ECOLOGY LETTERS

Ecology Letters, (2013) **16**: 214–218 doi: 10.1111/ele.12031

LETTER

Unrelated facultative endosymbionts protect aphids against a fungal pathogen

Piotr Łukasik,[1]*† Margriet van Asch,[1] Huifang Guo,[1,2] Julia Ferrari[1,3] and H. Charles J. Godfray[1]

Abstract

The importance of microbial facultative endosymbionts to insects is increasingly being recognized, but our understanding of how the fitness effects of infection are distributed across symbiont taxa is limited. In the pea aphid, some of the seven known species of facultative symbionts influence their host's resistance to natural enemies, including parasitoid wasps and a pathogenic fungus. Here we show that protection against this entomopathogen, *Pandora neoaphidis*, can be conferred by strains of four distantly related symbionts (in the genera *Regiella, Rickettsia*, *Rickettsiella* and *Spiroplasma*). They reduce mortality and also decrease fungal sporulation on dead aphids which may help protect nearby genetically identical insects. Pea aphids thus obtain protection from natural enemies through association with a wider range of microbial associates than has previously been thought. Providing resistance against natural enemies appears to be a particularly common way for facultative endosymbionts to increase in frequency within host populations.

Keywords

Acyrthosiphon pisum, endosymbiosis, *Hamiltonella defensa*, inclusive fitness, *Pandora neoaphidis*, resistance, secondary symbiont.

Ecology Letters (2013) **16**: 214–218

INTRODUCTION

The majority of arthropod species host different types of endosymbiotic bacteria and other microorganisms inside their tissues and cells (Hilgenboecker *et al.* 2008). Facultative endosymbioses are particularly common and involve a broad range of eubacterial taxa (Moran *et al.* 2008). These microbes, not essential for host survival and reproduction and usually occurring in only a fraction of host individuals, are normally maternally transmitted at high frequency, but are also capable of horizontal transmission within or between host species (Russell *et al.* 2003; Oliver *et al.* 2010). Different bacterial types can infect the same host species, and co-infections with more than one sort of facultative endosymbiont in a single host are not unusual (Duron *et al.* 2008). For example, the pea aphid *Acyrthosiphon pisum* Harris 1776, which has emerged as a model system for the study of endosymbiosis, can host at least seven genera of facultative endosymbionts (Oliver *et al.* 2010), and *c.* 80% of clones carry one or more of them (Ferrari *et al.* 2012). This is in addition to the obligate endosymbiont *Buchnera*, which provides nutrients missing in phloem sap and is thus essential for aphid survival and found in all individuals (Douglas 1998). The pea aphid is an Old World species spread around the globe by man and forms genetically differentiated populations on various species of legumes (Peccoud *et al.* 2009). Facultative endosymbiont prevalence varies across these host plant races and geographic areas (Tsuchida *et al.* 2002; Ferrari *et al.* 2012) but the factors which have shaped these patterns are not fully understood.

The positive effects of obligate endosymbiotic mutualists on their insect hosts have been appreciated for many decades (Buchner 1965). But it is only more recently that the varied effects that facultative endosymbionts may have on their hosts, which range from pathogenicity through commensalism to true mutualism, have been appreciated (Moran *et al.* 2008; Ferrari & Vavre 2011). In the pea aphid, different facultative endosymbionts increase resistance to parasitoid wasps (Oliver *et al.* 2003) and a pathogenic fungus (Scarborough *et al.* 2005) as well as help their hosts withstand heat shock (Montllor *et al.* 2002; Russell & Moran 2006). They can also influence aphid performance on different host plants (Tsuchida *et al.* 2004; McLean *et al.* 2011), affect the frequency of sexual reproduction (Leonardo & Mondor 2006; Simon *et al.* 2011) and body coloration (Tsuchida *et al.* 2010), though in the last two cases the fitness consequences for their hosts are less well understood. Facultative endosymbionts in other insect taxa also confer protection from natural enemies including predators, parasitoids, fungal pathogens and viruses (e.g. Teixeira *et al.* 2008; Jaenike *et al.* 2010). It is clear that defensive symbionts can have major effects on their hosts' ecology and evolution (Haine 2008; Brownlie & Johnson 2009), and their influence on host population dynamics (Oliver *et al.* 2008) may affect whole communities (Ferrari & Vavre 2011). However, we know little about the distribution of protective phenotypes across taxa of bacterial symbionts, or across insect species and communities.

