

Review

Bacterial symbionts in insects or the story of communities affecting communities

Julia Ferrari^{1,*} and Fabrice Vavre²

¹*Department of Biology, University of York, PO Box 373, York YO10 5YW, UK*

²*Université de Lyon, 69000 Lyon, Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, 69622 Villeurbanne, France*

Bacterial symbionts are widespread in insects and other animals. Most of them are predominantly vertically transmitted, along with their hosts' genes, and thus extend the heritable genetic variation present in one species. These passengers have a variety of repercussions on the host's phenotypes: besides the cost imposed on the host for maintaining the symbiont population, they can provide fitness advantages to the host or manipulate the host's reproduction. We argue that insect symbioses are ideal model systems for community genetics. First, bacterial symbionts directly or indirectly affect the interactions with other species within a community. Examples include their involvement in modifying the use of host plants by phytophagous insects, in providing resistance to natural enemies, but also in reducing the global genetic diversity or gene flow between populations within some species. Second, one emerging picture in insect symbioses is that many species are simultaneously infected with more than one symbiont, which permits studying the factors that shape bacterial communities; for example, horizontal transmission, interactions between host genotype, symbiont genotype and the environment and interactions among symbionts. One conclusion is that insects' symbiotic complements are dynamic communities that affect and are affected by the communities in which they are embedded.

Keywords: community genetics; ecological communities; heritable symbionts; insects; reproductive manipulation; secondary symbionts

1. INTRODUCTION

Heritable bacterial endosymbionts are very common in insects. The past decade has seen an explosion of studies characterizing the biology of these symbionts. It has become clear that bacterial symbionts vary tremendously in the effects on their hosts, ranging from the provision of essential nutrients [1] and protection from natural enemies [2,3] to altering the host's reproductive system [4], along with sometimes imposing substantial costs [5,6]. Symbionts have mostly been investigated for the effects on their host partner. However, their influence can extend to the community to which the host belongs, either directly by mediating interactions with other species, or indirectly by impacting the host genetic diversity.

Traditionally, bacterial symbionts are divided into two groups, usually described as obligate or primary symbionts and facultative or secondary symbionts. Obligate symbionts are mutualists that tend to have a nutritional function and typically occur in insects that feed on imbalanced diets such as plant saps or cellulose [7]. In contrast, facultative symbionts have a much broader array of effects, ranging from mutualism to

manipulation of reproduction [8]. In this review, we will not discuss separately primary and secondary symbionts, but rather distinguish between bacteria (primary or secondary), in which the symbiont has an effect on an ecologically important trait of the host and may directly impact the community level, and reproductive manipulators for which effects on the community are more indirect. Note that in some cases the same bacterium might be included in both categories.

One important feature of all these symbionts is that most of them are exclusively or predominantly vertically (and usually maternally) transmitted, and thus heritable. Heritable genetic variation in insects thus includes variation in the nuclear genome, but also variation in the genetic composition of the cytoplasm. When symbionts are not fixed in populations, genetic polymorphism thus includes the presence or absence of thousands of genes. In fact, some of the variation in phenotypes that has been interpreted as nuclear genetic variation in the past may actually be variation owing to heritable symbionts. Their vertical transmission suggests parallels between symbionts and insect genes. Variation among endosymbionts that affect their host's interactions with other species is likely to have similar effects on ecological communities as equivalent genetic variation in an ecologically relevant trait in the insect. In this review, we will explore the similarities and differences between

* Author for correspondence (jf557@york.ac.uk).

One contribution of 13 to a Theme Issue 'Community genetics: at the crossroads of ecology and evolutionary genetics'.

symbionts and insect genes, and argue that insect–bacterial symbioses may allow faster adaptation to changing environments.

Not all bacterial symbionts influence the host's interactions with other species directly, and many of these bacterial species affect the reproductive system of their hosts by either causing female-biased sex ratios or impacting on the ability of pairs of individuals to produce offspring. Through these mechanisms, reproductive manipulators increase their transmission and spread through populations, which is well documented and reviewed in Engelstädter & Hurst [4]. However, reproductive manipulators are likely to affect both the population dynamics and the genetic diversity of their hosts, and through this process might have an impact on community-level processes and especially on patterns of local adaptation.

Finally, in addition to their individual effects on hosts and their possible consequences on insect communities, insect symbionts may themselves form communities. Often several bacterial strains or species co-occur within the same host, and in some cases, the presence of multiple species is required for the survival of the insect while other associations are facultative. These systems are useful, because the composition of the community is heritable and has a clear phenotype. They therefore provide excellent examples of how selection can act on different levels of organization, especially at the community level.

In this review, we will provide an overview of the range of effects that bacterial symbionts have on their hosts and how these effects are likely to affect entire communities. We argue that some of the insect symbiont systems provide valuable model systems for community genetics, while others may add complications but will have to be taken into account for a complete understanding of the communities. We will then explore whether multi-species symbiont communities within a host species can serve as model systems for community genetics.

2. ARE HERITABLE ENDOSYMBIONTS AFFECTING IMPORTANT ECOLOGICAL TRAITS EQUIVALENT TO HOST GENES?

Ecologically important traits can be provided by endosymbiotic bacteria and, as discussed above, these bacteria represent an alternative system of inheritance in arthropods. In addition to the predominantly vertical and in most cases maternal transmission, horizontal transmission between individuals of the same species or between species can occur, and depending on the frequency of horizontal transmission, this may change the ecological and evolutionary dynamics of the system. This is similar to plasmids that encode functions such as antibiotic resistance or virulence in free-living or pathogenic bacteria. We will now discuss in which situations insect genes and symbionts may be ecologically equivalent to each other.

(a) *Obligate symbionts*

Obligate or primary symbionts are essential for their hosts' survival and reproduction, and are often housed in specialized organs inside the insects called

the bacteriome. They tend to supplement the hosts' diet with amino acids or vitamins that are rare or absent in the food source. For example, many blood-feeding arthropods harbour obligate symbionts providing B vitamins, and virtually all plant sap-feeding insects have bacterial symbionts providing essential amino acids. The bacterium *Buchnera aphidicola*, the primary symbiont of aphids, is probably the best studied: it synthesizes several amino acids that are required for the aphids' metabolism, and aphids in which the symbiont has been artificially removed have extremely low survival and fecundity [1,9]. Primary symbionts tend to be purely vertically transmitted, and many of the associations with their insect partners are ancient (reviewed in [8]); the oldest known insect symbiont *Sulcia muelleri*, for example, has been associated with the Auchenorrhyncha for the past 270 Myr [10]. Typically, the primary symbiont's genome is extremely reduced [11], and at least in some cases, their gene content can be predicted based on knowledge of their lifestyle [12]. Even though the genome of primary symbionts can be extremely stable in terms of chromosome arrangements and gene content, there is evidence of rapid sequence evolution between closely related strains [13]. When they were first acquired by their hosts, primary symbionts undoubtedly played a major role in the hosts' ecology by widening their host's niche and allowing their host's development on particular resources. The symbionts' integration in the host physiology thus probably had major impacts on communities. Indirect evidence comes from the stinkbugs *Megacopta punctatissima* and *Megacopta cribraria*. These bugs harbour primary symbionts that are transmitted by the ovipositing female depositing a symbiont-containing capsule, which is taken up by her offspring [14]. This mode of transmission can be exploited to exchange primary symbionts between species experimentally. *Megacopta punctatissima* can be a serious pest on pea, *Pisum sativum* [15], whereas *M. cribraria* does not usually feed on pea. When the symbionts between the two species are exchanged, the pattern of their ability to develop on pea reverses [14]. Because of the ancient coevolutionary history between hosts and symbionts and their fixation in these lineages, they may, however, play little role in shaping actual communities. Indeed, little ecologically relevant genetic variation within symbiont species has been found to date. However, there are exceptions to this: *Buchnera* in populations of the pea aphid, *Acyrtosiphon pisum*, display a polymorphism in the promoter of the heat shock gene *ibpA*, which determines their performance under different thermal conditions [16], which may in turn affect population and community dynamics [17].

