

Arthropod symbioses: a neglected parameter in pest- and disease-control programmes

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Summary

1. Arthropods are important players in biological control as pests, control agents and transmitters of invertebrate diseases. Arthropods are frequently infected with one or several micro-organisms, serving as micro-ecosystems in which multiple interactions can take place. These micro-organisms include disease agents and symbiotic micro-organisms. The latter are usually vertically transmitted and can have a broad spectrum of effects on their hosts, ranging from reproductive manipulations to protection against natural enemies. These interactions may directly or indirectly alter the biology of many arthropods in agriculturally, medically and ecologically relevant ecosystems.

2. Symbiotic micro-organism-induced reproductive manipulations such as cytoplasmic incompatibility and parthenogenesis induction can substantially affect the rearing of biological control agents. Many insects, and recently also mites and nematodes, have been found to be infected, displaying a wide range of effects. We discuss examples of arthropod-micro-organism interactions and effects, which could have consequences for the practical application of arthropods in biological control.

3. Symbiotic micro-organisms can also be involved in host protection against natural enemies such as parasitoids, pathogenic bacteria, fungi and viruses.

4. Symbiotic bacteria can influence the vectorial capacity of disease-vectoring arthropods and may be very helpful in decreasing the transmission of disease agents.

5. *Synthesis and applications.* The effect of micro-organisms on the outcome of biological control programmes is usually not considered in risk assessments and failure analyses. This review emphasizes the importance of endosymbiotic micro-organisms in comprehensive biological control programmes and provides recommendations on how to recognize, avoid or benefit from these influential tenants.

Key-words: arthropod biology, biological control, crop pest, disease vector, endosymbiont, reproduction manipulation, risk assessment, *Wolbachia*

Introduction

Arthropods are responsible for severe economic and ecological damage world-wide. In the United States, pest insects destroy approximately 13% of crop production, accounting for a loss of \$33 billion annually (Pimentel *et al.* 2003). Emerging or re-emerging pest species, such as the Argentine ant *Linepithema humile*, can also potentially affect whole ecosystems (e.g. Jenkins, Aber & Canham 1999; Gomez & Oliveras 2003; Fowler 2004). In addition, arthropods function as disease vectors, and as ecto- and endoparasites of humans and animals,

creating a world-wide health risk [World Health Organization, WHO (<http://www.who.int>) and World Organization for Animal Health, OIE (http://www.oie.int/eng/en_index.htm)]. A very prominent example are the hard ticks (Acari: Ixodidae), which can vector several human disease agents (Beugnet & Marie 2009) such as the bacterium *Borrelia burgdorferi*, causative agent of lyme disease and the viral agent of tick-borne encephalitis (Nazzi *et al.* 2010; Randolph 2010).

Arthropods are also the most frequently used organisms in augmentative biological control (BC; in numbers released – J. Klapwijk, pers. comm.), and most of them are hosts to one or several endosymbiotic bacteria (Zchori-Fein & Perlman 2004; Weinert *et al.* 2007; Duron *et al.* 2008; Hilgenboecker

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et al. 2008). The two most studied maternally inherited bacterial endosymbionts (ESs) are *Wolbachia* and *Cardinium*, which infect 66% and 6%–7% of insect species, respectively (Hurst & Jiggins 2000; Kittayapong *et al.* 2003; Zchori-Fein & Perlman 2004). For example, recent studies on large samples of ladybirds (Coccinellidae) showed that 52% of the species were infected with either *Wolbachia*, *Rickettsia* or *Spiroplasma* (Weinert *et al.* 2007). Given that arthropods represent a large part of our planet's biomass, maternally inherited ESs are probably the most common bacteria living in association with living organisms on Earth. Endosymbiotic bacteria can be divided into obligatory (primary) and facultative (secondary) symbionts. Obligatory symbionts are involved in and sometimes solely responsible for vital functions of their host (Baumann 2005). Thus, their hosts would not survive without them. One of the most studied examples is *Buchnera aphidicola*, the primary symbiont of the pea aphid *Acyrtosiphon pisum*, providing its host with essential amino acids (Douglas 1998). Facultative ESs, on the other hand, are not essential for host survival and their presence can be neutral, beneficial or detrimental to the host (Oliver *et al.* 2003; Perotti *et al.* 2006). Facultative ESs can be involved in their hosts' feeding (Gunduz & Douglas 2009; Hosokawa *et al.* 2010), reproductive (O'Neill, Hoffmann & Werren 1997; Werren, Baldo & Clark 2008) or defence strategies (Haine 2007; Brownlie & Johnson 2009). There is growing evidence that the interactions between facultative ESs and their hosts vary with environmental stressors such as the presence or absence of a natural enemy; in extreme cases, ESs being beneficial in one situation and costly in another (Haine 2007).

