella insecticola" in honor of the entomologist Reginald F. Chapman and found that *H. defensa* and *R. insecticola* are sister groups.

Host plant fitness is likely to be affected by aphid genomic variation (Leonardo, 2004), and also by *R. insecticola* (Tsuchida et al., 2004; 2011), but the effect rely on the host genotype. Actually, the fitness of *R. insecticola*-infected *A. pisum* on red clover *Trifolium pratense* L. varies depending on aphid clones (Ferrari et al., 2007).

R. insecticola can provide protection to aphids against pathogen and parasitoids. For instance, infected *A. pisum* showed a decreased mortality to a fungal entomopathogen *Pandora neoaphidis* (Ferrari et al., 2004; Scarborough et al., 2005; Łukasik et al., 2013c; 2015). Parasitoids resistance provided by *R. insecticola* was showed to be conferred by one of its specific isolate (Vorburger et al., 2009a), suggesting that different strains of the symbiont may provide various effects on its host aphid. Besides, *R. insecticola* play a role in inducing phenotypic variation of *A. pisum*, such as reducing winged offspring and altering the timing of sexual reproduction (Leonardo and Mondor, 2006). However, functions of *R. insecticola* are affected by temperature indicating the conditional benefit of the symbiont (Russell and Moran, 2006).

2.4 *Rickettsia*

Rickettsia has rods shape and is about 2 μm long and 0.5 μm thick (Sakurai et al., 2005). It was identified for the first time on *A. pisum* California clones and was named PAR (Pea Aphid Rickettsia) (Chen et al., 1996). PAR was then referred to as *Rickettsia* symbiont due to its belonging to the same named genus (Tsuchida et al., 2002). *Rickettsia* is localized in secondary mycetocytes and sheath cells (i.e., two types of endosymbiosis cells).

Rickettsia can increase the survival and fecundity of aphids. One clone of *A. pisum* infected with *Rickettsia* showed increased fitness and fecundity at 25℃ (Chen et al., 2000). Moreover, it can confer strong resistance to *P. neoaphidis* for *A. pisum* compared with uninfected ones (Łukasik et al., 2013c). However, when co-existing with *S. symbiotica*, heat-stress resistance provided by *Rickettsia* was not only cancelled but also heat sensitivity increased (Montllor et al., 2002).

In addition, *Rickettsia* infection can negatively affect its host fitness (Sakurai et al., 2005). For example, *Rickettsia*-infected *A. pisum* would have lower fitness on bur clover *Medicago hispida* Gaertn and sweet pea *Lathyrus odoratus* L. at 20℃compared to uninfected ones (Chen et al., 2000). The detrimental effect of *Rickettsia* on plant fitness is surprising regarding the high prevalence of this symbiont in some aphid populations (Simon et al., 2007). Several explanations have been proposed (Fukatsu et al., 2000; 2001): firstly, vertical transmission fidelity as it is the main pathway for most symbionts and *Rickettsia* is no exception; secondly, the frequency of horizontal transmission, that increases symbiont chance to spread (Sakurai et al., 2005) (see below for a description of symbiont transmission strategies); thirdly, fitness influence for their host, as symbionts endow benefits to aphids resulting in a better survival for themselves.

2.5 *Rickettsiella*

Rickettsiella can be located in secondary bacteriocytes, sheath cells, various tissues and the hemolymph (Tsuchida et al., 2010). Later the designation "*Candidatus* Rickettsiella viridis" was proposed for this symbiont in *A. pisum* (Tsuchida et al., 2014).

Rickettsiella can change the body color of *A. pisum* from red to green in natural populations by increasing blue green polycyclic quinones in the body, which consequently may confer protection from predators (Tsuchida et al., 2010). Thus it can be seen that the facultative symbionts can also affect important ecological traits such as phenotype of aphids except for biological traits. The body color of aphid tends to be greener by coinfection with *Hamiltonella* whereas the aphid fitness decreases compared with single infection of *Rickettsiella* (Tsuchida et al., 2014).

Rickettsiella also protects *A. pisum* against the important fungal pathogen *P. neoaphidis* by decreasing fungal sporulation on dead aphids (Łukasik et al., 2013c) although the symbiont has been considered as pathogens to other arthropods (Cordaux et al., 2007).

2.6 PAXS (X-type)

PAXS (pea aphid X-type symbiont) was a newly discovered symbiont from *A. pisum* and was found associated with *H. defensa* infection (Guay et al., 2009). Coinfection of PAXS and *H. defensa* may confer additional resistance to *A. ervi* compared with *H. defensa* alone and PAXS-*H. defensa* coinfection could remain high resistance under heat stress whereas *H. defensa* alone couldn't (Guay et al., 2009). What's more, PAXS-*H. defensa*/APSE coinfection protect *A. pisum* from parasitism by *A. ervi* even when superparasitised, however, protection against *A. ervi* endowed by *H. defensa*/APSE can be overcome by superparasitism (Donald et al., 2016). In addition, the study of Heyworth and Ferrari (2015) showed that PAXS can confer multiple ecological benefits to *A. pisum* including the resistance to parasitoid, *P. neoaphidis* and heat shock stress.

2.7 *Spiroplasma*

A symbiotic bacterium belonging to the genus *Spiroplasma* was firstly discovered from Japanese aphid strains and referred to as *Spiroplasma* symbiont (Fukatsu et al., 2001). To date, the genus *Spiroplasma* has been detected in several insect orders such as Diptera (Xie et al., 2010), Lepidoptera (Jiggins et al., 2000), Coleoptera (Majerus et al., 1999) and Hemiptera (Fukatsu et al., 2001).

In aphids, *Spiroplasma* infection has been found to induce male-killing phenotype (Simon et al., 2011) and confer fungi resistance by reducing mortality and decreasing fungal sporulation (Łukasik et al., 2013c). Conversely, *Spiroplasma* exhibited negative effects on growth, reproduction and longevity, especially in older adults (Fukatsu et al., 2001). However, when aphids were co-infected with *H. defensa-Spiroplasma* or *R. insecticola-Spiroplasma*, both the parasitism and mummification reduced significantly and the protection levels were even stronger than *H. defensa* or *R. insecticola* themselves (Nyabuga et al., 2010) implying the synergistic effect of *Spiroplasma* for the parasitoid resistance. Studied in *A. pisum*, *Spiroplasma* occurred more commonly among aphids collected from red clover and alfalfa *Medicago sativa* L. than pea *Pisum sativum* L. and broad bean *Vicia faba* L. (Frantz et al., 2009) suggesting the associations between facultative symbionts and aphid biotypes from different host plants.