Primary symbionts not only have nutritional effects, there are suggestions that they might also affect their hosts' interactions with natural enemies. Removing *Wigglesworthia* symbionts in tse-tse flies makes them more susceptible to trypanosome infection [18]. Recent investigations suggest that the interaction is mediated by a host peptidoglycan recognition protein that is involved both in host immune tolerance to the obligate symbiont and in the trypanosome infection

process [19]. *Blochmannia* symbionts in carpenter ants of the genus *Camponotus* provide their hosts with amino acids [20], but may also affect their encapsulation ability of foreign bodies such as parasitoid eggs [21]. Ants with higher bacterial densities display an increased encapsulation response, although it is unclear whether this is simply a consequence of their better nutritional condition. It is worth noting that colonies treated with antibiotics have a high encapsulation response, irrespective of bacterial densities, and this has been interpreted as a response to stress [21]. At present, the precise role of *Blochmannia* in defence against natural enemies is therefore unclear, but the above examples suggest that there may be an effect of primary symbionts on their hosts' interactions with other species. Whether there is sufficient genetic variation within primary symbionts within an insect species for these to be relevant for community genetics remains to be seen.

(b) Mutualistic secondary symbionts

Within species, there is usually variation in whether or not a particular mutualistic secondary symbiont species is found in an individual. This observation suggests that there are costs and benefits associated with harbouring secondary symbionts and that this balance between costs and benefits maintains intermediate frequencies in natural arthropod populations. The advantage provided by secondary symbionts generally depends on the environment, so that the benefit may vary greatly in space and time, which probably contributes to the maintenance of the polymorphism.

Secondary symbionts are typically vertically transmitted from mother to offspring and often the transmission rates are very near 100 per cent. Paternal transmission can also occur, at least in aphids [22], but its frequency is unknown. Additionally, secondary symbionts can be transferred horizontally between individuals and between species [23,24]. Again, the frequency and mechanism of this process are unknown and are likely to vary between systems, but the distribution of symbionts across species clearly shows that it must occur at least rarely.

Mutualistic secondary symbionts can affect almost any trait of their hosts, and here we will give examples for the diversity of these effects, but refer to Haine [3], Janson *et al.* [25], Moran *et al.* [8] and Oliver *et al.* [26] for a more complete overview. The effects of secondary symbionts on natural enemies of their hosts have been most actively studied. Perhaps the best understood example is the interaction between the secondary symbiont *Hamiltonella defensa* in aphids and the parasitoid wasp *Aphidius ervi*. Pea aphids, *A. pisum*, artificially infected with *Hamiltonella* are significantly less likely to die after attack by an ovipositing parasitoid than uninfected pea aphids of the same genotype [27]. The variation between symbiont strains in the degree of resistance they confer is associated with variation in a lysogenic lambdaoid bacteriophage, named *A. pisum* secondary endosymbiont (APSE), which encodes toxins that target eukaryotic tissue [28–30]. There is evidence that pea aphids with *Hamiltonella*

win in competition with *Hamiltonella*-free aphids of the same genotype in the presence of the parasitoid, but not in its absence [31]. Increased resistance to parasitoids conferred by secondary symbionts is not restricted to the *A. pisum*–*Hamiltonella*–APSE interaction. Other secondary symbiont species also increase resistance to parasitoids in several aphid species, but it is not known whether this is also caused by bacteriophages [27,32–35]. Other facultative endosymbionts have the opposite effect on parasitoids: *Wolbachia* infection in *Drosophila simulans* leads to reduced encapsulation ability and therefore increased susceptibility to the wasp *Leptopilina heterotoma* [36]. The same study did not find an effect of *Wolbachia* on susceptibility to the fungal pathogen *Beauveria bassiana*.

However, secondary symbionts can increase resistance to fungal pathogens. The secondary symbiont *Regiella insecticola* in pea aphids protects the aphids from the lethal fungus *Pandora neoaphidis* [37]. The presence of *R. insecticola* not only improves the aphids' survival after exposure to the pathogen, in aphids that die it also suppresses sporulation of the fungus, thereby protecting nearby aphids that are likely to be related [37]. Similarly, increased protection from external fungal infestation is provided to a range of taxa by several *Streptomyces* species [38–40]. Two recent reports have shown that *Wolbachia* infection of *Drosophila melanogaster* increases resistance to four types of RNA viruses, some of which are common in both the field and laboratory cultures [41,42], and there is variation between strains of *Wolbachia* [43]. Similar protection from natural enemies can be observed in other arthropods [44–46].

While the effects of secondary symbionts on their hosts' natural enemies are best studied, there is also evidence that they affect interactions with other organisms; for example, with the host plants of herbivorous insects. The pea aphid, *A. pisum*, consists of a series of host-adapted populations that are specialized on different host plant species or genera [47–49]. These host-adapted populations tend to carry different species of secondary symbionts, suggesting that the symbionts might play an active role in host use and specialization on certain plant species, although these differences are not fixed between populations [32,50–53]. Manipulation experiments have shown that the effects of the secondary symbionts on host use are complex and vary with aphid and/or symbiont genotype [54–56]. In most cases, harbouring the secondary symbiont that is most frequently found in aphids specialized on a given plant species improves fecundity of the aphid irrespective of the actual food plant, but it is very rare that there is an improvement of performance on the plant on which the aphid is specialized and not also on a universally suitable host [54–57]. Overall, this suggests that in pea aphids, secondary symbionts are likely to have effects on aphid performance and presumably on aphid population dynamics, but are less likely to affect the host-associated population structure.

Endosymbionts can also affect plant virus transmission by insect vectors. GroEL is a protein produced by bacteria. In aphids and whiteflies, GroEL

homologues are circulating in the haemolymph outside the bacterial cells [58]. It has been shown that virions bind to these GroEL homologues, thereby escaping destruction by the vector's immune system and allowing virus transmission [58,59]. It is not always clear whether GroEL of facultative or primary symbionts is involved in this process, and therefore it is unknown how much variability there is within the vector species. However, it seems clear that the bacteria are likely to have significant epidemiological consequences and also effects on organisms that interact with the plant. Incidentally, there are attempts to express these proteins in transgenic plants to trap viruses and avoid pathogenesis [60,61].

Finally, secondary symbionts influence other traits that could indirectly affect the ecological community. *Serratia symbiotica* in the pea aphid, for example, has been shown to provide tolerance to heat shock in a similar way as described for the primary symbiont *Buchnera* above [62], and the bacterium *R. insecticola* can alter the probability of the production of winged morphs and of sexual reproduction in aphids [63].

In this section, we have clearly seen that heritable symbionts have important effects on their hosts and on species with which the host interacts. Even though the direct consequences of secondary symbionts on population or community dynamics have been little studied, it is probable that these effects have major consequences for communities. For example, in the case of protection against natural enemies, it is likely that a population with a high incidence of protective symbionts would support a smaller population of natural enemies, while in turn, the presence of natural enemies selects for increased resistance and therefore higher frequencies of protective symbionts [31,35,64]. This arms race could therefore lead to observations of negative or positive associations between symbiont and natural enemy frequencies. So far, most of the evidence that heritable variation may affect populations and communities comes from clonal plant species because of the relative ease of manipulating genetic diversity in these systems [65]. Heritable symbionts provide similarly convenient model systems for studying the effects of heritable variation on ecological communities, and in some cases, it may be possible to manipulate the presence of symbionts in several of the interacting partners (e.g. hosts and parasitoids), substituting interactions between several genotypes and the environment with interactions between several symbionts and the environment. The equivalence of variation in symbionts and genetic variation in the host is even clearer when genetically distinct strains of the same symbiont species are employed (e.g. [30,66]).