Here we compare the defensive properties of strains of five genera of pea aphid facultative endosymbionts against an entomopathogenic fungus *Pandora neoaphidis* (Zygomycetes: Entomophthorales), one of the most important natural enemies of pea aphids (Pickering *et al.* 1989; Hufbauer 2002). We did this by transferring symbionts from different pea aphid clonal genotypes into aphids from a single clone of the same species which naturally lacked known facultative endosymbionts. The resulting aphid clonal lines, genetically identical

---

[1] *Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK*

[2] *Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Zhongling Street, Nanjing, 210014, China*

[3] *Department of Biology, University of York, York, YO10 5DD, UK*

† *Department of Biology, Drexel University, 3245 Chestnut St, Philadelphia, PA, 19104, USA*

* *Correspondence: E-mail: p.lukasik@gmail.com*

© 2012 Blackwell Publishing Ltd/CNRS

and differing only in the facultative endosymbiont they carried, were then exposed to the pathogen and their susceptibility assessed.

MATERIALS AND METHODS

Pea aphids reproduce asexually during the summer and genotypes can be maintained clonally in the laboratory. All pea aphid clones used in this study (the experimental clone and the original hosts of the experimental symbiont strains; Table 1) were collected in Southern England and maintained in laboratory culture until used in the experiments. DNA from the field-collected clones was extracted using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Aphids were typed at four microsatellite loci (Ferrari *et al.* 2008) and different, genetically distinct lineages were established. These were then tested for the presence of the seven known facultative endosymbionts of the pea aphid using polymerase chain reaction (PCR) with previously published diagnostic primers for the 16S ribosomal RNA gene, as well as universal bacterial primers 10F and 35R (Tsuchida *et al.* 2010; McLean *et al.* 2011). The resulting PCR products for each of the detected symbionts were sequenced using Big Dye Terminator v. 3.0. All sequences, manually edited and assembled with CodonCode Aligner v 2.06 (CodonCode Corporation, Centerville, MA, USA), showed at least 99% identity with a sequence of a strain of the bacterium previously reported from the pea aphid. The exception was *Spiroplasma*, which though very similar to bacteria previously found in the pea aphid had sequence traces which were polymorphic at up to 1.3% nucleotides. This suggested differences between 16S rRNA gene copies within a genome of a single strain (Pei *et al.* 2010), or else the presence of multiple *Spiroplasma* genotypes in the experimental clones. No attempt was made to investigate this further. All sequences were deposited in Genbank (accession numbers: JX943560–JX943568).

Nine strains of five species of facultative endosymbionts were used in the experiment. They were (1) *Rickettsia* sp. (Alphaproteobacteria: Rickettsiales; 2 strains), (2) *Rickettsiella* sp. (Gammaproteobacteria: Legionellales; 1 strain), (3) *Regiella insecticola* (Gammaproteobacteria: Enterobacteriales; 1 strain), (4) *Hamiltonella defensa* (Gammaproteobacteria: Enterobacteriales; 2 strains) and (5) *Spiroplasma* sp. (Mollicutes: Entomoplasmatales; 3 strains). Some of these strains originally came from aphids carrying two species of endosymbionts (Table 1), but following antibiotic treatment (clone reference numbers 141, 161, 208 and 333 – McLean *et al.* 2011) or passage through another symbiont-free aphid clone (reference number 185 – P. Łukasik, unpublished) the number was reduced to one and it was these manipulated, single-infected lines that were used as donors (Table 1). Nine experimental lines of the recipient clone (reference number 145), each originating from a single parthenogenetic female injected with haemolymph containing one of the symbiont strains, were set up. All lines were checked for successful establishment of the symbiont by diagnostic PCRs in the first generation post-injection and then at least three more times in subsequent generations. All lines were propagated for at least eight generations before they were used for experiments. Throughout the study, the aphids were kept on broad bean seedlings enclosed in polyethylene cylinders (30 cm high, 10 cm in diameter) with gauze-covered vents at 20 ± 2 °C, L16 : D8 light regime and a relative humidity of *c.* 70%.

