Hidden arsenal: endosymbionts in arthropods, their role and possible implications for biological control success

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Abstract Bacterial endosymbionts are common among arthropods including many important pest and beneficial insect species. These symbionts provide either an obligate function, performing essential reproductive or nutritive roles, or are facultative, influencing the ecology and evolution of their hosts in ways that are likely to impact biological control. This includes resistance against parasitoids and modification to parasitoid fecundity. Recent research has shown that endosymbionts are associated with exotic weevil pests found in New Zealand pasture, including the clover root weevil, *Sitona obsoletus* (= *S. lepidus*). The role of endosymbionts in insect biology and impacts on biological control is reviewed. For New Zealand plant protection scientists, endosymbiont research capability will provide important insights into interactions among insect pests, plant hosts and biological control agents, which may provide management opportunities for existing and future pest incursions in New Zealand.

Keywords symbiosis, arthropod symbiont diversity, arthropod pests, *Wolbachia*, symbiont-based control strategies, metagenomics, parasitism.

INTRODUCTION

Two exotic *Sitona* species have been accidentally introduced and established in New Zealand. *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) was first found in the 1970s where it proved to be a significant pest of lucerne (*Medicago sativa*) stands (Goldson et al. 1985). The introduction and release of a Moroccan strain of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) (Stufkens et al. 1987), an endoparasitoid of the adult weevil, led to a significant reduction in weevil populations and *M. sativa* yield losses (Kean & Barlow 2000).

However, a major negative impact was its wide non-target host range. Moroccan *M. aethiopoides* was able to parasitise and develop in a range of native and introduced beneficial weed biocontrol Curculionidae species (Barratt et al. 1997b), as well as the exotic hosts *Listronotus maculicollis* (Dietz) (McNeill et al. 1999) and *S. hispidulus* Fabricius (Sundaralingam et al. 2001).

Sitona obsoletus (Gmelin) (= *S. lepidus*) (Löbl and Smetana 2013) was first discovered in New Zealand in 1996 (Barratt et al. 1996). Of European origin, this pasture pest weevil species significantly

impacts on the production and persistence of white clover (*Trifolium repens*) (Gerard et al. 2007). Based on its success in controlling *S. discoideus* and wide host range, the suitability of Moroccan *M. aethiopoides* was evaluated against *S. obsoletus* in the laboratory. *Sitona obsoletus* proved to be an unsuitable host for Moroccan *M. aethiopoides* with emergence rates from exposed *S. obsoletus* of 1–6% (Barratt et al 1997a). A further study showed that although up to 50% of *S. obsoletus* exposed to Moroccan *M. aethiopoides* were subject to ovipositional attempts, almost all (up to 98%) parasitoid larvae removed from dissected weevils were non-viable (McNeill et al. 2000). While there are genetic differences between *M. aethiopoides* strains (Phillips et al. 2002), the result was intriguing given the ability of this parasitoid to successfully develop in a wide range of alternative hosts (Barratt et al. 1997b; McNeill et al. 1999; Sundaralingam et al. 2001). However, a possible causal agent was identified when preliminary screening in 2000 found that *S. obsoletus* was infected with either *Rickettsia* or *Wolbachia* bacterial endosymbionts (N.K. Richards, AgResearch, unpublished data). *Wolbachia* has previously been implicated in defending a weevil host species against parasitism (Hsiao 1996). Control of *S. obsoletus* was eventually achieved with the release of an Irish strain of *M. aethiopoides* (Gerard et al. 2006), but the reason behind resistance to parasitism by the Moroccan strain remained of interest due to its implications for future biological control programmes. In a recent genetic diversity profile study, *S. obsoletus* was found to be infected by one strain of *Wolbachia* and two strains of *Rickettsia*, none of which was found in *S. discoideus* (J.A. White, University of Kentucky, unpublished data). Although the function of these endosymbionts in relation to the biological control of these two *Sitona* species has yet to be examined in detail, the role of endosymbionts in insect biology and the implications for biological control in general are reviewed below.

What are endosymbionts?

An endosymbiont can be described as any organism that lives within the body or cells of another organism, i.e. forming an endosymbiosis.

