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REVIEW

Overview and relevance of *Wolbachia* bacteria in biocontrol research

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Abstract
Wolbachia bacteria are an unsuspected, but potentially important, component of many biocontrol programs. To heighten awareness of these bacteria, we review current knowledge of *Wolbachia* and their possible application in biocontrol research. *Wolbachia* promote their spread by altering the reproductive success of their arthropod hosts. This ability frequently is identified as having potential either to reduce populations of pest species, or to increase populations of beneficial species. However, only 19 and 1% of peer-reviewed research articles ($n = 844$) on *Wolbachia* appear in arthropod- and biocontrol-specific journals, respectively. Although *Wolbachia* will not have application for all programs, their prevalence cannot be denied. We screened for *Wolbachia* in populations of arthropods of current interest to biocontrol programs in Canada. Infections were detected in 47% of 177 populations, representing 46% of the 105 species tested. Greater awareness, in combination with the rapidly expanding knowledge base of *Wolbachia* and similar endosymbionts, offers new directions for research in biocontrol programs. We recommend that all arthropod species in biocontrol programs be screened for these bacteria.

Keywords: *Symbiont, biological control, survey, bacteria, arthropod*

Introduction

Wolbachia are obligate intracellular bacteria that infect arthropods and nematodes. Infections are common, occur in diverse taxa, and can profoundly alter host reproduction. For these reasons, there is growing interest in the potential application of *Wolbachia* in biocontrol programs, either to increase populations of biocontrol agents or to reduce populations of pest species (e.g., see reviews by Werren 1997; Stouthamer 2004). Interest has been further enhanced with the discovery of *Wolbachia* as essential symbionts of filarial nematodes that, in turn, cause diseases in humans.

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Targeting *Wolbachia* with antibiotics, rather than their host nematodes with nematocides, promises an indirect method to cure the human patient (refs. cited in Foster et al. 2005). Spurred onwards by book publications (O'Neill et al. 1997), international conferences every second year, and genome sequencing projects (Wu et al. 2004; Foster et al. 2005), research on *Wolbachia* continues to accelerate. More than 70% of the articles on these bacteria have been published since Werren's (1997) prediction that 'Research into Wolbachia is likely to undergo an explosive growth in the near future' (Figure 1).

Despite the rapid proliferation of literature, *Wolbachia* remain an unsuspected component of many biocontrol research programs. We attribute this lack of awareness to at least five main factors. First, much of the research on *Wolbachia* is still in the discovery phase. The diversity and phylogeny of *Wolbachia* 'strains' needs resolution, the cellular basis of *Wolbachia*-host interactions is largely unknown, and mechanisms for the movement of *Wolbachia* between host species remain to be clarified. Thus, there currently are very few tangible examples of applied *Wolbachia* research to capture the interest of the biocontrol community (but see, for example, Silva et al. 2000; Zabalou et al. 2004). Second, *Wolbachia* does not readily meet the traditional definition of a biocontrol agent; i.e., a parasite, pathogen or predator of the target species. Although *Wolbachia* can function as a parasite to reduce host fitness (e.g., Silva et al. 2000), it also may serve as a mutualist to benefit the host (e.g., Dedeine et al. 2001; Foster et al. 2005). Hence, its status as a biocontrol agent is ambiguous. Third, research on *Wolbachia* requires molecular techniques, equipment and expertise that are absent in most laboratories studying the ecology/biology of biocontrol agents and (or) their target species in the field. Thus, serious consideration to incorporate studies on *Wolbachia* into existing biocontrol research programs may be prevented by concerns regarding additional program costs.