2.8 *Wolbachia*

Wolbachia was first reported in the gonads of mosquito *Culex pipiens* Linnaeus (Hertig, 1936), whereas first found in aphids (*Toxoptera citricida* Kirkaldy and *Aphis craccivora* Koch) based on the sequencing of the *wsp* gene in 2000 (Jeyaprakash and Hoy, 2000). This symbiont can be located in head, thorax, abdomen, salivary glands, gastrointestinal tract and also reproductive tissues in *Drosophila* (Hadfield and Axton, 1999), whereas it may reside in reproductive tissues and muscles in aphids (Wang et al., 2014).

Wolbachia is usually considered to have a low prevalence in aphids. However, several studies recently focused on their mutualism. Augustinos et al. (2011) notably reported that 37 among 425 aphid samples collected from Spain, Portugal, Greece, Israel and Iran were infected with *Wolbachia*, while *Wolbachia* was also found to be widespread in Chinese aphids (Wang et al., 2014). Only a little progress has been made about the function of this bacteria in aphids so far. Research showed it is related with the high level of parthenogenesis in banana aphid *Pentalonia nigronervosa* Coquerel (De Clerck et al., 2014). Furthermore, co-obligatory symbiosis exists between *B. aphidicola* and *Wolbachia* in *P. nigronervosa*, favoring the supply of essential nutrients to the host aphid (De Clerck et al., 2015). But more work is needed to investigate if this symbiont has other effects on aphids.

2.9 *Arsenophonus*

Arsenophonus was initially discovered in the parasitoid wasp, *Nasonia vitripennis*, associated with son-killer trait and most commonly in straight rods shape (Huger et al., 1985), and then named it *Arsenophonus nasoniae* (Gherna et al., 1991). *Arsenophonus* genus has been identified in many insect species including aphids but not in *A. pisum* so far (Tsuchida et al., 2002; Russell et al., 2003; Nováková et al., 2009).

Arsenophonus provides general fitness benefits to aphids regardless of host plant resistance (Wulff and White, 2015), but no resistance to parasitoid and fungi (Wulff et al., 2013). Also, *Arsenophonus* can mediate dietary specialization of some herbivores. For instance, *A. craccivora* was found specialized on locust tree *Robinia pseudoacacia* L. when infected with the symbiont, on the contrary, uninfected individuals appeared alfalfa-biotype (Wagner et al., 2015). In addition, *Arsenophonus* are commonly infected with the bacteriophage APSE (Duron, 2014) demonstrating that APSE may play important role in protective symbiosis. However, research of *Arsenophonus* strains in aphid is still in the infancy stage and the infections spread patterns are unclear (Jousselin et al., 2013).

3 Interactions and evolution of facultative symbionts

3.1 Symbionts interactions with biotic and abiotic environment

Symbionts, aphids, plants, natural enemies and the environment are all directly or indirectly associated with each other composing a reticular structure (Figure 2) and the defensive symbionts may have the potential to alter the food web structure and dynamics (Rothacher et al., 2016; Hrček et al., 2016).

The interrelation study between these factors become more prevalent in recent years, such as host plant, parasitoid and symbiont (Mclean and Godfray, 2015); aphid, host plant and symbiont (Peccoud et al., 2015; Zytynska et al., 2016); host plant, parasitoid, natural enemies, symbiont and environment (Smith et al., 2015). First, as described above regarding *Arsenophonus*, facultative symbionts may influence the adaptation and distribution of their hosts through the colonization of new plants and climatic regions (Henry et al., 2013). Second, infection pattern of facultative symbionts are closely related to the host plant species. For example, *R. insecticola* emerge frequently in *A. pisum* collected from *Trifolium* whereas *S. symbiotica* with aphids collected from *Cytisus*, *Pisum* and *Vicia* (Ferrari et al., 2012). Third, parasitoids are negatively affected by defensive symbionts (implying a longer developmental time and reduced mass - Nyabuga et al., 2010) while the abundance of *H. defensa* is also influenced by parasitism (Martinez et al., 2014). Regarding the symbiont-parasitoid interaction, On one hand, different genotypes of parasitoids have different abilities to overcome *H. defensa*, generating a symbiont × parasitoid genotype interaction (Vorburger et al., 2009b). Some parasitoids exhibit rapid evolution when they are exposed to *H. defensa*, which finally allow them to reach a similar parasitism rate on infested aphids than on symbiont-free ones (Dion et al., 2011). On the other hand, different strains of *H. defensa* confer diverse levels of protection against parasitoids (Oliver et al., 2005), imposing different evolutionary challenges to the parasitoids. Thus, the host-parasite interaction mediated by symbionts may be exerted through line-by-line genetic specificity (Rouchet and Vorburger, 2014). Fourth, both the frequency (Smith et al., 2015) and protective level (Hrček et al., 2016) are negatively correlated to aphid mortality caused by other natural enemies that are not targeted by the symbionts. Finally, symbiotic functions and interactions often vary under different environment conditions. For example, defensive symbionts frequencies shift seasonally in order to adapt the seasonal changing of parasitoid pressures (Smith et al., 2015). Such variations act as an important role in the prevalence and distributions of symbiosis in nature (Russell and Moran, 2006).

3.2 Endosymbiont-host coevolution

The endosymbiotic diversity of aphids illuminates the dynamic of endosymbiotic evolution (Fukatsu, 1994). *H. defensa* of *A. pisum* and *B. tabaci* is estimated to have diverged 17 to 34 million years ago (Darby et al., 2001). Facultative symbionts can spread among different insect genotypes and exchange genes with other microbes which may expand ecological opportunities for insects (Janson et al., 2008). Also, *S. symbiotica* increases the heat tolerance of *A. pisum* demonstrating the abiotic environment adaptations conferred by symbionts (Russell and Moran, 2006), implying that symbionts may play a role in shaping the geographic distributions of host and consequently promote the host evolution on population level.