To improve their own fitness, endosymbiotic bacteria have to guarantee their maintenance in a host population. Many facultative ESs are maternally inherited. As vertical transmission rates (from mother to offspring) are usually < 100%, they would eventually be lost from the population in the absence of some measure of horizontal transmission (Lipsitch *et al.* 1995; Lively *et al.* 2005). To ensure its persistence in a host population, an ES can reduce the fitness of non-infected female hosts by manipulating their reproductive strategies (O'Neill, Hoffmann & Werren 1997). Such manipulations include cytoplasmic incompatibility (CI) between infected males and uninfected (or differently infected) females, selective male-killing in broods, feminization of genetic males or parthenogenesis induction.

Protecting their host against natural enemies is another strategy that allows ESs to spread in their host's population. The ES can provide the infected host with a selection advantage or increased fitness (Lively *et al.* 2005; Jones, White & Boots 2007), although surprisingly little evidence for this has been found in the field. Interestingly, some ESs have been shown to protect their hosts from predators, macroparasites (Olsen, Reynolds & Hoffmann 2001; Harcombe & Hoffmann 2004; Chiel *et al.* 2009a), bacteria and viruses (Davidson *et al.* 2001; Lopanik, Lindquist & Targett 2004; Brownlie & Johnson 2009) and to be involved in pesticide-resistance mechanisms (Kontsedalov *et al.* 2008). Such ES-mediated resistance to pathogens or chemicals can give an organism a substantial advantage over nonresistant conspecifics in an environment

where biotic or abiotic antagonists are present. The mechanisms underlying the various types of resistance are often unknown even if, in some cases, the ES produces toxic compounds that affect its host's enemies (Gil-Turnes, Hay & Fenical 1989; Gil-Turnes & Fenical 1992; Kellner 2002; Oliver *et al.* 2009). In other cases, ES-induced behavioural changes in the host (Haine, Boucansaud & Rigaud 2005; Rigaud & Haine 2005), such as deterrence effects that protect the prey from its predator (Davidson *et al.* 2001; Lopanik, Lindquist & Targett 2004), have been suggested or observed.

Endosymbionts can infect any beneficial arthropod species and may dramatically affect the outcome of a BC programme. By manipulating their host's biology, they have the potential to dramatically affect all phases of BC, from the rearing of a biological control agent (BCA) to its establishment in the field. In this review, we will explain how ESs can influence their arthropod hosts, give examples of known interactions among micro-organisms and their pest or disease-vectoring hosts, explain how ESs can play a role in the context of BC systems, and give practical advice on how to seek and recognize potential ES-mediated effects. Finally, we discuss how ES–host interactions can be profitably integrated into pest- and disease-control programmes.

Direct interactions among microbes and arthropods

The outcome of a BC programme can be positively or negatively influenced by many unpredictable biotic factors, ESs being one of them. Some bacteria are known to affect their host's reproduction strategies in a wide variety of ways. They can induce parthenogenic reproduction and thereby improve their own transmission to the next generation, as for example *Wolbachia* in a phytophagous pest mite of the genus *Bryobia* (Weeks & Breeuwer 2001). Another strategy is to disable crosses between infected males and uninfected females by causing CI, such as *Cardinium* in *Encarsia pergandiella* (Perlman, Kelly & Hunter 2008). Unidirectional or bidirectional CI may also occur between host populations carrying different strains of the bacterium (Bordenstein, O'Hara & Werren 2001). In another wasp, *Encarsia hispida*, *Cardinium* can turn genetic males into females (feminization; Giorgini *et al.* 2009). In arthropod hosts that lay their eggs in batches, freshly hatched siblings are often the first food source for young larvae. Vertically transmitted ES of the ladybeetle *Adalia bipunctata* can kill infected male embryos, an appreciated and crucial first meal for their sisters, providing them with a competitive advantage over larvae hatched from an uninfected brood (Schulenburg *et al.* 2002, among others). Remarkably, different species of *Wolbachia*, *Cardinium* and other endosymbiotic bacteria can be responsible for different reproductive manipulations in different hosts.