(c) *Symbionts versus host genes*

Clearly, there are differences between symbionts and insect genes. Symbionts are separate entities that carry an entire genome and while their carriage brings significant advantages to the host, the host will also have to provide nutrients for their upkeep. These costs are likely to be much greater than those occurred by carrying single genes and those genes' pleiotropic effects.

As defined above, the total heritable genetic variance in insects is the sum of the nuclear genetic variance and the cytoplasmic genetic variance (including presence or absence of the symbiont together with genetic variation among symbionts) and the interaction among the host and the symbiont. Usually, when a trait is determined by multiple genes, recombination may lead to a lower efficiency of selection, but this does not apply to symbionts that are mostly clonal. Selection on complex traits provided by symbionts should thus be an efficient process, leading to more rapid adaptation than the same trait coded in the insect's nuclear genome.

Compared with insect genes, in natural populations secondary symbionts can be acquired much more frequently by horizontal transfer between individuals or even different species. These horizontal acquisitions can be considered meta-mutations since, like other types of mutations, they occur randomly. Horizontal gene or symbiont transfer can be costly or neutral to the recipient, or provide ready-made functions, such as resistance to natural enemies, which could have immediate beneficial effects. Recently, some authors have argued that the acquisition of major traits through horizontal transfer within a single generation contradicts the Darwinian principle of slow and gradual change (e.g. [67,68]). We agree that the rate of change is greater than a point mutation is likely to be. However, we do not think that horizontal transfer is fundamentally different from other types of mutations, since the transferred gene or symbiont will be subject to the same population genetic processes as other mutations. Nevertheless, the likely greater effect of symbiont acquisition suggests that dynamics involving symbionts might be accelerated compared with those involving insect genes. The probable higher cost of their carriage indicates that there would be stronger selection against the symbionts in environments in which the trait that they improve is less relevant [69]. Of course, once infection has been lost within a population, migration of infected individuals or horizontal transmission is required for acquiring the symbiont. Phages such as the APSE, which confers parasitoid resistance in aphids, can be very mobile and aid the transfer of ecologically important functions between different symbionts, and are likely to accelerate these dynamics even more [8,26]. APSE, for example, has also been found in *Arsenophonus* [70], an endosymbiont distantly related to *Hamiltonella*, suggesting that lateral transfer of phages between symbionts may be quite common.

Depending on local conditions and on the net effect of symbiosis, there may be a conflict between the host and the symbiont, and this may lead to selection of hosts that are more resistant or more tolerant to the carriage of symbionts and to selection on symbionts that are less costly to the host. Genetic variation in symbionts can be low, especially in primary symbionts, and in these cases, most of the coevolutionary dynamics of the system could be mediated by the host [71]. This suggests that adaptation to specific conditions may depend on direct selection on symbionts expressing ecologically important traits and simultaneously on indirect selection on the host for

being more or less tolerant to the symbiont. This will have implications for the evolution of classic defence systems. Increased tolerance to a bacterial symbiont may require the immune system to be downregulated to accommodate extracellular symbionts. At the same time, if the symbiont fulfils a defensive function, some elements of the immune system may not be needed and are lost over time (see [69] for a thorough review). The potential loss of immune function is likely to affect the longer term dynamics of the interactions between the host and its natural enemies.

Over much longer timescales, it is possible that symbiont genes may be transferred into the insect nuclear genome, similarly to bacterial plasmid genes that are incorporated into the bacterial chromosome [8]. While this process is presumably not relevant on experimental timescales, it may be important in the natural evolutionary dynamics, and there are several examples where such a transfer has taken place and where the genes acquired from a symbiont are still expressed (reviewed in [72]).

3. EFFECTS OF REPRODUCTIVE MANIPULATORS ON POPULATIONS AND COMMUNITIES

In addition to bacterial mutualists, insects (and more generally arthropods) are also frequently infected by vertically transmitted symbionts that manipulate their reproduction (reviewed in [4]). Because symbionts are maternally transmitted, any strategy favouring infected matrilines will allow the spread of infection, even if this comes at the expense of the male's fitness. Phenotypes induced by reproductive manipulators fall into two broad categories: (i) induction of female-biased sex ratio in the offspring of infected females through feminization of genetic males, or induction of thelytokous parthenogenesis or male-killing, and (ii) reduction of female production by uninfected females through cytoplasmic incompatibility (CI). *Wolbachia* bacteria are the archetype of reproductive manipulators and can induce all these manipulations, but other bacteria like *Cardinium*, *Spiroplasma*, *Rickettsia* and *Arsenophonus* are also capable of playing with their hosts' reproduction, as do other organisms outside bacteria, such as microsporidia or viruses. Reproductive manipulators are not only diverse, but also exhibit high incidence among arthropods. A recent survey of the four most common reproductive manipulators showed that no less than 30 per cent of the arthropod species tested were infected by at least one of them [73], but estimations taking into account variable prevalence among species and limitation of sampling suggest that *Wolbachia* alone could infect around 70 per cent of insect species [74].

All the reproductive manipulations may impact considerably on the population dynamics and/or the genetic diversity of the hosts. We refer to Engelstädter & Hurst [4] for an excellent review on these effects and on the areas of ignorance that remain in this field. Despite available knowledge suggesting that reproductive manipulators may play an indirect role in the community structure around their host, very few experimental or theoretical studies have addressed this

question. In the following, we speculate on this hypothesis discussing separately sex-ratio distorters and CI-inducing bacteria.

(a) *Sex-ratio distorters*

Feminization of genetic males and induction of thelytokous parthenogenesis allow non-transmitting males to be converted into transmitting females, providing a direct advantage to infection. The advantage is less clear in the case of male-killing since it requires that killing sons brings an advantage to daughters (fitness compensation), a condition that can be met when there is competition among siblings or inbreeding depression. In any case, it is expected that production of more daughters may lead to an increase in the population size of the host, even though density dependence may limit this effect. For male-killing and feminization, scarcity of males can, however, limit this effect by leaving numerous unmated females in the population [75]. Such effects on the host population dynamics could affect the community to which the host belongs through over-exploitation of resources or modification of interspecific interactions like competition, prey-predator or host-parasitoid relationships. Because some of these interactions are unstable, such effects could lead to considerable modification of community structure through local extinction of some species. One example might be provided by the success of invasion of the amphipod *Crangonyx pseudogracilis* in the UK that could be mediated by its higher growth rate owing to its infection with feminizing microsporidia [76].

From a genetic point of view, sex-ratio distorters may decrease genetic diversity. In the case of feminization and male-killing, the effective population size will be greatly reduced as soon as the prevalence of the symbiont reaches sufficient frequencies because few males will get access to numerous females. In the case of male-killing, this effect will be even larger because effective size of the population will be reduced to the effective size of the uninfected part of the population [77]. Consequently, genetic diversity is reduced, the rate of fixation of deleterious mutation increased and fixation of beneficial mutations impeded. In addition, in both systems, nucleo-cytoplasmic conflicts select for nuclear resistance alleles to counteract the symbiont effect, and resistance has indeed been demonstrated both for feminization [78,79] and male-killing [80]. In the latter case, invasion of a resistant allele has been monitored in the butterfly *Hypolimnas bolina* and shown to be extremely rapid [81]. As these resistance alleles invade, selective sweeps on linked genes might lead to an important loss of genetic variability in the host species. If we add that these systems are highly dynamic [82], one can expect that male-killers and feminizers can regularly and deeply impact the level of genetic variation within one species.