All symbionts transmitted vertically suffer gene loss over evolutionary time leaving a smaller genome (Burke and Moran, 2011). Many symbionts remain only the most essential functions including genes that benefit hosts (McCutcheon and Moran, 2012). For example, the genome of *S. symbiotica* decayed dramatically compared with free-living relatives of the same genus through deletions, pseudogenes and rearrangements (Burke and Moran, 2011). *B. aphidicola* has experienced a reductive process of genome size, retaining only essential genes for its specialized lifestyle (Gil et al., 2002) and might be replaced by the coexisting facultative symbiont (Gómez-Valero et al., 2004; Pérez-Brocal et al., 2006; Lamelas et al., 2008; 2011). To conclude, the endosymbionts may go through a long term evolutionary transition from free-living microbe in the early stage to obligate mutualism in host.

Conclusions

Facultative symbionts are undoubtedly involved in aphid fitness and evolution. There exist abundant symbionts in aphid and more (except for these nine) will be studied in the future which consequently give us the implications for better control of aphid. Symbiont-based strategies are expected to defeat aphid or obstruct the transmission of pathogenic microorganisms to reach the purpose of pest and disease management (Douglas et al., 2006; Gross et al., 2009). Most of the work done at present is on pea aphid, investigation of the facultative symbionts on other aphid species is needed. Research of symbiont-mediated protection and the functional mechanism is on the way with the development of molecular technology and there still remains a lot of work.

Acknowledgement

This research was funded by the CARE AgricultureIsLife (University of Liège), and the Opening Fund of State Key Laboratory for Biology of Plant Diseases and Insect Pests (SKLOF201601).

Disclosure

The authors declare that they have no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of their manuscript.

References

Asplen M.K., Bano N., Brady C.M., Desneux N., Hopper K.R., Malouines C., Oliver K.M., White J.A., Heimpel G.E., 2014. Specialisation of bacterial endosymbionts that protect aphids from parasitoids. Ecol. Entomo. 39(6), 736-739.

Augustinos A.A., Santos-Garcia D., Dionyssopoulou E., Moreira M., Papapanagiotou A., Scarvelakis M., Doudoumis V., Ramos S., Aguiar A.F., Borges P.A.V. et al., 2011. Detection and characterization of *Wolbachia* infections in natural populations of aphids: Is the hidden diversity fully unraveled? PLoS ONE 6, 1-10.

Baumann P., 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu. Rev. Microbiol. 59, 155-189.

Burke G.R., Moran N.A., 2011. Massive genomic decay in *Serratia symbiotica*, a recently evolved symbiont of aphids. Genome Biol. Evol. 3, 195-208.

Castañeda L.E., Sandrock C., Vorburger C., 2010. Variation and covariation of life history traits in aphids are related to infection with the facultative bacterial endosymbiont *Hamiltonella defensa*. Biol. J. Linn. Soc. 100, 237-247.

Cayetano L., Vorburger C., 2015. Symbiont-conferred protection against Hymenopteran parasitoids in aphids: how general is it? Ecol. Entomol. 40, 85-93.

Cayetano L., Rothacher L., Simon J.C., Vorburger C., 2015. Cheaper is not always worse: strongly protective isolates of a defensive symbiont are less costly to the aphid host. P. Roy. Soc. B-Biol. Sci. 282(1799), 20142333.

Chen D.Q., Campbell B.C., Purcell A.H., 1996. A new *Rickettsia* from a herbivorous insect, the pea aphid *Acyrthosiphon pisum* (Harris). Curr. Microbiol. 33, 123-128.

Chen D.Q., Purcell A.H., 1997. Occurrence and transmission of facultative endosymbionts in aphids. Curr. Microbiol. 34, 220-225.

Chen D.Q., Montllor C.B., Purcell A.H., 2000. Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. Entomol. Exp. Appl. 95, 315-323.

Cordaux R., Pacesfessy M., Raimond M., Michelsalzat A., Zimmer M., Bouchon D., 2007. Molecular characterization and evolution of arthropod-pathogenic *Rickettsiella* bacteria. Appl. Environ. Microb. 73(15), 5045-5047.

Costopoulos K., Kovacs J., Kamins A., Gerardo N., 2014. Aphid facultative symbionts reduce survival of the predatory lady beetle *Hippodamia convergens*. BMC Ecol. 14, 80-91.

Darby A.C., Birkle L.M., Turner S.L., Douglas A.E., 2001. An aphid-borne bacterium allied to the secondary symbionts of whitefly. FEMS Microbiol. Ecol. 36, 43-50.

De Clerck C., Tsuchida T., Massart S., Lepoivre P., Francis F., Jijakli M.H., 2014. Combination of genomic and proteomic approaches to characterize the symbiotic population of the banana aphid (Hemiptera: Aphididae). Environ. Entomol. 43, 29-36.

De Clerck C., Fujiwara A., Joncour P., Léonard S., Félix M.-L., Francis F., Jijakli M.H., Tsuchida T., Massart S., 2015. A metagenomic approach from aphid’s hemolymph sheds light on the potential roles of co-existing endosymbionts. Microbiome 3, 1-11.

Degnan P.H., Moran N.A., 2008a. Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. Mol. Ecol. 17, 916-929.

Degnan P.H., Moran N.A., 2008b, Diverse phage-encoded toxins in a protective insect endosymbiont. Appl. Environ. Microb. 74, 6782-6791.

Dion E., ZÉLÉ F., Simon J.C., Outreman Y., 2011. Rapid evolution of parasitoids when faced with the symbiont-mediated resistance of their hosts. J. Evolution. Biol. 24, 741-750.

Donald K.J., Clarke H.V., Mitchell C., Cornwell R.M., Hubbard S.F., Karley A.J., 2016. Protection of pea aphids associated with coinfecting bacterial symbionts persists during superparasitism by a braconid wasp. Microb. Ecol. 71(1), 1-4.

Douglas A., 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annu. Rev. Entomol. 43, 17-37.

Douglas A.E., FranÇois C.L. M.J., Minto L.B., 2006. Facultative ‘secondary’ bacterial symbionts and the nutrition of the pea aphid, *Acyrthosiphon pisum*. Physiol. Entomol. 31(3), 262-269.

Duron O., 2014. *Arsenophonus* insect symbionts are commonly infected with APSE, a bacteriophage involved in protective symbiosis. FEMS Microbiol. Ecol. 90, 184-194.