Table 1 The pea aphid clones used in the present study, including the experimental clone 145 and nine 'donor' clones. The donor clones were originally infected with different combinations of facultative endosymbionts *Rickettsia* (Ri), *Hamiltonella defensa* (Ha), *Rickettsiella* (Rcl), *Regiella insecticola* (Reg) and *Spiroplasma* (Sp), but symbionts in brackets had been removed from the double-infected lines at least 10 months before the present study.

Clone code	Original symbionts	Collection plant	Collection county	Collection year
145	None	*Lathyrus pratensis*	Berkshire	2003
141	Ri, (Ha)	*Lotus pedunculatus*	Berkshire	2003
208	Ri, (Ha)	*Lotus pedunculatus*	Berkshire	2003
J240	Rcl	*Medicago lupulina*	Buckinghamshire	2010
126	Reg	*Trifolium pratense*	Berkshire	2003
161	Sp, (Ha)	*Medicago sativa*	Berkshire	2003
185	Sp, (Reg)	*Trifolium pratense*	Berkshire	2003
333	Sp, (Reg)	*Medicago sativa*	Berkshire	2008
101	Ha	*Ononis spinosa*	Buckinghamshire	2003
132	Ha	*Lotus pedunculatus*	Berkshire	2003

Aphid susceptibility to isolate X4 of *P. neoaphidis*, the entomopathogen used in previous studies on heritable variation in susceptibility among aphids (Ferrari *et al.* 2001; Stacey *et al.* 2003; Scarborough *et al.* 2005), was measured in a standardized assay. For each replicate, a group of twenty 9–10-day-old wingless aphids was exposed for 90 min to a spore shower emanating from two sporulating aphid cadavers before being transferred onto a fresh broad bean plant (Ferrari *et al.* 2001). After exposure, the aphids were kept at a relative humidity close to 100% for 24 h in order to facilitate germination of fungal spores; afterwards, humidity was allowed to drop. Aphid condition was assessed 6 days after the exposure, and all insects were classified as surviving, dead and showing fungal sporulation or dead but not sporulating. All non-sporulating dead aphids were transferred onto wet filter paper to test for later sporulation, and all surviving insects were kept for an additional 2 days on fresh broad bean leaves in order to assess the incidence of late sporulation. Six to ten replicates (mean 9.2) were performed for each line, all in a single temporal block.

The proportion of aphids dying, and of dead sporulating aphids, after exposure to the fungus were analyzed with the statistical package R v. 2.13.0 (R Development Core Team 2011) using logistic generalized linear modelling. Quasibinomial error variances were assumed to account for overdispersion, and Bonferroni corrections were made in assessing the significance of multiple comparisons.

RESULTS

We detected significant differences between genetically identical aphid lines carrying different symbionts in mortality following exposure to *Pandora* spores ($F_{9,82} = 65.9$, $P < 0.001$), as well as in the rate of pathogen sporulation occurring on any dead aphids ($F_{9,63} = 30.5$, $P < 0.001$). In the symbiont-free control line, over 95% of individuals died within 6 days of exposure to the pathogen (Fig. 1a) and in 98% of cases successful sporulation occurred on these dead individuals (Fig. 1b). The presence of *Hamiltonella* or two of the three isolates of *Spiroplasma* had no effect on aphid survival or fungal sporulation. However, carriage of *Rickettsiella* or either of the two isolates of *Rickettsia* led to almost complete immunity against the pathogen: survival 6 days after exposure to the fungus was more than 88%. Furthermore, *Pandora* successfully sporulated in only a single individual out of the 580 aphids carrying *Rickettsia* or *Rickettsiella* which had been exposed to spores.