How do parthenogenesis-inducing ESs affect BC? These ESs are only known from haplo-diploid organisms (Floate, Kyei-Poku & Coghlin 2006). The sex determination of Hymenoptera and Thysanoptera, as well as some Acarida, Hemiptera and Coleoptera (Normark 2003), is determined by the numbers of chromosome sets, i.e. males are haploid and develop from

unfertilized eggs, whereas females are diploid and the eggs are usually fertilized. Parasitoid wasps, beetle larvae and predatory mites can be very important BCAs. Parthenogenic reproduction may considerably increase population growth and facilitate the rearing of beneficial organisms by making the presence of males unnecessary and reducing reproductive costs. Parthenogenic reproduction induced by ESs will influence the sex ratio towards females, thereby considerably increasing the success of the programme if sexes differ in efficiency and effectiveness as BCAs. In many cases, only females act as BCAs by laying their eggs in the pest, e.g. in parasitoid wasps, females lay their eggs in or on their hosts and hatched larvae kill the host by consuming it. In this case, males are reared solely to fertilize females. In the case of parthenogenic parasitoids, theoretically, twice as many pest hosts can be parasitized by a similar BCA population size. Sexually reproducing BCAs may hybridize with native species in the field, affecting their genetic integrity and thereby having a dramatic nontarget effect on the environment (Yara 2004; Hopper, Brich & Wajnberg 2006). By using parthenogenic BCAs, BC practitioners can avoid this risk. However, there are also disadvantages with parthenogenetically reproducing populations. In some species, the reproductive rate can be higher in sexual lines compared to ES-induced parthenogenic lines, due, for example, to the high mortality of *Wolbachia*-infected offspring (Lamb & Willey 1979), or delays in development time (Corley & Moore 1999; Matsuura & Kobayashi 2007). Stouthamer (1993) compared the efficiency of parasitic wasp (*Trichogramma*) females of sexual and asexual lines in controlling pest moth populations and found that their relative efficiency depends on host density. At a high density of pest hosts, sexual females produce more offspring and parasitize more, whereas at low density, the asexual female wasps perform better. Ongoing discussions about the evolution and maintenance of sexual versus asexual reproduction highlight many theoretical advantages and disadvantages of both reproduction modes (Stouthamer 1993; Hurst & Peck 1996, among others).

To date, *Wolbachia* (Tagami, Miura & Stouthamer 2001; Weeks & Breeuwer 2001), *Cardinium* (Gotoh, Noda & Ito 2007) and *Rickettsia* (Hagimori *et al.* 2006) infection have been found to induce parthenogenesis. When rearing haplo-diploid species on a commercial scale, we recommend paying attention to potential parthenogenesis-inducing ES infections.

How do CI-inducing ESs affect BC? CI is another reproductive manipulation which can have severe consequences for BC programmes. CI suppresses the development of offspring from crosses between infected males and uninfected females. There have been attempts to use CI-inducing ESs directly in BC, to deplete uninfected pest populations by releasing an excess of males carrying CI-inducing ESs. This technique is analogous to the sterile insect technique, considered to be one of the only strategies that can successfully eradicate a detrimental insect population (Krafsur 1998). Zabalou *et al.* (2004, 2009) demonstrated a rapid decrease in laboratory Mediterranean fly *Ceratitis capitata* populations inundated with males artificially infected with a CI strain isolated from a closely related species, *Rhagoletis cerasi*. This approach could be taken much further