Reduction of genetic variation is even higher in the case of parthenogenesis-inducing (PI) bacteria. In most species, PI symbionts have reached fixation within populations or even in the entire species, and sexual reproduction is no longer possible (reviewed in [83]). In addition, PI is often induced through the

diploidization of early embryos through abortion of telophase during the first mitotic division, thus leading to the complete homozygosity of individuals. Selection thus acts on clones and genetic variation should rapidly decline in infected populations. Interestingly, despite complete dependence of the host upon its symbionts, there is no evidence for co-speciation between PI symbionts and their hosts. One possible explanation for this result is that absence of genetic variation and recombination impairs the adaptability of the host of the symbiont, leading to the extinction of the association. This explanation is all the more possible because many species infected by PI symbionts are parasitoid wasps that may be engaged in an arms race with their own hosts.

(b) *Cytoplasmic incompatibility-inducing symbionts*

In its simplest form, CI results in the sterility of crosses between uninfected females and infected males. Because infected females can mate with any type of male in the population, contrary to uninfected females, infection spreads. In addition, when mates are infected with different CI-inducing strains of bacteria, both reciprocal crosses are sterile, which may lead in particular circumstances to the coexistence, at the scale of the meta-population, of different CI-inducing bacteria (reviewed in [84]). Interestingly, as soon as a polymorphism is maintained at the meta-population level, either with coexistence of uninfected and infected sub-populations, or between populations infected with bi-directionally incompatible strains, gene flow is reduced between populations. This effect results from a reduction of effective migration owing to CI. While it has been shown that such reduction may allow maintenance of local adaptation in constant environments [85], consequences could be radically different when considered in variable environments or in the context of coevolution between species. Indeed, the migration rate between hosts and parasites is a major factor affecting patterns of local reciprocal adaptation [86]. By reducing effective migration of one partner, CI could thus perturb co-evolutionary dynamics among infected hosts and their interacting species.

(c) *Direct effects of reproductive manipulators*

We end this part with the specific association between the *Drosophila* parasitoid wasp *Leptopilina boulardi* and a symbiotic virus, suggesting that reproductive manipulators could also directly impact community structure. Parasitoid insects are key species in insect communities through their ability to regulate their host populations. Exploitation of the host resource depends crucially on the behaviour of the parasitoid and includes long- and short-range detection of the host, but also inspection of the sanitary state of the host. For solitary parasitoids (where only a single parasitoid can emerge from one host), laying an egg in an already parasitized host is a dangerous strategy because of the competition that will take place within the host and that will end with the death of one of the larvae (most frequently the latest that has been laid).

However, when unparasitized hosts are scarce, it might pay off to take that risk [87]. The ability of females to adapt their superparasitism strategy is critical in the stability of host–parasitoid systems [88]. Surprisingly, in the *Drosophila* parasitoid wasp *L. boulardi*, superparasitism is mainly mediated by the virus FvLb (for filamentous virus of *L. boulardi*) [89]. This virus is vertically transmitted, but can also be horizontally transmitted when multiple wasps are hosted within the same host individual, i.e. when superparasitism occurs. Favouring superparasitism thus allows the virus to complete its imperfect maternal transmission and to colonize new wasp lineages. However, the epidemiology of the virus depends highly on the opportunities of superparasitism, and thus on the local relative densities of *Drosophila* and its parasitoids [90]. Prevalence of the virus can be as high as 70 per cent in some populations [90,91], thus making it the main determinant of this behaviour and leading to potential conflicts between the two partners and modifications of the selective pressures acting on the superparasitism strategy on the host side [92,93]. Because it drastically modifies host exploitation strategy, presence of the virus could exert a major effect on the host–parasitoid couple. In addition, different species of parasitoids compete on the same hosts, and wastage of eggs through superparasitism has been shown to decrease the competitive ability of *L. boulardi* against *L. heterotoma* (J. Varaldi 2010, personal communication). Because *L. boulardi* is a very strong competitor, virus infection may be a major factor allowing coexistence of different wasp species. Thus, as parasites may be important actors in community structure [94], the role of reproductive manipulators should also be taken into account.

4. INSECTS AS SYMBIOTIC COMMUNITIES

One host species can harbour multiple symbionts, each of them being present in different individuals, or even within the same individual. However, systematic surveys are lacking to estimate the real incidence of these multiple infections (but see [73]). Some examples are particularly demonstrative of the complexity symbiotic communities can reach. In the pea aphid, *A. pisum*, in addition to the primary symbiont *B. aphidicola*, six common secondary symbionts are known [23,24,33,50,95]. In the whitefly *Bemisia tabaci*, the number of secondary symbionts also reaches six (e.g. [96]). For many of these bacterial species, several bacterial strains have been identified, increasing the variability of symbionts occurring in these insect species [28,97]. Existence of these multiple infections raises many questions related to their origin, maintenance and consequences on host evolution (see [98] for review).

In the following, we focus on the fact that symbiotic communities may constitute model systems for community genetics that allow studying how selection and adaptation occur at different levels of community structure. One important parameter to take into account in community genetics is θ , which measures the degree of co-inheritance of gene combinations. Initially introduced for studying epistasis within genomes, θ has

recently been applied to gene interactions across genomes in the context of community genetics [71,99]. Increased θ facilitates selection to act on gene combinations across genomes. Vertical transmission of symbionts increases θ if one considers genes present in the host and the symbiont, and facilitates coevolution towards mutualism. However, as previously mentioned, host sexual reproduction partially breaks apart host-symbiont gene combinations, which reduces θ and limits the action of selection at the super-organism level, unless population structure or mating systems maintain it at a high level. When focusing on the community of vertically transmitted symbionts present within the same host, θ is very high and tends to unity, which should make symbiotic communities particularly prone to the action of selection at the community level. This is all the more likely because there is a clear community phenotype that is expressed at the insect individual level. Given the diversity of symbionts' phenotypic effects and their profound consequence on the host, one can easily imagine that the composition of the symbiotic communities may mediate competition among individuals. Analyses of these situations may thus allow important advances in the field of community genetics.

(a) Generating variability at the symbiotic community level

Horizontal transmission of symbionts is probably the origin of multiple infections, and is an important factor in increasing the number of symbionts present in one host. On the other hand, sporadic inefficient transmission of some symbionts will tend to reduce symbiont diversity. Depending on the frequency of both these events, the value of θ will more or less depart from unity. However, while measuring the efficiency of vertical transmission is relatively easy, measuring the rate of horizontal transmission is far more difficult. These two factors have in common that they generate a polymorphism in the symbiotic community, and allow selection to sort out these communities if they impact the fitness of the insect individual they infect.

(b) Selection at the host individual level

The extended phenotype of the host is partly the expression of the phenotype of the symbiotic community. Variation in this community phenotype is heritable, and selection at the host individual level is therefore critical for the maintenance of multiple infections. Many examples show that the composition of the symbiotic community is far from neutral and thus that the notion of a community phenotype makes sense in these systems. On the one hand, symbionts within hosts share an environment that is limited in space and resources. Limitation of host resources has been repeatedly found in various systems where multiply infected hosts suffer higher fitness costs compared with uninfected and singly infected hosts (e.g. [6,100]). This higher infection cost is often associated with an increase in the total bacterial density in multiply infected hosts. This increase in infection cost leads to selection against multiple infections. On the other hand, multiple infections may be

stably maintained, because the benefit generated to the matriline exceeds the increased infection cost. There are three main ways for this benefit to occur. First, each symbiont confers an advantage to the host fitness. Multiple infection with *Buchnera* and *Hamiltonella* (and its phage) in aphids allows exploitation of plant resources together with increased resistance to parasitoids. Second, symbionts are CI inducers and bidirectionally incompatible. In those cases, only females infected by the whole symbiotic community are able to mate with any type of males within the population. Third, some symbionts provide a fitness advantage, while others induce reproductive alterations. So, as for the maintenance of single infections, both fitness advantage and reproductive manipulations may maintain multiple infections. However, this time, selection does not act on a single symbiotic genome, but on a community of symbionts. Within a population experiencing a stable environment (with regard to the symbiotic community phenotype), one can predict that one symbiotic community will be fitter and thus get fixed in the population.