© 2012 Blackwell Publishing Ltd/CNRS

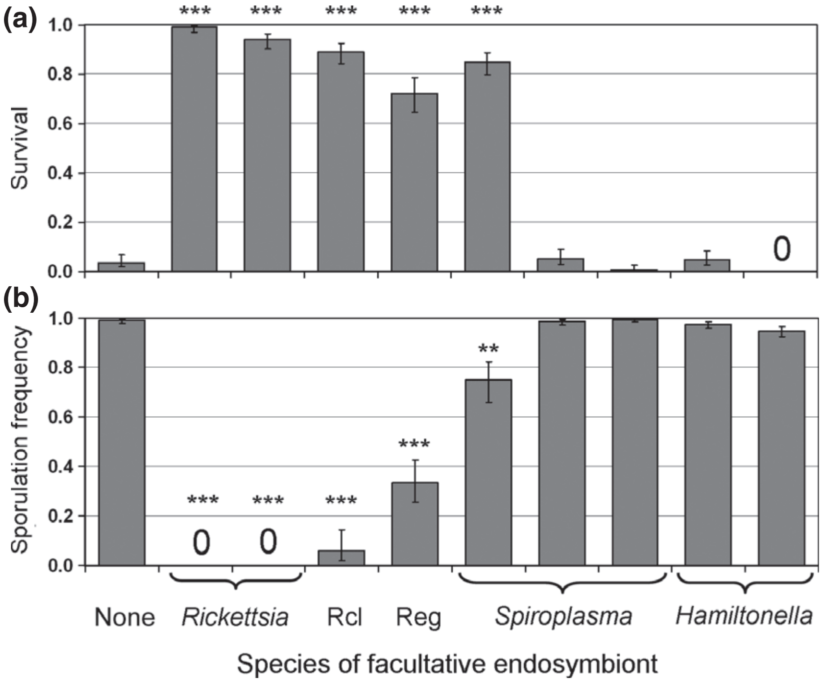

Figure 1 (a) Survival of pea aphids (all from a single clonal genotype) carrying no facultative endosymbiotic bacteria or one of nine strains of five different species, 6 days after exposure to spores of the pathogenic fungus *Pandora neoaphidis*. (b) The frequency of pathogen sporulation amongst aphids which died within 6 days of spore exposure. Symbiont strains are shown in the same order as in Table 1. Rcl refers to *Rickettsiella* and Reg to *Regiella*. Means and SEs among the replicates are shown. Significant differences compared to aphids with no facultative symbiont: $**P < 0.01$; $***P < 0.0001$.

Carrying *Regiella* led to partial resistance to the fungus, while a similar benefit was conferred by one of the three isolates of *Spiroplasma*. In the cases of *Regiella* and the single isolate of *Spiroplasma* where a significant number of aphids succumbed to the fungus, death and sporulation were often delayed relative to the controls: the percentage of all recorded deaths leading to sporulation within 6 days of exposure was 99.8% for the five susceptible lines, compared to 67% in the line carrying *Regiella* and 54% in this *Spiroplasma*-infected line. We also observed that *c.* 50% of the dying aphids that carried the resistance-conferring *Spiroplasma* isolate dropped off their host plant before sporulating while aphids from susceptible lines (and those carrying *Regiella*) always remained attached.

DISCUSSION

Our results show that strains of four unrelated endosymbiotic bacteria can all significantly reduce the aphid mortality caused by an important fungal pathogen, an effect that had previously only been reported for *Regiella* (Scarborough *et al.* 2005). The strongest protective effects were found with *Rickettsia* and *Rickettsiella*, two common pea aphid symbionts which had not previously been shown to have a clear benefit for their hosts (Simon *et al.* 2007; Tsuchida *et al.* 2010). Symbiont strains which do not confer complete protection may further decrease the rate of local disease transmission by reducing and delaying sporulation. Scarborough *et al.* (2005) suggested that this reduces the risk of infection to individuals from the same clone carrying the same symbiont. The observation that some dying aphids carrying *Spiroplasma* drop off the plant and sporulate away from the aphid's feeding site may have a similar explanation.