in the future. Given that the host's genes, located on mitochondrial DNA, will spread into a population in the same way (because mitochondria and ESs are both vertically transmitted), the use of CI-inducing ESs has been suggested for the introduction of a gene impeding malaria transmission into an *Anopheles* population (Curtis & Sinkins 1998). Brelsfoard, St Clair & Dobson (2009) discuss the use of CI-inducing ESs in combination with a low dose of radiation for the control of lymphatic filariasis-transmitting mosquitoes. The repeated release of incompatible males only could deplete the mosquito population. It is crucial not to release any females infected with the CI ES into the population to avoid a spread of infection that would lead to population replacement (uninfected to infected). To prevent accidental release of females, low-dose irradiation of BCAs was suggested to sterilize any females present in the material to be freed. CI-inducing ESs may also cause indirect negative effects in an augmentative BC programme. If the aim is to artificially increase a pre-existing population of beneficial arthropods, the presence of CI-inducing ESs in the mass-reared and released individuals may not allow them to produce any offspring in the field. If the released and native populations are both infected but with different strains, reproductive isolation might be near complete, although in most cases CI is not 100% efficient, leaving a few uninfected offspring of infected males to reach adulthood.

Endosymbionts can be crucial for host egg production. In the braconid wasp genus *Asobara*, the symbiont *Wolbachia* does not only manipulate reproduction – in some strains it is indispensable for oogenesis. The exact mechanism is not clear: without the symbiont, either females fail to produce oocytes at all or the offspring generated by the oocytes do not develop properly (Dedeine, Bouletreau & Vavre 2005). Although this example is fairly unique in current research, its occurrence should still be considered when using Hymenopteran BCAs.

Symbionts can protect their hosts from abiotic stress, thus increasing the survival of pests and vectors in the environment. A striking example of a direct interaction between a symbiont and its host comes from the tick *Ixodes scapularis*, in which the bacterium *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, induces the expression of antifreeze glycoprotein which helps the host survive in cold temperatures (Neelakanta *et al.* 2010). In this case, the symbiont is also a horizontally transmitted mammalian pathogen, protecting its vector in stressful environments and enhancing its vectorial capacity. In the pea aphid *Acyrtosiphon pisum*, the symbiont *Serratia symbiotica* enables its host to survive under heat shock by providing a rapid supply of essential metabolites to the aphid or to the essential primary symbiont, through its own lysis (Burke, Fiehn & Moran 2009). In contrast, a symbiotic *Rickettsia* in the whitefly *Bemisia tabaci* was shown to reduce the whitefly's resistance to specific pesticides, an important component of the environment (Kontsedalov *et al.* 2008). This *Rickettsia* was shown to be transferred to the immature stages of the whitefly parasitoids *Eretmocerus mundus* and *Encarsia pergandiella*; however, its effects are not known and it does not persist later in adulthood (Chiel *et al.* 2009b; Fig. 1). Infection of the parasitoids during larval

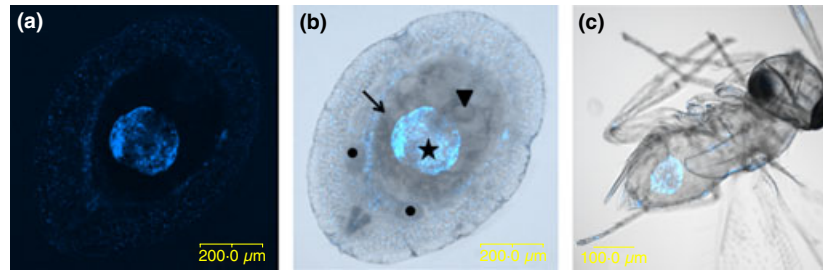


Fig. 1. Demonstration of the multitrophic interaction: Pest-BCA-ES using fluorescent *in situ* hybridization (FISH) of *Rickettsia* endosymbiont (blue) in the whitefly *Bemisia tabaci* and in its parasitoid *Eretmocerus emiratus*. (a) *B. tabaci* larva parasitized with *E. emiratus* larvae, fluorescent channel showing *Rickettsia* only. (b) Same as (a), fluorescent and bright field channel, arrow – parasitoid larvae, arrowhead – *E. emiratus* eggshell, star – *E. emiratus* gut filled with *Rickettsia*, dots – *B. tabaci* bacteriomes (c) *E. emiratus* adult showing *Rickettsia* in its abdomen, fluorescent and bright field channels. For more details please see Chiel *et al.* 2009b.