(c) Populations of hosts are metacommunities of symbionts

Migration of insect individuals among populations involves the migration of the insect and the entire symbiont community with which it is associated. Insect populations can thus be viewed as metacommunities of symbiont communities. We have shown above that many symbionts confer advantages that depend on the environment. Under variable environments, the fittest symbiotic community may vary in space or in time, because of interactions between multiple genotypes and the environment, leading either to the fixation of different communities in different populations or to a polymorphism of symbiont communities within populations. This pattern is evident in the aphid *A. pisum* where populations specialized on different plant species tend to harbour different complements of secondary symbionts. These differences between host specialists are, however, not fixed and there is probably some gene flow as well as symbiont exchange between these populations [48,52]. As mentioned above, it is unclear whether and why different symbiont complements are favoured in these host-specialized populations [54–57]. In *B. tabaci*, the different biotypes of this phytophagous insect are all associated with different symbiotic communities [97,101]. Unfortunately, the effects of these symbionts are not known, but this situation may suggest that the differentiation of this species complex has been, and is still, influenced by these communities. Interestingly, coexistence of inter-fertile individuals harbouring different symbiont communities has been found in some populations, which will allow monitoring of the outcome of competition that may be mediated by the symbiont communities (G. Gueguen 2010, personal communication).

(d) Are there adaptations at the community level?

If communities of vertically transmitted symbionts are a major level of selection, then one should expect

specific adaptations to have evolved at the community level. Interestingly, different kinds of adaptations may be suspected depending on the dynamics of multiple infections. Two extreme situations can be envisioned. First, frequent horizontal transmission and low stability of multiple infections should select for increased competitive ability of symbionts, because, as said earlier, hosts offer limited space and resources. In such circumstances, symbionts monopolizing resources should be selected for at the within-host level, if this increases the efficiency of their own vertical transmission. Examples of such competitive interactions have been illustrated between secondary symbionts [6,102], but also in situations of co-infections between primary and secondary symbionts [103,104]. If symbionts evolve higher competitive ability, this may further limit the maintenance of multiple infections. Second, horizontal transmission is sufficiently rare and multiple infections are stably maintained because they provide an advantage to the host matriline. In this case, specific adaptations may evolve to limit competition among symbionts sharing hosts. Indeed, highly competitive symbionts may be selected for within the host, but may be selected against at the individual host level. On the contrary, traits favouring coexistence and co-transmission should be selected for at this level. Theoretical analyses of this question have indeed shown that as soon as multiple infections are stably maintained, selection acts only to increase the production of multiply infected daughters by multiply infected females [105]. The increased production may be achieved by increasing the co-transmission of the symbiotic community, notably through cooperation among symbionts. Direct evidence for such direct interactions is scarce. One possible case has been repeatedly found in individuals infected by different CI-inducing *Wolbachia*, where each strain within a host has a specific density that is independent of the presence of other strains [100,106]. Such specific regulation of density may limit competition among symbionts, but may also limit stochastic variation in specific density and reduce loss of some symbionts during transmission.

Another consequence highlighted in Vautrin *et al.* [105] is that multiple infections may facilitate the evolution of dependence among symbionts. Through a process analogous to what has been discussed for inter-genome gene transfer [71,107], increasing θ may facilitate gene transfer or gene loss across co-transmitted genomes. Because θ is very high for vertically transmitted symbionts, such a phenomenon could be very frequent among symbionts sharing the same host. Examples of complex interdependent communities have been found in sharpshooters where two primary symbionts are present [108] and in some nematodes [109]. An interesting example is how the usually facultative symbiont *S. symbiotica* has become obligatory to the aphid *Cinara cedri* because it complements some functions that have been lost by the primary symbiont *Buchnera* [110,111]. Evolution of dependence will inevitably lead to a stabilization of the symbiont community, which should further facilitate the evolution of specific adaptation at the community level.

5. CONCLUSIONS

Heritable symbionts have a broad array of effects on their hosts. We argue that they are useful tools for community genetic research: secondary symbionts and at least some primary symbionts cause significant variation in ecologically important traits, either through their presence and absence or through genetic variation between strains of the same symbiont species. Some of these symbionts can be experimentally manipulated and may be useful model systems for studying the effects of heritable variability on species interactions and community dynamics. As seen above, many insect individuals represent entire symbiont communities, and these are tractable systems for researching ecological and evolutionary dynamics at different levels of organization. However, reproductive manipulators are more likely to be a complication in community genetic research, since they are likely to alter the genetic structure and diversity of their hosts in a way that would be unpredictable if their presence and actions were ignored.

From an applied perspective, symbionts have recently been used for developing control strategies of pest insect populations. Interestingly, some of these developments are based on a community approach. The best example is probably the recent introduction of a *Wolbachia* strain named *wMelPop* in the mosquito *Aedes aegypti*, a major vector of important pathogens such as dengue virus [112]. The strategy is based on the effect of this *Wolbachia* strain inducing CI and shortening the life of mosquitoes. Because older individuals play a crucial role in the epidemiology of the virus, modification of the age structure of the population could reduce the transmission of the virus to humans. Infection has also been shown to influence blood-feeding behaviour of infected mosquitoes [113] and to interfere directly with pathogen infection through activation of the host immune system [114]. All these effects could act in synergy in this system to reduce the vector capacity of mosquitoes. This example clearly shows that the whole range of effects of symbionts, both on the infected individual and on the species with which the host interacts, must be taken into account to evaluate the overall action of symbionts in the dynamics of the host and the community with which it interacts.

REFERENCES

- 1 Douglas, A. E. 1998 Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* **43**, 17–37. (doi:10.1146/annurev.ento.43.1.17)
- 2 Brownlie, J. C. & Johnson, K. N. 2009 Symbiont-mediated protection in insect hosts. *Trends Microbiol.* **17**, 348–354. (doi:10.1016/j.tim.2009.05.005)
- 3 Haine, E. R. 2008 Symbiont-mediated protection. *Proc. R. Soc. B* **275**, 353–361. (doi:10.1098/rspb.2007.1211)
- 4 Engelstädter, J. & Hurst, G. D. D. 2009 The ecology and evolution of microbes that manipulate host reproduction. *Annu. Rev. Ecol. Evol. Syst.* **40**, 127–149. (doi:10.1146/annurev.ecolsys.110308.120206)
- 5 Fleury, F., Vavre, F., Ris, N., Fouillet, P. & Bouletreau, M. 2000 Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila*