Two broad categories of insect endosymbionts have been identified, obligate (primary) or facultative (secondary), and both can significantly influence the ecology, biology and evolution of the host (Table 1). Obligate symbionts are maternally inherited and generally have a nutritional basis, supplying essential nutrients that are lacking in host diets (e.g. Douglas 1998; Baumann 2005) with symbiosis going back millions of years (Moran et al. 1993; Wernegreen & Moran 2001). Approximately 10% of all insect species carry nutritional symbionts (Buchner 1965). Primary symbionts typically occupy specialized host organs called bacteriomes (Baumann 2005) and, because the symbiosis is obligate, neither the bacteria nor the insect is viable without the other. Two well known obligate endosymbionts include *Buchnera aphidicola*, which infects the pea aphid *Acyrtosiphon pisum* and provides its host with essential amino acids (Douglas 1998), and *Wigglesworthia glossinidia*, which is a symbiont of the tsetse fly *Glossina morsitans morsitans* and aids its host in vitamin synthesis (Nogge 1981). Weevils are ancestrally infected with the primary endosymbiont *Nardonella*. In the West Indian sweet potato weevil, *Euscepes postfasciatus*, removal of this endosymbiont resulted in significantly lighter body weight and lower growth rate than the control insects, an effect that persisted in the offspring (Kuriwada et al. 2010).

In comparison, facultative or secondary endosymbionts are not essential for host survival and their presence can be neutral, beneficial or detrimental to the host (Oliver et al. 2010; Vorburger & Gouskov 2011). Facultative endosymbionts exhibit a more recently developed association, are sometimes horizontally transferred between hosts and live in the haemolymph of the insects (not specialised bacteriocytes). Common examples include *Wolbachia*, *Rickettsia*, *Cardinium*, *Spiroplasma*, *Hamiltonella*, *Regiella* and *Serratia*, and can be found in the host as single species (e.g. Lachowska et al. 2010) or as multiple species infections (e.g. Toju & Fukatsu 2011; Ishii et al. 2013).

Role of facultative endosymbionts in defence

Facultative endosymbionts have been documented to significantly impact on the success of biological

control (e.g. Hedges et al. 2008; Oliver et al. 2010; Xie et al. 2010; Table 1). For example, the extensively studied aphid endosymbionts have been shown to provide defence against parasitoids and fungi, improve heat tolerance and increase fecundity (Oliver et al. 2010). *Rickettsiella* has been shown to have an important role in aphid colouration (Tsuchida et al. 2010), which in turn potentially influences attractiveness to natural enemies (Tsuchida et al. 2014). *Hamiltonella defensa* and *Serratia symbiotica* improve defensive properties in the pea aphid, *Acyrtosiphon pisum* (Oliver et al. 2003), as does *Regiella insecticola* in the peach-potato aphid, *Myzus persicae* (Vorburger et al. 2010). It is the toxins encoded by a bacteriophage of *H. defensa* (APSE) that kill larvae of the solitary endoparasitoid wasp *Aphidius ervi*, thereby allowing the pea aphid

(*A. pisum*) hosts to survive and reproduce. Strains of *H. defensa* have been found to vary in the degree of protection conferred against *A. ervi* (Degnan & Moran 2008). Thus strains that have lost the phage exhibit decreased protection against the parasitoid (Oliver et al. 2009), while conversely, the presence of the endosymbiont can influence parasitoid behaviour. Oliver et al. (2012) showed that *A. ervi* discriminates between symbiont-infected and uninfected aphids and changes its oviposition behaviour accordingly, depositing a single egg in uninfected aphids, but two or more eggs in *H. defensa*-infected aphids. The suggestion that oviposition choices may be largely determined by infection status led to the comment that that an evolutionary arms race between *A. pisum* and the parasitoid may be mediated by a bacterial symbiosis (Oliver et al. 2012).

Table 1 Endosymbiont functions and taxonomic distributions (not exhaustive).

Bacterial taxa	Host taxa	Described functions	References
Obligate endosymbionts			
Numerous taxa	Plant sap feeders blood feeders	Host nutrition	Baumann 2005, Moran et al. 2008
Facultative endosymbionts			
<i>Wolbachia</i>	>30% of arthropod species	Reproductive manipulations Host defense, Host fitness	Hsiao 1996, Duron et al. 2008, Hedges et al. 2008, Zug & Hammerstein 2012
<i>Cardinium</i>	~4% of arthropod species, including mites, spiders, and parasitic wasps	Reproductive manipulations	Zchori-Fein & Perlman 2004, Duron et al. 2008
<i>Arsenophonus</i>	~4% of arthropod species, including ticks, whiteflies, triatomine bugs	Reproductive manipulations Host nutrition	Duron et al. 2008, Novakova et al. 2009
<i>Spiroplasma</i>	~1% of arthropod species, including weevils	Reproductive manipulations Host defense	Duron et al. 2008, Xie et al. 2010
<i>Rickettsia</i>	<1% of arthropod species, including ticks and beetles	Reproductive manipulations Host fitness	Perlman et al. 2006, Duron et al. 2008, Himler et al. 2011
<i>Hamiltonella</i>	Aphids, whiteflies	Host defense	Oliver et al. 2010
<i>Regiella</i>	Aphids	Host defense	Oliver et al. 2010
<i>Serratia</i>	Aphids	Host defense Protection from environmental stress	Oliver et al. 2010
<i>Rickettsiella</i>	Pea aphid	Host colouration	Tsuchida et al. 2010