development may nevertheless affect them later as adults either by modifying their commensal bacterial community or directly by inflicting a cost of infection carried throughout the life of the parasitoid. Developmental media were demonstrated to affect the mating behaviour of *Drosophila* by influencing their commensal bacteria (Sharon *et al.* 2010). Therefore, feeding media and multitrophic interactions need to be further investigated and taken into consideration when mass rearing pests and their BCA.

Microbial interactions within the arthropod host

Microbes can interact to protect their host against its natural enemies. In the case of the pea aphid and its ES, the presence of the ES alters the host's interaction with *Aphidius ervi*, protecting it from the parasitoid (Oliver, Moran & Hunter 2005). *Aphidius ervi* is used world-wide to control aphid populations in legumes and ornamentals. Pea aphid clones, however, vary greatly in their resistance to *A. ervi*, which oviposits in an adult aphid with the developing larva slowly killing it from the inside. Oliver *et al.* (2003) demonstrated that the development of resistant populations is owing to differences in secondary symbiont infections. Both *Hamiltonella defensa* and *Regiella insecticola* conferred resistance to *A. ervi* by decreasing its chances of completing development within the aphid host. Recent studies have shown that the bacteriophage associated with *Hamiltonella defensa* is responsible for the resistance, encoding a toxin capable of killing the parasitoid larvae (Oliver *et al.* 2009). As a bacterial strain can lose its associated phage, the levels of protection and resulting fitness of the aphids, and thus the success of a BC programme using *A. ervi*, may vary greatly in the field.

Nematodes are macroparasites of many arthropods. Females of the North American *Drosophila neotestacea* become completely sterile when infected with the nematode *Howardula aoronymphibium*. Recently, however, the protective effect of *Spiroplasma*, one of two symbionts found in this fly, has been described. While *Spiroplasma* does not change the fecundity of unparasitized flies, it can rescue most of the eggs in females infected with *H. aoronymphibium* (Jaenike *et al.* 2010). Because of the selective advantage provided by

the ES to its host in the presence of the nematode, rapid spread of *Spiroplasma* infection can be observed across continents.

Having beneficial and pathogenic bacteria as roommates may lead to conflicts of interest. The ES depends on the host for its own reproduction, while the pathogen uses it as a resource and may harm or kill it. It is thus advantageous for the ES to protect the host from the pathogen. Microbial pathogens are typically protozoa (including microsporidia), fungi, bacteria or viruses, and sometimes even occupy the same location as the symbiont within the host.

Beauveria bassiana is a fungal pathogen infecting a diverse range of insect hosts (Riedel & Steenberg 1998). It lives in the soil, often infecting species that are in close contact with the ground for part of their life cycle. It is known to be an important natural mortality factor in insect populations and it has been suggested or used as a BCA of pests of many insect orders (Quesada-Moraga *et al.* 2006; Akello *et al.* 2008; Espinel *et al.* 2008; Pardey 2009). In a laboratory study, females of *Drosophila melanogaster* with identical genetic backgrounds but different *Wolbachia* infection status (W+ or W-) showed differences in their resistance to *B. bassiana* (Pantelev *et al.* 2007). Overall, the proportion of surviving females 7 days after infection with *B. bassiana* was three times greater in the infected (W+) female group than in the non-infected (W-) female group. Infected (W+) females also exhibited behavioural changes, such as variation in oviposition substrate preference. Moreover, infected (W+) males exhibited greater reproduction success than non-infected (W-) males (Pantelev *et al.* 2007). *Beauveria bassiana* is considered a potential control agent of the Asian ladybird *Harmonia axyridis* as it causes a significant reduction in the fertility of infected individuals. Roy *et al.* (2008) found that native ladybird populations are much more susceptible to *B. bassiana* than populations collected in Britain, which is part of the invasive range. Different genetic backgrounds could explain this pattern (Roy *et al.* 2008), but the potential influence of ESs infecting *H. axyridis* (Aebi & Zindel 2010) remains an open question that warrants evaluation.