In addition to the four facultative endosymbionts which can protect pea aphids against a fungal pathogen, three others – the gammaproteobacteria *H. defensa*, *Serratia symbiotica* and 'X-type' – have all been shown or suggested to confer protection against hymenopterous parasitoids (Oliver *et al.* 2003; Guay *et al.* 2009). A strain of *Regiella*, genetically rather distant from those that protect pea aphids against fungal pathogens (Hansen *et al.* 2012), naturally protects the peach–potato aphid *Myzus persicae* against parasitoids and has the same effect when injected into another species, black bean aphid *Aphis fabae* (Vorburger *et al.* 2010). Thus probably all of the seven known types of pea aphid facultative endosymbionts can provide some protection against their host's natural enemies. The recent sequencing of the pea aphid genome has shown that compared with other insects, aphids have a markedly depauperate set of defense-related genes (Gerardo *et al.* 2010) which may provide an opportunity for symbionts to substitute for their functions. There is also evidence that *A. pisum* is relatively susceptible to *Pandora* compared to other aphid species (Shah *et al.* 2004); this could have resulted from, or promoted, its associations with diverse defensive symbionts, perhaps to a different extent than in these other species. Our results also indicate that the ability to defend their hosts is a particularly effective way for a facultative symbiont to increase in frequency within pea aphid populations. *Rickettsia*, *Spiroplasma* and *Rickettsiella* infect a wide range of other arthropods where they have typically been regarded as pathogens and reproductive manipulators (Cordaux *et al.* 2007; Duron *et al.* 2008; Engelstadter & Hurst 2009). The possibility that these (and other) bacteria have proliferated because of their ability to protect their hosts against natural enemies deserves further consideration (Haine 2008; Jaenike *et al.* 2010; Jones *et al.* 2011).

The mechanistic basis of the fungus resistance due to *Regiella* infection is not yet known despite the availability of the symbiont's genome (Degnan *et al.* 2010; Hansen *et al.* 2012). It will be interesting to see whether the four unrelated symbiont species have evolved the trait independently or whether it has spread laterally via a mobile genetic element. The protection against parasitoids conferred by *Hamiltonella* is due to the lysogenic bacteriophages it carries (Oliver *et al.* 2009) and which encode several distinct eukaryotic toxins (Degnan & Moran 2008a,b). However, these phages have not been found in other symbionts and the *Regiella* strain isolated from peach-potato aphid that confers parasitoid resistance appears to have evolved this phenotype independently (Hansen *et al.* 2012). There is also a possibility that facultative endosymbionts enhance aphid resistance to the pathogen indirectly, by priming the host immune system, upregulating defensive pathways, outcompeting the pathogen for essential nutrients, or increasing the energy reserves available for host immune response (Haine 2008). It will also be interesting to explore whether all *P. neoaphidis* isolates are affected in the same way by the different endosymbionts. There is evidence from before the time symbionts were implicated in defence that different fungal isolates varied in their ability to attack different pea aphid clones (Milner 1982). Distinct symbiont-conferred defensive mechanisms in a single species might be maintained by negative frequency-dependent selection between hosts and natural enemies (Schmid *et al.* 2012).

The ecological consequences of hosting facultative endosymbionts are determined not only by the benefits they confer but also by the costs their carriage incurs. In our experiments we did not compare the fitness of aphids with and without symbionts in the absence of fungus exposure, but our previous work indicates that the conditions in a susceptibility assay in the absence of the fungus are benign and mortality is low (< 10%). Previous studies of the facultative endosymbionts of pea aphids have shown that *Hamiltonella*, *Regiella*, *Rickettsia* and *Spiroplasma* can all negatively affect different components of fitness including fecundity, longevity and survival (e.g. Simon *et al.* 2011), though these effects vary amongst symbiont and host genotypes (Vorburger & Gouskov 2011) and may be more pronounced when the aphid is stressed (Oliver *et al.* 2008). Similarly, the strength of protection against natural enemies conferred by particular symbiont genotypes may depend on environmental factors such as temperature (Guay *et al.* 2009), host characteristics such as age (Schmid *et al.* 2012), and could also vary between host genotypes. A full understanding of the roles of facultative endosymbionts in natural populations will require better measurements of both the costs and benefits of carrying symbionts under different conditions encountered by aphids in the field, ability of host genotypes and species to harbour and benefit from alternative symbionts, as well as the frequency with which the aphids encounters different abiotic and biotic stressors.