Dykstra H.R., Weldon S.R., Martinez A.J., White J.A., Hopper K.R., Heimpel G.E., Asplen M.K., Oliver K.M., 2014. Factors limiting the spread of the protective symbiont *Hamiltonella defensa* in *Aphis craccivora* aphids. Appl. Environ. Microb. 80, 5818-5827.

Elsik C.G., 2010. The pea aphid genome sequence brings theories of insect defense into question. Genome Biol. 11, 1-3.

Ferrari J., Darby A.C., Daniell T.J., Godfray H.C.J., Douglas A.E., 2004. Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. Ecol. Entomol. 29, 60-65.

Ferrari J., Scarborough C., Godfray H.C., 2007. Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. Oecologia 153, 323-329.

Ferrari J., West J.A., Via S., Godfray H.C.J., 2012. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. Evolution 66, 375-390.

Frantz A., Calcagno V., Mieuzet L., Plantegenest M., Simon J.C., 2009. Complex trait differentiation between host-populations of the pea aphid *Acyrthosiphon pisum* (Harris): implications for the evolution of ecological specialisation. Biol. J. Linn. Soc. 97, 718-727.

Fukatsu T., 1994. Endosymbiosis of aphids with microorganisms: a model case of dynamic endosymbiotic evolution. Plant Spec. Biol. 9, 145-154.

Fukatsu T., Nikoh N., Kawai R., Koga R., 2000. The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). Appl. Environ. Microb. 66, 2748-2758.

Fukatsu T., Tsuchida T., Nikoh N., Koga R., 2001. *Spiroplasma* symbiont of the pea aphid, *Acyrthosiphon pisum* (Insecta: Homoptera). Appl. Environ. Microb. 67, 1284-1291.

Gehrer L., Vorburger C., 2012. Parasitoids as vectors of facultative bacterial endosymbionts in aphids. Biol. Letters 8, 613-615.

Gerardo N.M., Altincicek B., Anselme C., Atamian H., Barribeau S.M., De Vos M., Duncan E.J., Evans J.D., Gabaldón T., Ghanim M., 2010. Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. Genome Biol. 11, R21.

Gherna R.L., Werren J.H., Weisburg W., Cote R., Woese C.R., Mandelco L., Brenner D.J., 1991. NOTES: *Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the son-killer trait in the parasitic wasp *Nasonia vitripennis*. Int. J. Syst. Bacteriol. 41, 563-565.

Gil R., Sabater-Muñoz B., Latorre A., Silva F.J., Moya A., 2002. Extreme genome reduction in *Buchnera* spp.: toward the minimal genome needed for symbiotic life. Proc. Natl. Acad. Sci. 99, 4454-4458.

Gómez-Valero L., Soriano-Navarro M., Pérez-Brocal V., Heddi A., Moya A., García-Verdugo J.M., Latorre A., 2004. Coexistence of *Wolbachia* with *Buchnera aphidicola* and a secondary symbiont in the aphid *Cinara cedri*. J. Bacteriol. 186, 6626-6633.

Gross R., Vavre F., Heddi A., Hurst G.D.D., Zchori-Fein E., Bourtzis K., 2009. Immunity and symbiosis. Mol. Microbiol. 73(5), 751-759.

Guay J.F., Boudreault S., Michaud D., Cloutier C., 2009. Impact of environmental stress on aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. J. Insect Physiol. 55, 919-926.

Hadfield S.J., Axton J.M., 1999. Reproduction: Germ cells colonized by endosymbiotic bacteria. Nature 402, 482.

Haine E.R., 2008. Symbiont-mediated protection. P. Roy. Soc. B-Biol. Sci. 275, 353-361.

Henry L.M., Peccoud J., Simon J.C., Hadfield J.D., Maiden M.J.C., Ferrari J., Godfray H.C.J., 2013. Horizontally transmitted symbionts and host colonization of ecological niches. Curr. Biol. 23, 1713-1717.

Hertig M., 1936. The Rickettsia, *Wolbachia pipientis* (gen. et sp.n.) and associated inclusions of the mosquito, *Culex pipiens*. Parasitology 28, 453-486.

Heyworth E.R., Ferrari J., 2015. A facultative endosymbiont in aphids can provide diverse ecological benefits. J. Evolution. Biol. 28, 1753-1760.

Houk E.J., Griffiths G.W., 1980. Intracellular symbiotes of the Homoptera. Annu. Rev. Entomol. 25, 161-187.

Hrček J., McLean A.H.C., Godfray H.C.J., 2016. Symbionts modify interactions between insects and natural enemies in the field. J. Anim. Ecol. DOI: 10.1111/1365-2656.12586.

Huger A.M., Skinner S.W., Werren J.H., 1985. Bacterial infections associated with the son-killer trait in the parasitoid wasp *Nasonia* (= *Mormoniella*) *vitripennis* (Hymenoptera: Pteromalidae). J. Invertebr. Pathol. 46(3), 272-280.

Jaenike J., 2012. Population genetics of beneficial heritable symbionts. Trends Ecol. Evol. 27, 226-232.

Janson E.M., Stireman J.O., Singer M.S., Abbot P. 2008. Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. Evolution 62, 997-1012.

Janzen D.H., 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20, 249-275.

Jeyaprakash A., Hoy M.A., 2000. Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. 9, 393-405.

Jiggins F.M., Hurst G.D.D., Jiggins C.D., v.d. Schulenburg J.H.G., Majerus M.E.N., 2000. The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. Parasitology 120, 439-446.

Jousselin E., Cœur d'Acier A., Vanlerberghe-Masutti F., Duron O., 2013. Evolution and diversity of *Arsenophonus* endosymbionts in aphids. Mol. Ecol. 22, 260-270.

Koga R., Tsuchida T., Fukatsu T., 2003. Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. P. Roy. Soc. B-Biol. Sci. 270, 2543-2550.

Koga R., Tsuchida T., Sakurai M., Fukatsu T., 2007. Selective elimination of aphid endosymbionts: effects of antibiotic dose and host genotype, and fitness consequences. FEMS Microbiol. Ecol.

60, 229-239.

Lamelas A., Pérez-Brocal V., Gómez-Valero L., Gosalbes M.J., Moya A., Latorre A., 2008. Evolution of the secondary symbiont "*Candidatus* Serratia symbiotica" in aphid species of the subfamily Lachninae. Appl. Environ. Microb. 74, 4236-4240.