The fourth reason is that the scientific literature on these bacteria has not targeted the biocontrol community. Excluding several score research reports, theses, book chapters, and reviews, we identified 844 original scientific research articles pertaining

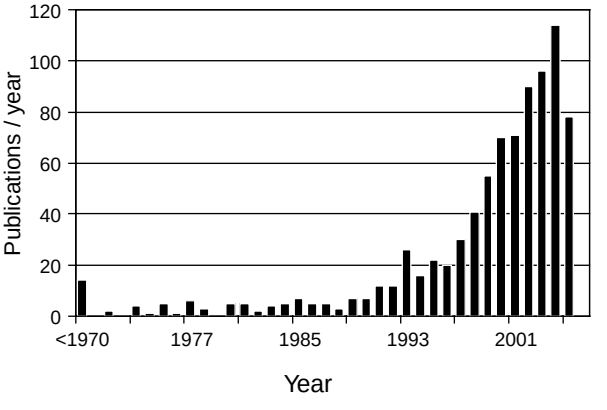

Figure 1. Annual publication of original, peer-reviewed research articles pertaining to *Wolbachia* for the period ending December 2005. Data was generated using database searches of CAB Abstracts, Biological Abstracts, and PubMed (http://www.ncbi.nlm.nih.gov/) using '*Wolbachia*' as the search term. Several score research reports, theses, book chapters, and reviews have been excluded.

to *Wolbachia* published prior to January 2006, using literature searches of CAB Abstracts, Biological Abstracts, and PubMed (http://www.ncbi.nlm.nih.gov/) (Figure 1). Of this total, 19.5% (165 articles) appeared in arthropod-specific journals. Only 1.3% (11 articles) appeared in journals specific to biological control; i.e., *Biocontrol* (1), *Biocontrol Science and Technology* (3), *Biological Control* (6), *Chinese Journal of Biological Control* (1). Research on *Wolbachia* was published most often in *Heredity* (54 articles), *Proceedings of the Royal Society of London – Series B* (49); *Genetics* (43), *Journal of Invertebrate Pathology* (37), and *Insect Molecular Biology* (28). Hence, readers browsing biocontrol or entomology journals seldom encounter papers on *Wolbachia*.

The fifth reason for limited awareness of *Wolbachia*, is that most of the research on these bacteria has been restricted to only a few arthropod taxa. We identified 582 original articles reporting on *Wolbachia* infections in arthropods, which we classified by the host's taxonomic family. Cases were excluded where this distinction was unclear, or included more than one family; e.g., surveys of *Wolbachia* across numerous families, or general discussions of *Wolbachia* without emphasis on a particular family. Articles ($n = 510$) on insect hosts were most often on Drosophilidae (25.5%), Culicidae (19.4%), Trichogrammatidae (8.2%) and Pteromalidae (5.1%). Articles ($n = 43$) on arachnid hosts primarily were on Tetranychidae (39.5%). Articles ($n = 26$) on isopod hosts primarily were on Armadillidiidae (76.9%). Readers would be largely unaware of such publications unless these taxa, or *Wolbachia*, were of specific interest.

To heighten awareness of the potential relevance of these bacteria in biocontrol research, here we provide an overview of research on *Wolbachia* with an emphasis on their application for pest control. Specifically, we present: (1) information on the classification of *Wolbachia*, (2) a brief summary of reproductive effects caused by *Wolbachia* in arthropod hosts, (3) results of a survey to determine the prevalence of *Wolbachia* in commercial, field, and laboratory populations of arthropods of biocontrol interest in Canada, and (4) examples to illustrate the relevance of *Wolbachia* in biocontrol research.

Although we restrict the current review to *Wolbachia*, we note that they are only one of several types of bacteria that affect the reproduction of arthropod hosts. Other such types of bacteria include *Arsenophonus* (γ-Proteobacteria), *Cardinium* (Bacteroidetes), *Rickettsia* (α-Proteobacteria), and *Spiroplasma* (Mollicutes). Much less information is available on these latter groups, but they should not go overlooked. A recent survey detected infections of *Cardinium* in 6% of 99 arthropod species (Zchori-Fein & Perlman 2004).

Wolbachia classification and nomenclature

Wolbachia is a genus of bacteria in the Family Rickettsaceae within Phylum α-Proteobacteria. First described from the tissues of the mosquito *Culex pipiens* (Hertig & Wolbach 1924), Hertig named the genus after his collaborator, Wolbach (Hertig 1938). *Wolbachia pipientis* currently is the only species in the genus (see Dumler et al. 2001 and refs. therein). Other named taxa include *W. persica*, *W. popcorn*, *W. postica* and *W. trichogrammae*. However, the latter three taxa have no official standing, and *Wolbachia persica* has been shown to be a member of the γ-Proteobacteria. Hence, reference to *Wolbachia* currently is synonymous with *W. pipientis*.