The well studied insect endosymbiont *Wolbachia* has been shown to have a range of impacts on its hosts, including cytoplasmic reproductive incompatibility, thelytoky or parthenogenesis, feminisation of males, male (son) - killing, increase in mating success of infected males via sperm completion and complete dependence on bacteria for egg production (see Lachowska et al. 2010). Its role in parasitoid defence was first documented in a study of the western strain of alfalfa weevil, *Hypera postica* (Gyllenhal) where the presence of *Wolbachia* caused high mortality of the braconid wasp larvae, *M. aethiopoides* (Hsiao 1996). *Wolbachia* was absent in Eastern and Egyptian strains of *H. postica*, which are permissive hosts for the parasitoid (Hsiao 1996). The presence of *Wolbachia* in the Western strain also blocks cross breeding with individuals from the Eastern and Egyptian strains through cytoplasmic incompatibility (Hsiao 1996).

Transfer between species

Endosymbionts can have significant phenological and ecological consequence on their hosts (e.g. Oliver et al. 2010; White 2011), ones that can have potential use in biological control of arthropod pests (Zindel et al. 2011). The survival and persistence of facultative endosymbionts in insects primarily depends on vertical maternal transmission, but horizontal transmission also can occur. The transfer of facultative endosymbionts between individuals of the same aphid species and across aphid species has been demonstrated in numerous laboratory studies (see Oliver et al. 2010), as well as between parasitoids (Watanabe et al. 2013). Transmission from host to parasitoid has also been demonstrated in the laboratory (Chiel et al. 2009; Gehrer & Vorburger 2012). For instance, the parasitoids *Aphidius colemani* and *Lysiphlebus fabarum* have been shown to transfer *H. defensa* and *R. insecticola* by sequentially stabbing infected and uninfected individuals of their host, black bean aphid, *Aphis fabae* (Gehrer & Vorburger 2012). However, no parasitoids that emerged from symbiont-infected aphids were infected with symbionts (Gehrer & Vorburger 2012). In another study, the potential of plant-

mediated transmission of endosymbionts was demonstrated whereby *Rickettsia* was transferred from the whitefly (*Bemisia tabaci*) to a plant, moved inside the phloem, and then acquired by other whiteflies (Caspi-Fluger et al. 2012). The consequence for biological control programmes are that potential failures may occur through a previously permissive host acquiring 'resistance' through the acquisition of defensive endosymbionts (e.g. Zindel et al. 2011).

Multiple endosymbiont infections

Hosts are often infected with multiple endosymbiont species, making it difficult to unravel which strain(s) are playing key roles in the host's ecology. For example, a survey of endosymbionts in natural populations of the chestnut weevil *Curculio sikkimensis* in Japan identified six distinct endosymbiont lineages, at different infection frequencies and with different geographical distribution patterns (Toju & Fukatsu 2011). Multiple endosymbiont infections were very common, with endosymbiont infection frequencies significantly correlated with climatic and ecological factors (Toju & Fukatsu 2011). Studies on the virulence of leafhoppers for rice plants are a good example of the complexity of endosymbiont interactions and host response. The *Macrosteles* leafhoppers, *M. striifrons* and *M. sexnotatus*, known vectors of phytopathogenic phytoplasmas, were found to support two obligate endosymbionts and five facultative endosymbionts (Ishii et al. 2013). The authors proposed that interactions between the obligate and facultative endosymbionts, and the phytopathogenic phytoplasmas within the same host insects, may affect vector competence of the leafhoppers. Endosymbionts may also allow leafhoppers to rapidly develop virulence to resistant rice varieties (Ferrater et al. 2013).

In terms of the dynamics of endosymbiont-arthropod associations, symbiont-driven evolution can be quite rapid (White 2011). Examples include an evolutionary shift in the consequence of *Wolbachia* infection on fruit fly (*Drosophila simulans*). Initially, this symbiont reduced fly fecundity, but after only 20 years of coadaptation with the host, infected flies exhibited a fecundity advantage over uninfected females

(Weeks et al. 2007). In a similar example, the spread of *Rickettsia* in field populations of sweet potato whitefly (*Bemisia tabaci*) in south-western USA occurred within 6 years (Himler et al. 2011). This was attributed to substantial fitness benefits to infected hosts including higher survival to adulthood and faster development times.