Endosymbionts can provide their hosts with antibiotic components. The European beewolf *Philanthus triangulum* engages in a close association with a *Streptomyces* species that protects

the offspring in the brood cells from fungal pathogens. The bacteria reside in the antennae of female digger wasps and are added to brood cells prior to oviposition. The hatched larva spins a cocoon, into which it integrates the bacteria. As the brood cells are also ideal habitats for pathogens, the bacteria provide the bees with a selective advantage (Kaltenpoth *et al.* 2006). Recently, antibiotic substances have been found in the brain of cockroaches which are now being considered for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) (<http://www.smartplanet.com>). To the best of our knowledge, there are no records of antibiotic metabolites produced by insects or other animals that support the hypothesis of symbiotically living micro-organisms in the brains of these cockroaches.

Arthropods are often harmed by viruses, and *Wolbachia* has been found to alter arthropod resistance to viruses. *Drosophila melanogaster* has been used as a model organism to study resistance to viruses in arthropods. Two research groups (Hedges *et al.* 2008; Teixeira, Ferreira & Ashburner 2008) independently demonstrated that *Wolbachia* infection increases its host's resistance to *Drosophila* C Virus (DCV), a single-stranded RNA virus, but also to three other RNA viruses: Cricket Paralysis Virus (Hedges *et al.* 2008), Nora Virus and Flock House (FH) Virus (Teixeira, Ferreira & Ashburner 2008). The mechanisms involved in the ES-mediated protection against viruses are not fully understood. In the case of DCV and Nora virus, W+ flies contained up to 10⁴-fold less viruses. To date, no *Wolbachia*-induced resistance to a DNA virus has been reported (Teixeira, Ferreira & Ashburner 2008). Interactions between ESs and viruses are quite likely to be common (see below), as ESs and viruses are often found together in the intracellular space. Any found resistance to a virus can theoretically be attributed to an ES, although only ES-mediated resistance to RNA viruses has been reported to date.

Interaction between arthropod symbiont and vectored pathogen

The ability of an arthropod host to serve as a vector for pathogenic agents can be described in two ways: 'vector competence' or 'vectorial capacity' (Reisen 2002). The former term refers to the ability of the vector to support pathogen infection, replication and/or development (depending on the pathogen group) and transmission (nearly always by bite). The latter term includes vector competence as a factor, but is a field-derived estimate obtained through vector biting rates and survival, which can greatly influence disease transmission. Thus, vectorial capacity is the more comprehensive and relevant factor in describing the ability of a potential vector to transmit pathogens. ESs may thus have an influence on vectorial capacity by two means: interactions between the vector and its symbionts, and interactions between the symbionts (enduring microbes) and the pathogens (transient microbes) within the vector. Here, we will elaborate on microbial interactions and their potential to reduce vectorial capacity by directly influencing the survival of the disease agent within its vector.

Vertically transmitted symbionts are usually considered beneficial to their host and are thus expected to have an advantage over nonbeneficial, potentially harmful, transient microbes such as vectored pathogenic agents. For example, the causative agent of Rocky Mountain spotted fever, *Rickettsia rickettsii*, is transmitted by the tick *Dermacentor andersoni* (Burgdorfer, Hayes & Mavros 1981) and is also pathogenic to the tick itself (Niebylski, Peacock & Schwan 1999). Competitive displacement of *R. rickettsii* by the nonvectored, symbiotic *Rickettsia peacockii* (Niebylski *et al.* 1997) not only protects the tick from the harmful effects of *R. rickettsii*, it also prevents its transmission. In contrast, ESs can increase the vectorial capacity of their hosts by contributing to the transmission of the pathogen. For example, in the B biotype of the whitefly *Bemisia tabaci*, plant viruses have been shown to positively (Jiu *et al.* 2007) or negatively (Rubinstein & Czosnek 1997) affect vector fitness. Recently, Gottlieb *et al.* (2010) showed that the efficacy of Tomato Yellow Leaf Curl Virus (TYLCV) transmission by *B. tabaci* depends on the presence of the whitefly symbiont *Hamiltonella*. The interaction between a specific *Hamiltonella* GroEL and the virus coat protein protects the virus from proteolysis in the haemolymph, enhancing its chances of infecting the whitefly salivary gland and increasing its probability of being transmitted to the next plant. Symbionts can be viewed as part of their host's immune system, a statement which has recently gained some experimental support (see examples in section Microbial interactions within the arthropod host). The defence mechanisms explained above could, in the near future, be used for the control of vector-borne diseases. Tracking the quantity of three symbionts infecting tsetse flies showed active and dynamic colonization of the host which was dependent on host or environmental factors (Rio *et al.* 2006). Challenging the host with a transient microbe, *Trypanosoma brucei rhodesiense*, the agent of African trypanosomiasis (sleeping sickness), only affected the density of a facultative symbiont, *Wolbachia*, but had no significant effect on the density of the obligatory symbionts (Rio *et al.* 2006). Although the response to trypanosome infection was shown to be cellular (Hao *et al.* 2001; Boulanger *et al.* 2002; Hao, Kasumba & Aksoy 2003), the contribution of symbionts to the immune response cannot be ruled out.