Wolbachia isolates are identified using specific DNA gene sequences. Commonly targeted genes for this purpose include 16*S* rDNA (partial small subunit ribosomal

DNA), *ftsZ* (cell division gene), *groEl* (heat shock protein gene) and *wsp* (outer surface coat protein gene). Sequences for 16*S* rDNA are the least variable and only identify isolates as *Wolbachia*. The more variable *ftsZ* sequences segregate *Wolbachia* isolates into 'Supergroups' of which eight have been reported thus far (Bordenstein and Rosengaus 2005 and refs. therein). Supergroups A and B mainly infect arthropod hosts. Supergroups C and D infect filarial nematodes. Supergroup E has been reported from springtails (Collembola). Supergroup F has been reported from filarial nematodes, termites (Isoptera), true bugs (Hemiptera) and weevils (Coleoptera). Infections of Supergroup G and H have been reported from spiders (Araneae) and termites, respectively. *Wsp* gene sequences have been most widely used and subdivide Supergroups into 'Groups' (sequences differing by at least 2.5%) and 'Strains' (sequences differing by at least 1.0%) (Zhou et al. 1998).

Strains are informally named following the general format of '*w*host'; e.g., *w*Mel denotes a strain of *Wolbachia* isolated from the vinegar fly, *Drosophila melanogaster* (Zhou et al. 1998). This system has been modified to distinguish multiple strains in the same host species. Strains from different populations of *Drosophila simulans* have been named after geographic locality; i.e., *w*Cof (Coffs Harbour), *w*Ha (Hawaii), and *w*Ri (Riverside) (Zhou et al. 1998). Alternatively, strains from the same host population may be designated with superscript numbers. Hence, *w*Ppub$_1$, *w*Ppub$_2$ and *w*Ppub$_3$ infect the same population of the pubic louse, *Pthirus pubis* (Kyei-Poku et al. 2005). Strain names usually are accompanied by an accession number that identifies the DNA sequence from which the strain designation was determined. Many journals require DNA sequence information to be submitted to a genetic database to permit the assignation of accession numbers prior to publication. Genbank is one such database, and is accessible via the homepage for the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/).

Interpretation of phylogenetic relationships between *Wolbachia* isolates can vary with the number and selection of genes used for comparisons. This interpretation can be further confounded by recombination between genes (Jiggins et al. 2001; Werren & Bartos 2001) or by the exchange of genetic material between *Wolbachia* strains (see Bordenstein & Wernegreen 2004 and refs. therein). Non-ambiguous interpretations are desired to clarify the evolutionary history of *Wolbachia* with their host species, and to possibly predict the effect of infections on host reproduction. Hence, a two-tier system has been proposed to ensure greater phylogenetic rigour (Bandi et al. 2004). In Tier 1, an isolate described using one or few genes would be termed a 'sequence variant' (seqvar) and be given a unique name. Thus, strain *w*Ppub$_1$ (see above) would be renamed as *Wolbachia pipientis* Seqvar Ppub$_1$. In Tier 2, seqvar isolates would be considered members of the same strain if, for example, they shared identical sequences for five of seven genes.

Consequences of *Wolbachia* infections to the host

Effects on host reproduction

Wolbachia are obligate symbionts that live in vacuoles within the cells of their host. Infections are transmitted vertically in egg cytoplasm, from infected mothers to their offspring. Transmission by infected males is prohibited by the low volume of cytoplasm in mature sperm, although rare cases may occur (Hoffmann & Turelli 1988). Because *Wolbachia* are maternally transmitted, infections only can spread in

the host population if infections favor the production of offspring by infected females. *Wolbachia* induce four known categories of effect on their hosts to achieve this goal; i.e., feminization, parthenogenesis, male-killing, and cytoplasmic incompatibility.