- parasitoid *Leptopilina heterotoma*. *Parasitology* **121**, 493–500. (doi:10.1017/S0031182099006599)
- 6 Oliver, K. M., Moran, N. A. & Hunter, M. S. 2006 Costs and benefits of a superinfection of facultative symbionts in aphids. *Proc. R. Soc. B* **273**, 1273–1280. (doi:10.1098/rspb.2005.3436)
 - 7 Baumann, P. 2005 Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* **59**, 155–189. (doi:10.1146/annurev.micro.59.030804.121041)
 - 8 Moran, N. A., McCutcheon, J. P. & Nakabachi, A. 2008 Genomics and evolution of heritable bacterial symbionts. *Annu. Rev. Genet.* **42**, 165–190. (doi:10.1146/annurev.genet.41.110306.130119)
 - 9 Akman Gündüz, E. & Douglas, A. E. 2009 Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proc. R. Soc. B* **276**, 987–991. (doi:10.1098/rspb.2008.1476)
 - 10 Moran, N. A., Tran, P. & Gerardo, N. M. 2005 Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Appl. Environ. Microbiol.* **71**, 8802–8810. (doi:10.1128/AEM.71.12.8802-8810.2005)
 - 11 Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H. E., Moran, N. A. & Hattori, M. 2006 The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. *Science* **314**, 267–267. (doi:10.1126/science.1134196)
 - 12 Pal, C., Papp, B., Lercher, M. J., Csermely, P., Oliver, S. G. & Hurst, L. D. 2006 Chance and necessity in the evolution of minimal metabolic networks. *Nature* **440**, 667–670. (doi:10.1038/nature04568)
 - 13 Tamas, I., Klasson, L., Canbäck, B., Näslund, A. K., Eriksson, A. S., Wernegreen, J. J., Sandström, J. P., Moran, N. A. & Andersson, S. G. E. 2002 50 million years of genomic stasis in endosymbiotic bacteria. *Science* **296**, 2376–2379. (doi:10.1126/science.1071278)
 - 14 Hosokawa, T., Kikuchi, Y., Shimada, M. & Fukatsu, T. 2007 Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. B* **274**, 1979–1984. (doi:10.1098/rspb.2007.0620)
 - 15 Tomokuni, M. 1993 *A field guide to Japanese bugs*. Tokyo, Japan: Zenkoku Noson Kyoiku Kyokai.
 - 16 Dunbar, H. E., Wilson, A. C. C., Ferguson, N. R. & Moran, N. A. 2007 Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biol.* **5**, 1006–1015. (doi:10.1371/journal.pbio.0050096)
 - 17 Harmon, J. P., Moran, N. A. & Ives, A. R. 2009 Species response to environmental change: impacts of food web interactions and evolution. *Science* **323**, 1347–1350. (doi:10.1126/science.1167396)
 - 18 Pais, R., Lohs, C., Wu, Y. N., Wang, J. W. & Aksoy, S. 2008 The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl. Environ. Microbiol.* **74**, 5965–5974. (doi:10.1128/AEM.00741-08)
 - 19 Wang, J. W., Wu, Y. N., Yang, G. X. & Aksoy, S. 2009 Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proc. Natl Acad. Sci. USA* **106**, 12 133–12 138. (doi:10.1073/pnas.0901226106)
 - 20 Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M. J. & Gross, R. 2007 Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biol.* **5**, 48. (doi:10.1186/1741-7007-5-48)
 - 21 de Souza, D. J., Bezier, A., Depoix, D., Drezen, J. M. & Lenoir, A. 2009 *Blochmannia* endosymbionts improve colony growth and immune defence in the ant *Camponotus fellah*. *BMC Microbiol.* **9**, 29. (doi:10.1186/1471-2180-9-29)
 - 22 Moran, N. A. & Dunbar, H. E. 2006 Sexual acquisition of beneficial symbionts in aphids. *Proc. Natl Acad. Sci. USA* **103**, 12 803–12 806. (doi:10.1073/pnas.0605772103)
 - 23 Russell, J. A., Latorre, A., Sabater-Munoz, B., Moya, A. & Moran, N. A. 2003 Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Mol. Ecol.* **12**, 1061–1075. (doi:10.1046/j.1365-294X.2003.01780.x)
 - 24 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. (doi:10.1046/j.1365-294X.2001.01189.x)
 - 25 Janson, E. M., Stireman, J. O., Singer, M. S. & Abbot, P. 2008 Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution* **62**, 997–1012. (doi:10.1111/j.1558-5646.2008.00348.x)
 - 26 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. (doi:10.1146/annurev-ento-112408-085305)
 - 27 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. (doi:10.1073/pnas.0335320100)
 - 28 Degnan, P. H. & Moran, N. A. 2008 Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Mol. Ecol.* **17**, 916–929. (doi:10.1111/j.1365-294X.2007.03616.x)
 - 29 Moran, N. A., Degnan, P. H., Santos, S. R., Dunbar, H. E. & Ochman, H. 2005 The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. *Proc. Natl Acad. Sci. USA* **102**, 16 919–16 926. (doi:10.1073/pnas.0507029102)
 - 30 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. (doi:10.1126/science.1174463)
 - 31 Oliver, K. M., Campos, J., Moran, N. A. & Hunter, M. S. 2008 Population dynamics of defensive symbionts in aphids. *Proc. R. Soc. B* **275**, 293–299. (doi:10.1098/rspb.2007.1192)
 - 32 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. (doi:10.1111/j.1365-2311.2004.00574.x)
 - 33 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. (doi:10.1016/j.jinsphys.2009.06.006)
 - 34 von Burg, S., Ferrari, J., Müller, C. B. & Vorburger, C. 2008 Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae*—no evidence for trade-offs. *Proc. R. Soc. B* **275**, 1089–1094. (doi:10.1098/rspb.2008.0018)
 - 35 Vorburger, C., Gehrler, L. & Rodriguez, P. 2010 A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biol. Lett.* **6**, 109–111. (doi:10.1098/rsbl.2009.0642)
 - 36 Fytrou, A., Schofield, P. G., Kraaijeveld, A. R. & Hubbard, S. F. 2006 *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proc. R. Soc. B* **273**, 791–796. (doi:10.1098/rspb.2005.3383)