Correlations between trypanosome infections and the presence of *Wolbachia* have also been described in the bug *Rhodnius pallescens* (Espino *et al.* 2009), a vector of *Trypanosoma cruzi*, the agent of American trypanosomiasis, or Chagas disease (Calzada *et al.* 2006), and *Trypanosoma rangeli*, a non-human pathogen (Guhl & Vallejo 2003). In that work, all field-collected triatomines were infected with *Wolbachia*, and the prevalence of trypanosome infection was between 25% and 56% for single infection (only one trypanosome species), whereas it was only 12% for double infection with the two trypanosome species. Thus, *Wolbachia* infection may reduce the number of double trypanosome infections via a competitive mechanism among all microbes, or by affecting the immune system of its host to prevent trypanosome development. The first hypothesis may be supported by the fact that *Wolbachia* has been found in the gonads, gut, salivary glands and faeces,

indicating the likelihood of its interaction with trypanosomes as both organisms share common locations in their host (Espino *et al.* 2009). The latter hypothesis may be supported by the recent work of Kambris *et al.* (2009) showing a reduction in the filarial nematode *Brugia pahangi*'s development in *Aedes aegypti* after artificial infection with the strain wMelPop of *Wolbachia* because of upregulation of immune system genes. These hypotheses, however, require further study. Symbiont manipulation (paratransgenesis) was used by Durvasula *et al.* (1997) to interfere with trypanosome transmission. The obligate symbiont of another triatomine, *Rhodnius prolixus*, was engineered to produce antimicrobial peptide against *T. cruzi*, and introduction of the engineered bacterium *Rhodococcus rhudnii* prevented *T. cruzi* establishment in several individuals in the laboratory. The ability to infect *R. prolixus* with modified symbionts via stercoaria (transmission through the faeces), the symbionts' natural mode of transmission, has great applicative potential. The paratransgenesis model was also tested for controlling Pierce's disease in grapevines caused by *Xylella fastidiosa*, a bacterium transmitted by the glassy-winged sharpshooter, *Homalodisca vitripennis*. The sharpshooter symbiont, *Alcaligenes xylosoxidans denitrificans*, was genetically modified to express single-chain antibodies that were specific to *X. fastidiosa* (Ramirez, Perring & Miller 2007), thus preventing persistence of the plant pathogen in the vector and reducing the vectorial capacity.

Gut microbes are expected to be the first barrier against transient microbes. Moreover, these microbiota are believed to actively inhibit pathogen transmission (summarized in Azambuja, Garcia & Ratcliffe 2005). Studies on the gut microbiota of malaria vectors have shown that the host immune genes modulate the symbiotic bacteria's gut community after intake of a blood meal (Meister *et al.* 2009). Natural responses of the microbiota in the mosquito gut may mediate antimicrobial immune responses against *Plasmodium* (Dong, Manfredini & Dimopoulos 2009). The interaction between *Aedes* mosquito microbiota, its defence response and infections with *Plasmodium* could then be targeted for specific control of malaria.