Endosymbionts as a pest management tool

The use of endosymbionts as a novel tool in the biological control of insect pests has also been the subject of recent research. In particular, a European-funded research programme (EU COST Action FA0701 2012) has investigated the potential to target either the symbionts within a target pest or introduce an endosymbiont into a novel host to disrupt the traits contributing to the insects pest status (e.g. capacity to vector diseases, natural enemy resistance (Douglas 2007; Hoffmann et al. 2011)). However, while having great potential in controlling major pests, developing a robust methodology that permits transfer of an endosymbiont into a novel host, and the rapid multiplication in the target population while maintaining infection and persistence across generations, are challenges to achieving a viable endosymbiont pest management tool.

New Zealand endosymbiont research

In contrast to the rapidly evolving research taking place in the USA and Europe, local research capability on endosymbiont-insect interactions is only just emerging. This is surprising given the importance of biological control to New Zealand's broad-acre agriculture and primary plant-based industries. Preliminary investigations have recently confirmed that both obligate and facultative endosymbionts occur in exotic and native weevils found in New Zealand pasture (J.A. White, University of Kentucky, unpublished data). However, research to determine their possible implications in relation to weevil phenology and impacts on biological control agents is the target for future work. The initial poor establishment, and the low parasitism levels by Irish *M. aethiopoides* of *S. obsoletus* in Northland, New Zealand (Gerard et al. 2010), plus high rates of parasitoid mortality in weevils containing only a single parasitoid compared to

healthy larvae containing multiple parasitoid eggs (P.J. Gerard, AgResearch, unpublished data), suggests that an endosymbiont-induced parasitoid defence response may be taking place, similar to that provided by *H. defensa* in the pea aphid. Furthermore, Northland *S. obsoletus* samples were infected with *Wolbachia* and higher levels of *Rickettsia* strain 2 compared to weevils sampled in Canterbury (J.A. White, University of Kentucky, unpublished data). Thus, it is possible that one or both of these endosymbionts may play a protective role against the parasitoid. The elimination of endosymbionts through heat treatment or selected antibiotics (e.g. Hagimori et al. 2006; White et al. 2009), followed by the investigation of subsequent development responses in the target insect populations would be the standard methodologies to apply to such a study.

Additional studies will include exploring the effect of environmental stresses, such as temperature and age of the host, on endosymbiont titre within the insect. This will require designing suitable primers to detect the target endosymbionts and to determine detection thresholds. Recent research has shown that certain *Wolbachia*-infections are capable of escaping standard PCR screening methods by 'hiding' as low-titre infections below the detection threshold of standard PCR detection techniques. The use of highly sensitive PCR technology can enhance the symbiont detection limits substantially (Schneider et al. 2013). In addition, quantitative PCR in conjunction with real-time detection of the 16S rRNA gene can be used to investigate titres of endosymbiont within the target organism (e.g. Watanabe et al. 2013). It is intended to make use of fluorescence *in situ* hybridization (FISH) to identify the location of endosymbiont bacteria in the host insect as demonstrated in Dhami et al. (2012).

A key step in characterising the relationship of identified endosymbionts is through the application of phylogenetics. Molecular methods that allow the study of both culturable and non-culturable bacteria combined with the development of cheap high-throughput sequencing technologies, known as next-gen sequencing (NGS), have greatly enhanced microbial community diversity research (Fakruddin & Mannan 2013). Such methods

have been recently used to study the diversity and evolutionary pathways of endosymbiotic associations between bacteria and insects (Merville et al. 2013; Toju et al. 2013). However, as the database of sequencing information grows, so does the bioinformatics and phylogenetics expertise required to help make sense of the data. Choosing the right sequence to build the tree is crucial as well as which sequencing platform, bioinformatics pipeline, phylogenetics software and settings are best for a specific project (Solomon et al. 2014). Hence, our experience has shown it is essential to develop a close collaboration between biologists and bioinformaticians to build a scientifically valid tree that accurately represents the links between the organisms of interest. Without these relationships it is very easy to create trees that are nonsensical.

Conclusion

Preliminary investigations have confirmed that both obligate and facultative endosymbionts occur in exotic and native weevils found in New Zealand pasture, although their role is yet to be elucidated. However, as is evident in this contribution, many other insect groups are infected with endosymbionts that can enhance their pest status, impacts and resistance to biological control agents. For New Zealand cropping, forestry and horticulture industries, the impact of sector insect pests may well be influenced by the presence of endosymbionts, examples of which include the potato psyllid, *Bactericera cockerelli* (Casteel et al. 2012; Nachappa et al. 2011) and aphids (Oliver et al. 2010). Developing research capability in endosymbionts within New Zealand's plant protection research community would seem prudent, particularly in the face of the inevitable new pest invasions and climate change.

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