- 37 Scarborough, C. L., Ferrari, J. & Godfray, H. C. J. 2005 Aphid protected from pathogen by endosymbiont. *Science* **310**, 1781–1781. (doi:10.1126/science.1120180)
- 38 Currie, C. R., Scott, J. A., Summerbell, R. C. & Malloch, D. 1999 Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* **398**, 701–704. (doi:10.1038/19519)
- 39 Kaltenpoth, M., Gottler, W., Herzner, G. & Strohm, E. 2005 Symbiotic bacteria protect wasp larvae from fungal infestation. *Curr. Biol.* **15**, 475–479. (doi:10.1016/j.cub.2004.12.084)
- 40 Scott, J. J., Oh, D. C., Yuceer, M. C., Klepzig, K. D., Clardy, J. & Currie, C. R. 2008 Bacterial protection of beetle–fungus mutualism. *Science* **322**, 63. (doi:10.1126/science.1160423)
- 41 Hedges, L. M., Brownlie, J. C., O'Neill, S. L. & Johnson, K. N. 2008 *Wolbachia* and virus protection in insects. *Science* **322**, 702–702. (doi:10.1126/science.1162418)
- 42 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. (doi:10.1371/journal.pbio.1000002)
- 43 Osborne, S. E., Leong, Y. S., O'Neill, S. L. & Johnson, K. N. 2009 Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog.* **5**, e1000656. (doi:10.1371/journal.ppat.1000656)
- 44 Gil-Turnes, M. S. & Fenical, W. 1992 Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biol. Bull.* **182**, 105–108. (doi:10.2307/1542184)
- 45 Gil-Turnes, M. S., Hay, M. E. & Fenical, W. 1989 Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. *Science* **246**, 116–118. (doi:10.1126/science.2781297)
- 46 Haïne, E. R., Boucansaud, K. & Rigaud, T. 2005 Conflict between parasites with different transmission strategies infecting an amphipod host. *Proc. R. Soc. B* **272**, 2505–2510. (doi:10.1098/rspb.2005.3244)
- 47 Ferrari, J., Via, S. & Godfray, H. C. J. 2008 Population differentiation and genetic variation in performance on eight hosts in the pea aphid complex. *Evolution* **62**, 2508–2524. (doi:10.1111/j.1558-5646.2008.00468.x)
- 48 Peccoud, J., Ollivier, A., Plantegenest, M. & Simon, 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. (doi:10.1073/pnas.0811117106)
- 49 Via, S. 1991 The genetic structure of host plant adaptation in a spatial patchwork—demographic variability among reciprocally transplanted pea aphid clones. *Evolution* **45**, 827–852. (doi:10.2307/2409692)
- 50 Frantz, A., Calcagno, V., Mieuze, L., Plantegenest, M. & Simon, J. C. 2009 Complex trait differentiation between host-populations of the pea aphid *Acyrtosiphon pisum* (Harris): implications for the evolution of ecological specialisation. *Biol. J. Linn. Soc.* **97**, 718–727. (doi:10.1111/j.1095-8312.2009.01221.x)
- 51 Leonardo, T. E. & Muiro, G. T. 2003 Facultative symbionts are associated with host plant specialization in pea aphid populations. *Proc. R. Soc. Lond. B* **270**, S209–S212. (doi:10.1098/rsbl.2003.0064)
- 52 Simon, J. C., Carre, S., Boutin, M., Prunier-Leterme, N., Sabater-Munoz, B., Latorre, A. & Bournoville, R. 2003 Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proc. R. Soc. Lond. B* **270**, 1703–1712. (doi:10.1098/rspb.2003.2430)
- 53 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, *Acyrtosiphon pisum*. *Mol. Ecol.* **11**, 2123–2135. (doi:10.1046/j.1365-294X.2002.01606.x)
- 54 Ferrari, J., Scarborough, C. L. & Godfray, H. C. J. 2007 Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. *Oecologia* **153**, 323–329. (doi:10.1007/s00442-007-0730-2)
- 55 Leonardo, T. E. 2004 Removal of a specialization-associated symbiont does not affect aphid fitness. *Ecol. Lett.* **7**, 461–468. (doi:10.1111/j.1461-0248.2004.00602.x)
- 56 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. B* **278**, 760–766. (doi:10.1098/rspb.2010.1654)
- 57 Tsuchida, T., Koga, R. & Fukatsu, T. 2004 Host plant specialization governed by facultative symbiont. *Science* **303**, 1989–1989. (doi:10.1126/science.1094611)
- 58 van den Heuvel, J. F. J. M., Verbeek, M. & van der Wilk, F. 1994 Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. *J. Gen. Virol.* **75**, 2559–2565. (doi:10.1099/0022-1317-75-10-2559)
- 59 Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M. & van den Heuvel, J. F. J. M. 1999 A *GroEL* homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. *Virology* **256**, 75–84. (doi:10.1006/viro.1999.9631)
- 60 Akad, F., Eybishtz, A., Edelbaum, D., Gorovits, R., Dar-Issa, O., Iraki, N. & Czosnek, H. 2007 Making a friend from a foe: expressing a *GroEL* gene from the whitefly *Bemisia tabaci* in the phloem of tomato plants confers resistance to tomato yellow leaf curl virus. *Arch. Virol.* **152**, 1323–1339. (doi:10.1007/s00705-007-0942-0)
- 61 Edelbaum, D., Gorovits, R., Sasaki, S., Ikegami, M. & Czosnek, H. 2009 Expressing a whitefly *GroEL* protein in *Nicotiana benthamiana* plants confers tolerance to tomato yellow leaf curl virus and cucumber mosaic virus, but not to grapevine virus A or tobacco mosaic virus. *Arch. Virol.* **154**, 399–407. (doi:10.1007/s00705-009-0317-9)
- 62 Montllor, C. B., Maxmen, A. & Purcell, A. H. 2002 Facultative bacterial endosymbionts benefit pea aphids *Acyrtosiphon pisum* under heat stress. *Ecol. Entomol.* **27**, 189–195. (doi:10.1046/j.1365-2311.2002.00393.x)
- 63 Leonardo, T. E. & Mondor, E. B. 2006 Symbiont modifies host life-history traits that affect gene flow. *Proc. R. Soc. B* **273**, 1079–1084. (doi:10.1098/rspb.2005.3408)
- 64 Herzog, J., Muller, C. B. & Vorburger, C. 2007 Strong parasitoid-mediated selection in experimental populations of aphids. *Biol. Lett.* **3**, 667–669. (doi:10.1098/rsbl.2007.0362)
- 65 Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N. & Vellend, M. 2008 Ecological consequences of genetic diversity. *Ecol. Lett.* **11**, 609–623. (doi:10.1111/j.1461-0248.2008.01179.x)
- 66 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**, 12 795–12 800. (doi:10.1073/pnas.0506131102)
- 67 Boto, L. 2010 Horizontal gene transfer in evolution: facts and challenges. *Proc. R. Soc. B* **277**, 819–827. (doi:10.1098/rspb.2009.1679)
- 68 Koonin, E. V. 2009 The Origin at 150: is a new evolutionary synthesis in sight? *Trends Genet.* **25**, 473–475. (doi:10.1016/j.tig.2009.09.007)
- 69 Hurst, G. D. D. & Darby, A. C. 2009 The inherited microbiota of arthropods, and their importance in