Lamelas A., Gosalbes M.J., Manzano-Marín A., Peretó J., Moya A., Latorre A., 2011. *Serratia symbiotica* from the aphid *Cinara cedri*: a missing link from facultative to obligate insect endosymbiont. PLoS Genet. 7, e1002357.

Laughton A.M., Fan M.H., Gerardo N.M., 2013. The combined effects of bacterial symbionts and ageing on life history traits in the pea aphid, *Acyrthosiphon pisum*. Appl. Environ. Microb. 80, 470-477.

Laughton A.M., Garcia J.R., Gerardo N.M., 2016. Condition-dependent alteration of cellular immunity by secondary symbionts in the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol. 86, 17-24.

Leclair M., Polin S., Jousseaume T., Simon J. C., Sugio A., Morlière S., Fukatsu T., Tsuchida T., Outreman Y., 2016a. Consequences of coinfection with protective symbionts on the host phenotype and symbiont titres in the pea aphid system. Insect Sci. DOI, 10.1111/1744-7917.12380.

Leclair M., Pons I., Mahéo F., Morlière S., Simon J.C., Outreman Y., 2016b. Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host. Evol. Ecol. 30(5), 925-941.

Leonardo T.E., Muiru G.T., 2003. Facultative symbionts are associated with host plant specialization in pea aphid populations. P. Roy. Soc. B-Biol. Sci. 270 (Suppl 2), S209-S212.

Leonardo T.E., 2004. Removal of a specialization-associated symbiont does not affect aphid fitness. Ecol. Lett. 7, 461-468.

Leonardo T.E., Mondor E.B., 2006. Symbiont modifies host life-history traits that affect gene flow. P. Roy. Soc. B-Biol. Sci. 273, 1079-1084.

Łukasik P., Dawid M., Ferrari J., Godfray H.C., 2013a. The diversity and fitness effects of infection with facultative endosymbionts in the grain aphid, *Sitobion avenae*. Oecologia 173, 985-996.

Łukasik P., Guo H., van Asch M., Ferrari J., Godfray H.C.J., 2013b. Protection against a fungal pathogen conferred by the aphid facultative endosymbionts *Rickettsia* and *Spiroplasma* is expressed in multiple host genotypes and species and is not influenced by co-infection with another symbiont. J. Evolution. Biol. 26, 2654-2661.

Łukasik P., van Asch M., Guo H., Ferrari J., Godfray H.C.J., 2013c. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. Ecol. Lett. 16, 214-218.

Łukasik P., Guo H., van Asch M., Henry L.M., Godfray H.C.J., Ferrari J., 2015. Horizontal transfer of facultative endosymbionts is limited by host relatedness. Evolution 69, 2757–2766.

Majerus T., Der Schulenburg V., Graf J., Majerus M., Hurst G., 1999. Molecular identification of a male-killing agent in the ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Insect Mol. Biol. 8, 551-555.

Manzano-Marín A., Latorre A., 2014. Settling down: The genome of *Serratia symbiotica* from the aphid *Cinara tujafilina* zooms in on the process of accommodation to a cooperative intracellular life. Genome Biol. Evol. 6, 1683-1698.

Martinez A.J., Weldon S.R., Oliver K.M., 2014. Effects of parasitism on aphid nutritional and protective symbioses. Mol. Ecol. 23, 1594-1607.

McCutcheon J.P., Moran N.A., 2012. Extreme genome reduction in symbiotic bacteria. Nat. Rev. Microbiol. 10, 13-26.

McLean D.L., Houk E.J., 1973. Phase contrast and electron microscopy of the mycetocytes and symbiotes of the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol. 19, 625-633.

Mclean A.H., Godfray H.C., 2015. Evidence for specificity in symbiont-conferred protection against parasitoids. P. Roy. Soc. B-Biol. Sci. 282(1811).

Montllor C.B., Maxmen A., Purcell A.H., 2002. Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol. Entomol. 27, 189-195.

Moran N.A., Russell J.A., Koga R., Fukatsu T., 2005. Evolutionary relationships of three new species of Enterobacteriaceae living as symbionts of aphids and other insects. Appl. Environ. Microb. 71, 3302-3310.

Moran N.A., McCutcheon J.P., Nakabachi A., 2008. Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet. 42, 165-190.

Nault L.R., 1997. Arthropod transmission of plant viruses: a new synthesis. Ann. Entomol. Soc. Am. 90, 521-541.

Nováková E., Hypša V., Moran N.A., 2009. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. Bmc Microbiol. 9, 1-14.

Nyabuga F.N., Outreman Y., Simon J.C., Heckel D.G., Weisser W.W., 2010. Effects of pea aphid secondary endosymbionts on aphid resistance and development of the aphid parasitoid *Aphidius* *ervi*: a correlative study. Entomol. Exp. Appl. 136, 243-253.

Oliver K.M., Russell J.A., Moran N.A., Hunter M.S., 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc. Natl. Acad. Sci. 100, 1803-1807.

Oliver K.M., Moran N.A., Hunter M.S., 2005. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. Proc. Natl. Acad. Sci. USA 102, 12795-12800.

Oliver K.M., Moran N.A., Hunter M.S., 2006. Costs and benefits of a superinfection of facultative symbionts in aphids. P. Roy. Soc. B-Biol. Sci. 273, 1273-1280.

Oliver K.M., Campos J., Moran N.A., Hunter M.S., 2008. Population dynamics of defensive symbionts in aphids. P. Roy. Soc. B-Biol. Sci. 275, 293-299.

Oliver K.M., Degnan P.H., Hunter M.S., Moran N.A., 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325, 992-994.

Oliver K.M., Degnan P.H., Burke G.R., Moran N.A., 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol. 55, 247-266.

Oliver K.M., Smith A.H., Russell J.A., 2014. Defensive symbiosis in the real world-advancing ecological studies of heritable, protective bacteria in aphids and beyond. Funct. Ecol. 28, 341-355.

Peccoud J., Bonhomme J., Mahéo F., de la Huerta M., Cosson O., Simon J.C., 2014. Inheritance patterns of secondary symbionts during sexual reproduction of pea aphid biotypes. Insect Sci. 21, 291-300.

Peccoud J., Mahéo F., de la Huerta M., Laurence C., Simon J.C., 2015. Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. Insect Conserv. Diver. 8(5), 484-492.