An important challenge in symbiont biology is to understand how a diverse community of multiple bacterial types can evolve and persist in the same host or host population despite competition between symbionts for host resources. If spread and persistence are due to the benefits for the host provided by the symbionts then elementary population theory would suggest that species carrying the same beneficial trait would compete with the elimination of the least efficient. Thus other more subtle and as yet unknown effects must be in operation to maintain the large number of symbionts associated with the pea aphid and with other arthropods.

ACKNOWLEDGEMENTS

We thank L. Henry, J. Jaenike, F. Jiggins, X. Liu, A. McLean, J. Russell and three anonymous referees for discussion and helpful comments on the manuscript. We are grateful to Judith Pell (Rothamsted Research, UK) for the pathogen isolate used in the study. The work was supported by the UK Biotechnology and Biology Research Council award BB/E010857/1 and by a Sir Richard Southwood (Christensen Foundation) Graduate Scholarship to P.Ł.

AUTHOR CONTRIBUTIONS

All authors participated in designing the study; PŁ, MvA and HG developed the experimental lines; PŁ and MvA conducted the experiments; PŁ analysed the data and wrote the first draft of the manuscript, and all authors contributed to revisions.

REFERENCES

Brownlie, J.C. & Johnson, K.N. (2009). Symbiont-mediated protection in insect hosts. *Trends Microbiol.*, 17, 348–354.

Buchner, P. (1965). *Endosymbiosis of Animals with Plant Microorganisms*. Interscience Publishers, John Wiley and Sons, Inc., New York.

Cordaux, R., Paces-Fessy, M., Raimond, M., Michel-Salzat, A., Zimmer, M. & Bouchon, D. (2007). Molecular characterization and evolution of arthropod-pathogenic *Rickettsiella* bacteria. *Appl. Environ. Microbiol.*, 73, 5045–5047.

Degnan, P.H. & Moran, N.A. (2008a). Diverse phage-encoded toxins in a protective insect endosymbiont. *Appl. Environ. Microbiol.*, 74, 6782–6791.

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

Degnan, P.H., Leonardo, T.E., Cass, B.N., Hurwitz, B., Stern, D., Gibbs, R.A. *et al.* (2010). Dynamics of genome evolution in facultative symbionts of aphids. *Environ. Microbiol.*, 12, 2060–2069.

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

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L.Q., Engelstadter, J. *et al.* (2008). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol.*, 6, 12.

Engelstadter, J. & Hurst, G.D.D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annu. Rev. Ecol. Evol. Syst.*, 40, 127–149.

Ferrari, J. & Vavre, F. (2011). Bacterial symbionts in insects or the story of communities affecting communities. *Philos. Trans. R. Soc. B-Biol. Sci.*, 366, 1389–1400.

Ferrari, J., Muller, C.B., Kraaijeveld, A.R. & Godfray, H.C.J. (2001). Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution*, 55, 1805–1814.

Ferrari, J., Via, S. & Godfray, H.C.J. (2008). Population differentiation and genetic variation inperformance on eight hosts in the pea aphid complex. *Evolution*, 62, 2508–2524.

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.

Gerardo, N.M., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S.M., De Vos, M. *et al.* (2010). Immunity and other defenses in pea aphids. *Acyrthosiphon pisum*. *Genome Biol.*, 11, R21.

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.

Haine, E.R. (2008). Symbiont-mediated protection. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 275, 353–361.

Hansen, A.K., Vorburger, C. & Moran, N.A. (2012). Genomic basis of endosymbiont-conferred protection against an insect parasitoid. *Genome Res.*, 22, 106–114.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. & Werren, J.H. (2008). How many species are infected with *Wolbachia*? - a statistical analysis of current data. *FEMS Microbiol. Lett.*, 281, 215–220.