- understanding resistance and immunity. In *Insect infection and immunity—evolution, ecology, and mechanisms* (eds J. Rolff & S. E. Reynolds), pp. 119–136. Oxford, UK: Oxford University Press.
- 70 Darby, A. C., Choi, J. H., Wilkes, T., Hughes, M. A., Werren, J. H., Hurst, G. D. D. & Colbourne, J. K. 2010 Characteristics of the genome of *Arsenophonus nasoniae*, son-killer bacterium of the wasp *Nasonia*. *Insect Mol. Biol.* **19**, 75–89. (doi:10.1111/j.1365-2583.2009.00950.x)
- 71 Wade, M. J. & Goodnight, C. J. 2006 Cyto-nuclear epistasis: two-locus random genetic drift in hermaphroditic and dioecious species. *Evolution* **60**, 643–659.
- 72 Ros, V. I. D. & Hurst, G. D. D. 2009 Lateral gene transfer between prokaryotes and multicellular eukaryotes: ongoing and significant? *BMC Biol.* **7**, 20. (doi:10.1186/1741-7007-7-20)
- 73 Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J. & Hurst, G. D. 2008 The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol.* **6**, 27. (doi:10.1186/1741-7007-6-27)
- 74 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. (doi:10.1111/j.1574-6968.2008.01110.x)
- 75 Charlat, S., Reuter, M., Dyson, E. A., Hornett, E. A., Duploux, A., Davies, N., Roderick, G. K., Wedell, N. & Hurst, G. D. D. 2007 Male-killing bacteria trigger a cycle of increasing male fatigue and female promiscuity. *Curr. Biol.* **17**, 273–277. (doi:10.1016/j.cub.2006.11.068)
- 76 Slothouber Galbreath, J. G. M., Smith, J. E., Terry, R. S., Becnel, J. J. & Dunn, A. M. 2004 Invasion success of *Fibrillanosema crangonycis*, n.sp., n.g.: a novel vertically transmitted microsporidian parasite from the invasive amphipod host *Crangonyx pseudogracilis*. *Int. J. Parasitol.* **34**, 235–244. (doi:10.1016/j.ijpara.2003.10.009)
- 77 Engelstädter, J. & Hurst, G. D. D. 2007 The impact of male-killing bacteria on host evolutionary processes. *Genetics* **175**, 245–254. (doi:10.1534/genetics.106.060921)
- 78 Rigaud, T. & Juchault, P. 1992 Genetic-control of the vertical transmission of a cytoplasmic sex factor in *Armadillidium vulgare* Latr (Crustacea, Oniscidea). *Heredity* **68**, 47–52. (doi:10.1038/hdy.1992.6)
- 79 Rigaud, T. & Juchault, P. 1993 Conflict between feminizing sex-ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics* **133**, 247–252.
- 80 Hornett, E. A., Charlat, S., Duploux, A. M. R., Davies, N., Roderick, G. K., Wedell, N. & Hurst, G. D. D. 2006 Evolution of male-killer suppression in a natural population. *PLoS Biol.* **4**, 1643–1648. (doi:10.1371/journal.pbio.0040283)
- 81 Charlat, S., Hornett, E. A., Fullard, J. H., Davies, N., Roderick, G. K., Wedell, N. & Hurst, G. D. D. 2007 Extraordinary flux in sex ratio. *Science* **317**, 214–214. (doi:10.1126/science.1143369)
- 82 Hornett, E. A., Charlat, S., Wedell, N., Jiggins, C. D. & Hurst, G. D. D. 2009 Rapidly shifting sex ratio across a species range. *Curr. Biol.* **19**, 1628–1631. (doi:10.1016/j.cub.2009.07.071)
- 83 Huigens, M. E. & Stouthamer, R. 2003 Parthenogenesis associated with *Wolbachia*. In *Insect symbiosis* (eds K. Bourtzis & T. A. Miller), pp. 247–266. Boca Raton, FL: CRC Press.
- 84 Engelstädter, J. & Telschow, A. 2009 Cytoplasmic incompatibility and host population structure. *Heredity* **103**, 196–207. (doi:10.1038/hdy.2009.53)
- 85 Telschow, A., Hammerstein, P. & Werren, J. H. 2002 The effect of *Wolbachia* on genetic divergence between populations: models with two-way migration. *Am. Nat.* **160**, S54–S66. (doi:10.1086/342153)
- 86 Greischar, M. A. & Koskella, B. 2007 A synthesis of experimental work on parasite local adaptation. *Ecol. Lett.* **10**, 418–434. (doi:10.1111/j.1461-0248.2007.01028.x)
- 87 van Alphen, J. J. M. & Visser, M. E. 1990 Superparasitism as an adaptive strategy for insect parasitoids. *Annu. Rev. Entomol.* **35**, 59–79. (doi:10.1146/annurev.en.35.010190.000423)
- 88 Sirot, E. & Krivan, V. 1997 Adaptive superparasitism and host–parasitoid dynamics. *Bull. Math. Biol.* **59**, 23–41. (doi:10.1007/BF02459469)
- 89 Varaldi, J., Fouillet, P., Ravallec, M., Lopez-Ferber, M., Bouletreau, M. & Fleury, F. 2003 Infectious behavior in a parasitoid. *Science* **302**, 1930–1930. (doi:10.1126/science.1088798)
- 90 Patot, S., Martinez, J., Allemand, R., Gandon, S., Varaldi, J. & Fleury, F. 2010 Prevalence of a virus inducing behavioural manipulation near species range border. *Mol. Ecol.* **19**, 2995–3007.
- 91 Patot, S., Lepetit, D., Charif, D., Varaldi, J. & Fleury, F. 2009 Molecular detection, penetrance, and transmission of an inherited virus responsible for behavioral manipulation of an insect parasitoid. *Appl. Environ. Microbiol.* **75**, 703–710. (doi:10.1128/AEM.01778-08)
- 92 Gandon, S., Rivero, A. & Varaldi, J. 2006 Superparasitism evolution: adaptation or manipulation? *Am. Nat.* **167**, E1–E22. (doi:10.1086/498398)
- 93 Varaldi, J., Patot, S., Nardin, M. & Gandon, S. 2009 A virus-shaping reproductive strategy in a *Drosophila* parasitoid. *Adv. Parasitol.* **70**, 333–363. (doi:10.1016/S0065-308X(09)70013-2)
- 94 Prenter, J., MacNeil, C., Dick, J. T. A. & Dunn, A. M. 2004 Roles of parasites in animal invasions. *Trends Ecol. Evol.* **19**, 385–390. (doi:10.1016/j.tree.2004.05.002)
- 95 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. (doi:10.1111/j.1574-6941.2001.tb00824.x)
- 96 Gottlieb, Y., Ghanim, M., Gueguen, G., Kontsedalov, S., Vavre, F., Fleury, F. & Zchori-Fein, E. 2008 Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. *FASEB J.* **22**, 2591–2599. (doi:10.1096/fj.07-101162)
- 97 Gueguen, G. *et al.* 2010 Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Mol. Ecol.* **19**, 4365–4376. (doi:10.1111/j.1365-294x.2010.04775.x)
- 98 Vautrin, E. & Vavre, F. 2009 Interactions between vertically transmitted symbionts: cooperation or conflict? *Trends Microbiol.* **17**, 95–99. (doi:10.1016/j.tim.2008.12.002)
- 99 Wade, M. J. 2007 The co-evolutionary genetics of ecological communities. *Nat. Rev. Genet.* **8**, 185–195. (doi:10.1038/nrg2031)
- 100 Mouton, L., Dedeine, F., Henri, H., Bouletreau, M., Profizi, N. & Vavre, F. 2004 Virulence, multiple infections and regulation of symbiotic population in the *Wolbachia*–*Asobara tabida* symbiosis. *Genetics* **168**, 181–189. (doi:10.1534/genetics.104.026716)
- 101 Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M. & Ghanim, M. 2007 Biotypic-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bull. Entomol. Res.* **97**, 407–413. (doi:10.1017/S0007485307005159)

- 102 Goto, S., Anbutsu, H. & Fukatsu, T. 2006 Asymmetrical interactions between *Wolbachia* and *Spiroplasma* endosymbionts coexisting in the same insect host. *Appl. Environ. Microbiol.* **72**, 4805–4810. (doi:10.1128/AEM.00416-06)
- 103 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. *Proc. R. Soc. Lond. B* **270**, 2543–2550. (doi:10.1098/rspb.2003.2537)
- 104 Sakurai, M., Koga, R., Tsuchida, T., Meng, X. Y. & Fukatsu, T. 2005 *Rickettsia* symbiont in the pea aphid *Acyrtosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Appl. Environ. Microbiol.* **71**, 4069–4075. (doi:10.1128/AEM.71.7.4069-4075.2005)
- 105 Vautrin, E., Genieys, S., Charles, S. & Vavre, F. 2008 Do vertically transmitted symbionts co-existing in a single host compete or cooperate? A modelling approach. *J. Evol. Biol.* **21**, 145–161. (doi:10.1111/j.1420-9101.2007.01460.x)
- 106 Mouton, L., Henri, H., Bouletreau, M. & Vavre, F. 2003 Strain-specific regulation of intracellular *Wolbachia* density in multiply infected insects. *Mol. Ecol.* **12**, 3459–3465. (doi:10.1046/j.1365-294X.2003.02015.x)
- 107 Brandvain, Y., Barker, M. S. & Wade, M. J. 2007 Gene co-inheritance and gene transfer. *Science* **315**, 1685–1685. (doi:10.1126/science.1134789)
- 108 Wu, D. *et al.* 2006 Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biol.* **4**, 1079–1092. (doi:10.1371/journal.pbio.0040188)
- 109 Woyke, T. *et al.* 2006 Symbiosis insights through metagenomic analysis of a microbial consortium. *Nature* **443**, 950–955. (doi:10.1038/nature05192)
- 110 Gosalbes, M. J., Lamelas, A., Moya, A. & Latorre, A. 2008 The striking case of tryptophan provision in the cedar aphid *Cinara cedri*. *J. Bacteriol.* **190**, 6026–6029. (doi:10.1128/JB.00525-08)
- 111 Perez-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. (doi:10.1126/science.1130441)
- 112 McMeniman, C. J., Lane, R. V., Cass, B. N., Fong, A. W. C., Sidhu, M., Wang, Y. F. & O'Neill, S. L. 2009 Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* **323**, 141–144. (doi:10.1126/science.1165326)
- 113 Turley, A. P., Moreira, L. A., O'Neill, S. L. & McGraw, E. A. 2009 *Wolbachia* infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. *PLoS Neglect. Trop. Dis.* **3**, e516. (doi:10.1371/journal.pntd.0000516)
- 114 Kambris, Z., Cook, P. E., Phuc, H. K. & Sinkins, S. P. 2009 Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* **326**, 134–136. (doi:10.1126/science.1177531)