Pérez-Brocal V., Gil R., Ramos S., Lamelas A., Postigo M., Michelena J.M., Silva F.J., Moya A., Latorre A., 2006. A small microbial genome: The end of a long symbiotic relationship? Science 314, 312-313.

Polin S., Simon J.C., Outreman Y., 2014. An ecological cost associated with protective symbionts of aphids. Ecol. Evol. 4, 826-830.

Polin S., Le Gallic J.F., Simon J.C., Tsuchida T., Outreman Y., 2015. Conditional reduction of predation risk associated with a facultative symbiont in an Insect. PLoS ONE 10, e0143728.

Rothacher L., Ferrer‐Suay M., Vorburger C., 2016. Bacterial endosymbionts protect aphids in the field and alter parasitoid community composition. Ecology 97(7), 1712-1723.

Rouchet R., Vorburger C., 2014. Experimental evolution of parasitoid infectivity on symbiont-protected hosts leads to the emergence of genotype specificity. Evolution 68, 1607-1616.

Roux V., Bergoin M., Lamaze N., Raoult D., 1997. Reassessment of the taxonomic position of *Rickettsiella grylli*. Int. J. Syst. Bacteriol. 47,1255– 1257.

Russell J.A., Latorre A., Sabater-Muñoz B., Moya A., Moran N.A., 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol. 12, 1061-1075.

Russell J.A., Moran N.A., 2005. Horizontal transfer of bacterial symbionts: Heritability and fitness effects in a novel aphid host. Appl. Environ. Microb. 71, 7987-7994.

Russell J.A., Moran N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. P. Roy. Soc. B-Biol. Sci. 273, 603-610.

Russell J.A., Weldon S., Smith A.H., Kim K.L., Hu Y., Łukasik P., Doll S., Anastopoulos I., Novin M., Oliver K.M., 2013. Uncovering symbiont-driven genetic diversity across North American pea aphids. Mol. Ecol. 22, 2045-2059.

Sakurai M., Koga R., Tsuchida T., Meng X.Y., Fukatsu T., 2005. *Rickettsia* symbiont in the pea aphid *Acyrthosiphon pisum*: Novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. Appl. Environ. Microb. 71, 4069-4075.

Sandström J.P., Russell J.A., White J.P., Moran N.A., 2001. Independent origins and horizontal transfer of bacterial symbionts of aphids. Mol. Ecol. 10, 217-228.

Scarborough C.L., Ferrari J., Godfray H.C.J., 2005. Aphid protected from pathogen by endosymbiont. Science 310, 1781.

Schmid M., Sieber R., Zimmermann Y.S., Vorburger C., 2012. Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. Funct. Ecol. 26, 207-215.

Shigenobu S., Hattori H.W.M., Sakaki Y., Ishikawa H., 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. Nature 407, 81-86.

Simon J.C., Sakurai M., Bonhomme J., Tsuchida T., Koga R., Fukatsu T., 2007. Elimination of a specialised facultative symbiont does not affect the reproductive mode of its aphid host. Ecol. Entomol. 32, 296-301.

Simon J.C., Boutin S., Tsuchida T., Koga R., Le Gallic J.-F., Frantz A., Outreman Y., Fukatsu T., 2011. Facultative symbiont infections affect aphid reproduction. PLoS ONE 6, e21831.

Smith A.H., Łukasik P., O'Connor M.P., Lee A., Mayo G., Drott M.T., Doll S., Tuttle R., Disciullo R.A., Messina A., 2015. Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. Mol. Ecol. 24(5), 1135-1149.

Tsuchida T., Koga R., Shibao H., Matsumoto T., Fukatsu T., 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. Mol. Ecol. 11, 2123-2135.

Tsuchida T., Koga R., Fukatsu T., 2004. Host plant specialization governed by facultative symbiont. Science 303, 1989.

Tsuchida T., Koga R., Horikawa M., Tsunoda T., Maoka T., Matsumoto S., Simon J.C., Fukatsu T., 2010. Symbiotic bacterium modifies aphid body color. Science (New York, N.Y.) 330(6007), 1102-1104.

Tsuchida T., Koga R., Matsumoto S., Fukatsu T., 2011. Interspecific symbiont transfection confers a novel ecological trait to the recipient insect. Biol. Letters 7, 245-248.

van der Wilk F., Dullemans A.M., Verbeek M., van den Heuvel J.F.J.M., 1999. Isolation and characterization of APSE-1, a bacteriophage infecting the secondary endosymbiont of *Acyrthosiphon pisum*. Virology 262, 104-113.

Vorburger C., Gehrer L., Rodriguez P., 2009a. A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. Biol. Letters 6, 109-111.

Vorburger C., Sandrock C., Gouskov A., Castañeda L.E., Ferrari J., 2009b. Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. Evolution 63, 1439-1450.

Vorburger C., Gouskov A., 2011. Only helpful when required: a longevity cost of harbouring defensive symbionts. J. Evolution. Biol. 24, 1611-1617.

Vorburger C., Ganesanandamoorthy P., Kwiatkowski M., 2013. Comparing constitutive and induced costs of symbiont‐conferred resistance to parasitoids in aphids. Ecol. Evol. 3, 706-713.

Vorburger C., 2014. The evolutionary ecology of symbiont-conferred resistance to parasitoids in aphids. Insect Sci. 21, 251-264.

Wagner S.M., Martinez A.J., Ruan Y.m., Kim K.L., Lenhart P.A., Dehnel A.C., Oliver K.M., White J.A., 2015. Facultative endosymbionts mediate dietary breadth in a polyphagous herbivore. Funct. Ecol. 29, 1402-1410.

Wang Z., Su X.M., Wen J., Jiang L.Y., Qiao G.X., 2014. Widespread infection and diverse infection patterns of *Wolbachia* in Chinese aphids. Insect Sci. 21, 313-325.

Werren J.H., Zhang W., Guo L.R., 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. P. Roy. Soc. B-Biol. Sci. 261, 55-63.

Wulff J.A., Buckman K.A., Wu K., Heimpel G.E., White J.A., 2013. The endosymbiont *Arsenophonus* is widespread in soybean aphid, *Aphis glycines*, but does not provide protection from parasitoids or a fungal pathogen. PLoS ONE 8, e62145.

Wulff J.A., White J.A., 2015. The endosymbiont *Arsenophonus* provides a general benefit to soybean aphid (Hemiptera: Aphididae) regardless of host plant resistance (Rag). Environ. Entomol. 44, 574-581.