© 2012 Blackwell Publishing Ltd/CNRS

Hufbauer, R.A. (2002). Aphid population dynamics: does resistance to parasitism influence population size? *Ecol. Entomol.*, 27, 25–32.

Jaenike, J., Unckless, R., Cockburn, S.N., Boelio, L.M. & Perlman, S.J. (2010). Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science*, 329, 212–215.

Jones, E.O., White, A. & Boots, M. (2011). The evolution of host protection by vertically transmitted parasites. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 278, 863–870.

Leonardo, T.E. & Mondor, E.B. (2006). Symbiont modifies host life-history traits that affect gene flow. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 273, 1079–1084.

McLean, A.H.C., van Asch, M., Ferrari, J. & Godfray, H.C.J. (2011). Effects of bacterial secondary symbionts on host plant use in pea aphids. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 278, 760–766.

Milner, R.J. (1982). On the occurrence of pea aphids, *Acyrthosiphon pisum*, resistant to isolates of the fungal pathogen *Erynia neoaphidis*. *Entomol. Exp. Appl.*, 32, 23–27.

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., McCutcheon, J.P. & Nakabachi, A. (2008). Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet.*, 42, 165–190.

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. USA*, 100, 1803–1807.

Oliver, K.M., Campos, J., Moran, N.A. & Hunter, M.S. (2008). Population dynamics of defensive symbionts in aphids. *Proc. R. Soc. Lond. Ser. 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, 247–66.

Peccoud, J., Ollivier, A., Plantegenest, M. & Simon, J.C. (2009). A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proc. Natl Acad. Sci. USA*, 106, 7495–7500.

Pei, A.Y., Oberdorf, W.E., Nossa, C.W., Agarwal, A., Chokshi, P., Gerz, E.A. *et al.* (2010). Diversity of 16S rRNA genes within individual prokaryotic genomes. *Appl. Environ. Microbiol.*, 76, 3886–3897.

Pickering, J., Dutcher, J.D. & Ekbom, B.S. (1989). An epizootic caused by *Erynia neoaphidis* and *Erynia radicans* (Zygomycetes, Entomophthoraceae) on *Acyrthosiphon pisum* (Homoptera, Aphididae) on legumes under overhead irrigation . *J. Appl. Entomol.-Z Angew. Entomol.*, 107, 331–333.

R Development Core Team (2011). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: [http://www.R-project.org].

Russell, J.A. & Moran, N.A. (2006). Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 273, 603–610.

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

Scarborough, C.L., Ferrari, J. & Godfray, H.C.J. (2005). Aphid protected from pathogen by endosymbiont. *Science*, 310, 1781–1781.

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.

Shah, P.A., Clark, S.J. & Pell, J. (2004). Assessment of aphid host range and isolate variability in *Pandora neoaphidis* (Zygomycetes: Entomophthorales). *Biol. Control*, 29, 90–99.

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. *et al.* (2011). Facultative symbiont infections affect aphid reproduction. *PLoS ONE*, 6, e21831.

Stacey, D.A., Thomas, M.B., Blanford, S., Pell, J.K., Pugh, C. & Fellowes, M.D. E. (2003). Genotype and temperature influence pea aphid resistance to a fungal entomopathogen. *Physiol. Entomol.*, 28, 75–81.

Teixeira, L., Ferreira, A. & Ashburner, M. (2008). The Bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol.*, 6, 2753–2763.

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, 1989–1989.

Tsuchida, T., Koga, R., Horikawa, M., Tsunoda, T., Maoka, T., Matsumoto, S. *et al.* (2010). Symbiotic bacterium modifies aphid body color. *Science*, 330, 1102–1104.

Vorburger, C. & Gouskov, A. (2011). Only helpful when required: a longevity cost of harbouring defensive symbionts. *J. Evol. Biol.*, 24, 1611–1617.

Vorburger, C., Gehrer, L. & Rodriguez, P. (2010). A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biol. Lett.*, 6, 109–111.

Editor, Wim van der Putten
Manuscript received 20 August 2012
First decision made 26 September 2012
Manuscript accepted 15 October 2012

© 2012 Blackwell Publishing Ltd/CNRS