Analyses of the microbiota of other dipteran vectors of important veterinary and human diseases could reveal more natural interactions or potential candidates for manipulation. The sand fly *Phlebotomus argentipes*, vector of Kala-Azar, harbours specific bacteria (*Bacillus megaterium* and *Brevibacterium linens*) that are suitable for a paratransgenesis approach to controlling leishmaniasis. These bacteria can cause persistent infections and can be cultured (Hillesland *et al.* 2008). Comparisons between biting midges (*Culicoides*) vectoring bluetongue virus and nonvectoring midges revealed significant differences in microbial community composition (Campbell *et al.* 2004). These findings suggest that the microbial community may naturally determine vectorial capacity. This is also supported by the differing bacterial species richness of the flea vector *Ctenocephalides felis* when infected or not infected with *Rickettsia felis* (Pornwiroon *et al.* 2007).

To date, there is no conclusive evidence for a natural role for *Wolbachia* in direct protection against transient microbes, but a study has shown that it can be a target for manipulation,

specifically when introduced into a novel host. In a pioneering work, McMeniman *et al.* (2009) injected a life-shortening *Wolbachia* strain into the *Dengue* virus vector, *Aedes aegypti*, and directly reduced its vectorial capacity by influencing its survival and biting ability. Other works (e.g. Dobson, Marsland & Rattanadechakul 2009; Espino *et al.* 2009) showing natural *Wolbachia* infection in important vector species may open the way for direct manipulation of the symbiont or its environment to prevent transmission.

Conclusions and implications

Several biotic and abiotic factors affecting the BCA or the target crop-pest species can influence the success of a BC programme. One of the most variable and commonly underestimated factors is endosymbiotically living organisms such as bacteria, fungi or viruses. In addition to drastically influencing the dynamics and structure of BCA or pest/vector populations, ESs can (i) be involved in the evolution of resistance against natural enemies commonly used in BC programmes, (ii) induce resistance to pesticides and (iii) influence the vectorial capacity of some disease vectors. Evidence for ES-mediated protection strategies against very different enemies or chemicals is accumulating, even if it is still not clear how often ESs are actually responsible for an observed effect. A determination of the presence and influence of ESs in arthropods involved in BC programmes should be included in the risk assessment protocol prior to the BCA's release. Although ESs fall into the definition of contaminants that could potentially affect the efficacy of BCAs, defining their associated risk is an arduous task. In risk-assessment procedures, risk is usually defined as 'hazard x probability', hazard being any identifiable adverse effect that has a probability or likelihood of occurring. In the case of ESs infecting BCAs, both hazards and probabilities remain poorly described in BCAs, and it is virtually impossible to calculate the probability of a hazard occurring as most of the above-mentioned examples have been described in only a limited number of biological systems. Although there are currently no quality-control standards for contaminants associated with BCAs, we strongly encourage BC practitioners to perform a survey of potential ESs infecting the BCA, possibly threatening the success of a BC programme (Goettel & Inglis 2006). However, the effort invested in the detection of potentially adverse ESs should be in direct proportion to the risk they pose to the BCA or to the outcome of a BC programme.

Different methods of investigating the presence of ESs or other associated bacteria are available. Whole bacterial communities associated with a given species can be described by denaturing gradient gel electrophoresis (DGGE) (<http://www.eeescience.utoledo.edu/faculty/sigler/research/protocols/dgge/dgge.pdf>) or clone library analysis on bacterial 16S rDNA gene product, followed by a sequencing procedure. If the presence of bacteria is suspected (sex-ratio bias, unexplained resistance to natural enemies, unexplained rearing crashes, incompatibility between strains), polymerase chain reaction (PCR) with specific primers can, in some cases, help

confirm its presence (Enigl & Schausberger 2007; Weinert *et al.* 2007; Duron *et al.* 2008). An online, open-access catalogue of widely used BCAs (EPPO Standards on Safe Use of Biological Control – PM 6/3 – Version 2010), known bacterial associations and their potential effects is available on the following webpage: <http://www.symbiontsincontrol.ch>. The aim of this catalogue is to guide and inform BC practitioners on ES–arthropod interactions.

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