Xie J., Vilchez I., Mateos M., 2010. Spiroplasma Bacteria Enhance Survival of Drosophila hydei

Attacked by the Parasitic Wasp Leptopilina heterotoma. PLoS ONE 5(8), e12149.

Zytynska S.E., Meyer S.T., Sturm S., Ullmann W., Mehrparvar M., Weisser W.W., 2016. Secondary bacterial symbiont community in aphids responds to plant diversity. Oecologia 180(3), 735-747.

Table 1 Nine facultative symbionts and their attribute

Symbionts	Other names	Bacterial group	References
Serratia symbiotica	PASS; R-type	*γ-proteobacteria*	(Chen et al., 1997)
Hamiltonella defensa	PABS; T-type	*γ-proteobacteria*	(Darby et al., 2001)
Regiella insecticola	PAUS; U-type	*γ-proteobacteria*	(Sandström et al., 2001)
Rickettsia	PAR; S-type	*α-proteobacteria*	(Chen et al., 1996)
Rickettsiella		*γ-proteobacteria*	(Roux et al., 1997)
PAXS	X-type	*γ-proteobacteria*	(Guay et al., 2009; Ferrari et al., 2012)
Spiroplasma		*Mollicutes*	(Fukatsu et al., 2001)
Wolbachia		*α-proteobacteria*	(Werren et al., 1995)
Arsenophonus		*γ-proteobacteria*	(Gherna et al., 1991)

Table 2 Reported impacts of nine facultative symbionts in aphids

Symbionts	Function	References
Serratia symbiotica	Heat shock resistance Parasitoid resistance Nutrition supply	(Chen et al., 2000; Russell and Moran, 2006) (Oliver et al., 2003; 2005) (Koga et al., 2003; 2007)
Hamiltonella defensa	Parasitoid resistance Heat shock resistance Increase the body mass and fecundity Longevity ↓ Defensive behavior ↓	(Oliver et al., 2003; 2005) (Russell and Moran, 2006) (Castañeda et al., 2010) (Vorburger et al., 2013) (Polin et al., 2014)
Regiella insecticola	Fungi resistance Parasitoids resistance Enhance host plant fitness Induce phenotypic variation	(Scarborough et al., 2005) (Vorburger et al., 2009a) (Tsuchida et al., 2004; 2011) (Leonardo and Mondor, 2006)
Rickettsia	Heat shock resistance Fungi resistance Host plant fitness ↓	(Chen et al., 2000) (Łukasik et al., 2013c) (Chen et al., 2000; Sakurai et al., 2005; Simon et al., 2007)
Rickettsiella	Change body color Fungi resistance	(Tsuchida et al., 2010; 2014) (Lukasik et al., 2013c)
PAXS	Parasitoids resistance Heat shock resistance Fungi resistance	(Donald et al., 2016; Heyworth and Ferrari, 2015) (Guay et al., 2009; Heyworth and Ferrari, 2015) (Heyworth and Ferrari, 2015)
Spiroplasma	Fungi resistance Male-killer Growth, reproduction and longevity ↓	(Łukasik et al., 2013c) (Simon et al., 2011) (Fukatsu et al., 2001)
Wolbachia	Asexual reproduction Nutrition supply	(De Clerck et al., 2014) (De Clerck et al., 2015)
Arsenophonus	Host plant specialization	(Wagner et al., 2015)

↓ means negative effect on host.

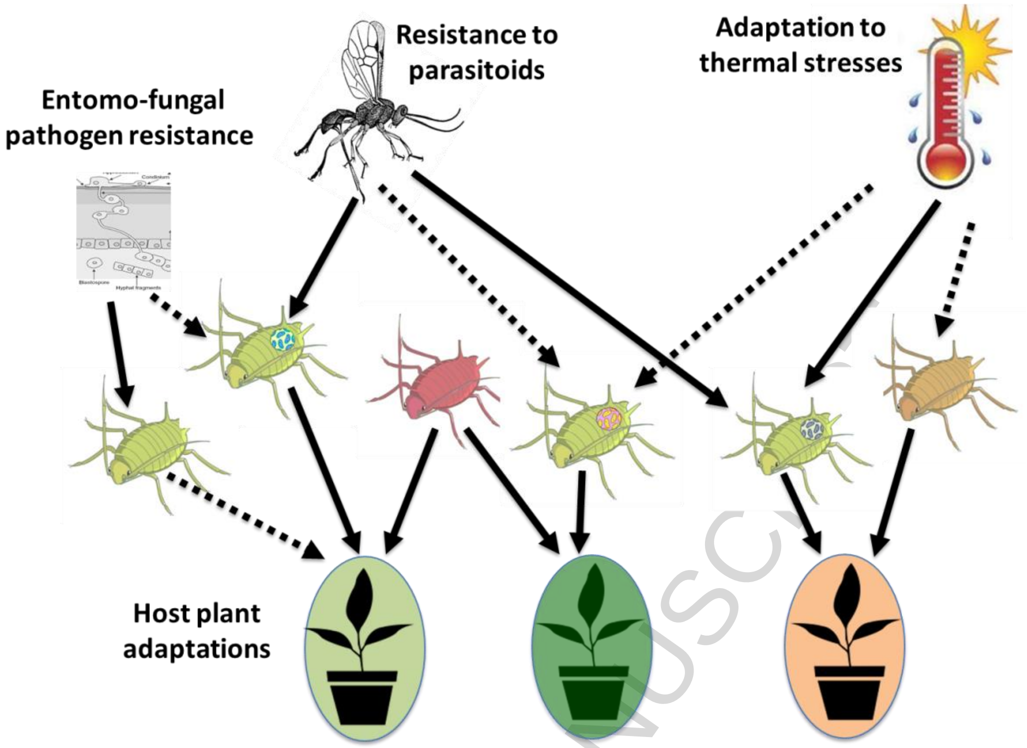


Figure 1 Main fitness effects on aphids of facultative symbionts.

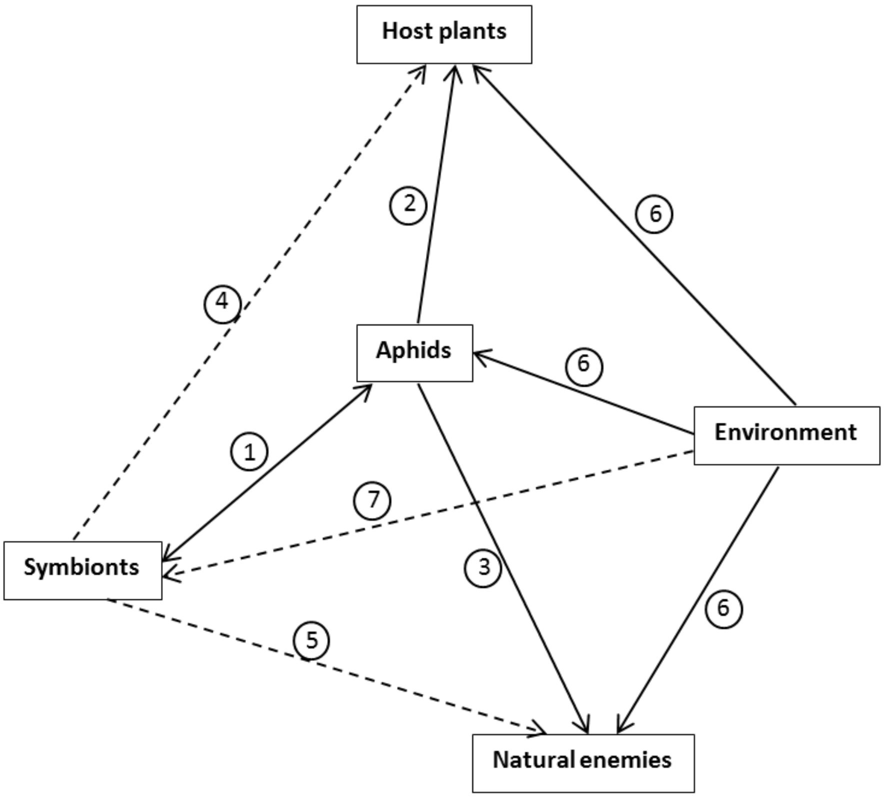
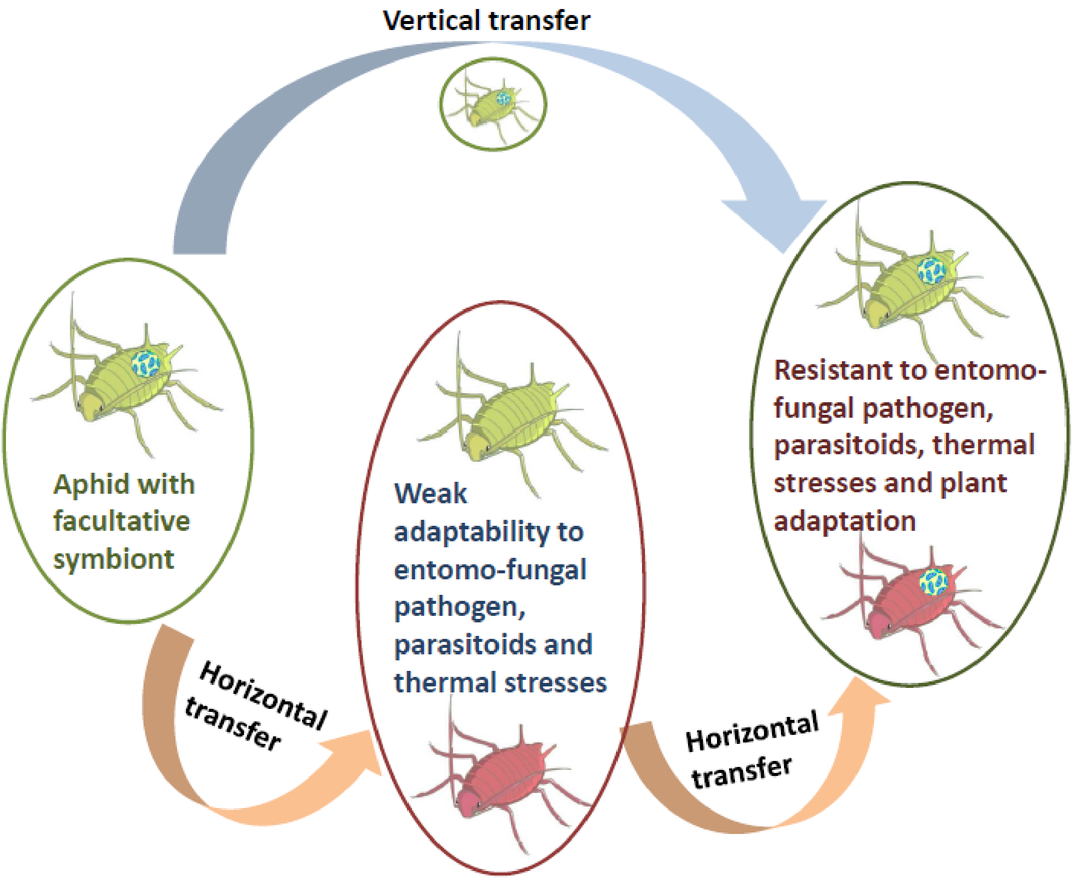


Figure 2 Reticular structure of symbionts, aphids, plant, natural enemies and environment.

Legend of figure

Figure 1 Main fitness effects on aphids of facultative symbionts. Different colors are related to different species (whole host plants, whole aphids or endosymbionts in a single aphid species), full and dot lines are related to positive and negative adaptations respectively.

Figure 2 Reticular structure of symbionts, aphids, plant, natural enemies and environment. ↔ mutualistic relations; → effect of/on organism behavior; —→ effect of/on symbiont function. ①: mutualistic relationship; ②: aphids feed on plant; ③: aphids are killed by natural enemies; ④: symbionts are involved in host plant specialization (Tsuchida et al., 2004) or host plant fitness (Sakurai et al., 2005) of aphid; ⑤: symbionts confer resistance to natural enemies for aphid (Oliver et al., 2003; 2005); ⑥: host plants, natural enemies and aphids are influenced by environmental conditions; ⑦: symbionts functions can be affected by the environment (Russell and Moran, 2006).



Graphical abstract

Highlights

- Described the infection frequency and cost of facultative symbionts in aphids.
- Reviewed the functions of nine facultative symbionts one by one.
- Discussed the interactions between symbionts and aphids, plants and environment.