Feminization. Feminizing strains of *Wolbachia* cause genetic male hosts to develop into functional females. Such infections are common in terrestrial isopods of which those in the woodlouse, *Armadillidium vulgare* have been best studied (Cordaux et al. 2004 and refs. therein). Sex in this and other crustaceans is determined by the action of a male hormone that suppresses female development. *Wolbachia* is thought to inhibit development of the androgenic gland that produces this hormone and also may block receptor sites required for hormone activity. Hence, infected isopods produce female-biased sex-ratios regardless of their sex chromosome complement (WZ = females; ZZ = males) (reviewed in Rigaud 1997).

Parthenogenesis induction (PI). PI *Wolbachia* only are known from haplo-diploid taxa. Reproduction by arrhenotoky is common in these taxa, whereby males develop from unfertilized (haploid) eggs and females develop from fertilized (diploid) eggs. Two forms of 'gamete duplication' (Stouthamer & Kazmer 1994) explain cases of *Wolbachia*-induced parthenogenesis in Hymenoptera. In *Leptopilina clavipes* (Figitidae) (Pannebakker et al. 2004) and *Trichogramma* spp. (Trichogrammatidae) (Stouthamer & Kazmer 1994), infections interfere with the separation of chromosomes in anaphase of the first mitotic division. The unfertilized egg thus contains a diploid nucleus with two identical sets of chromosomes and develops into a female. In *Muscidifurax uniraptor* (Pteromalidae) (Gottleib et al. 2002) and *Diplolepis rosae* (Cynipidae) (Stille & Dävring 1980), the first mitotic division is normal and produces haploid nuclei, but these subsequently fuse to restore the diploid condition. Females arising from gamete duplication are homozygous for all alleles (Stouthamer & Kazmer 1994).

PI *Wolbachia* also have been reported in two species of phytophagous mites (Acari: Tetranychidae) in the genus *Bryobia* (Weeks & Breeuwer 2001). In contrast to products of gamete duplication, mite offspring are genetically identical to the female parent, which may be heterozygous for alleles. PI *Wolbachia* also have been reported for the predatory thrip, *Franklinothrips vespiformis* (Thysanoptera), but the mechanism of parthenogenesis is unknown (Arakaki et al. 2001).

Male-killing (MK). MK *Wolbachia* are one of several types of maternally-inherited bacteria that kill males during embryonic development. Hence, infected females may lay a mixed brood of male and female eggs, but only the latter survive to become adults. Cases of MK *Wolbachia* have been reported in taxa including Coleoptera (Coccinellidae, Tenebrionidae), Diptera (Drosophilidae) and Lepidoptera (Nymphalidae). The cytological mechanisms causing male-killing are unknown. Studies of male-killing most recently have been reviewed in Stevens et al. (2001).

Cytoplasmic incompatibility (CI). Cytoplasmic incompatibility (CI) is the most common of the *Wolbachia*-induced phenotypes that affect host reproduction. It has been reported in Coleoptera, Diptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera, mites (Acari: Phytoseiidae, Tetranychidae), and woodlice (Isopoda: Porcellionidae) (refs. cited in Stouthamer et al. 1999). CI arises in matings between

Wolbachia-infected males versus uninfected females, or between partners infected with different strains of *Wolbachia*. Most recently reviewed by Tram et al. (2003), the presence of *Wolbachia* in males is thought to introduce a factor into their sperm that prevents embryogenesis in the fertilized egg, unless the female partner is infected with the same *Wolbachia* strain to allow the sperm's 'rescue'. Unidirectional CI is most frequent and usually occurs between males infected with a single strain of *Wolbachia* and uninfected females. However, hosts may be infected with more than one strain of *Wolbachia*. Hence, cases of bidirectional CI have been reported with offspring arising only when both partners are infected with the same *Wolbachia* strains.

CI causes the loss of paternal chromosomes with different outcomes depending upon whether the host is a diplo-diploid or haplo-diploid species. In diplo-diploid species, paternal chromosome loss causes the haploidization of fertilized eggs that subsequently die. When the same process occurs in haplo-diploid species, potentially all of the haploid eggs develop into males; i.e., male-development (MD) type CI. Alternatively, CI in haplo-diploid species may be characterized by the death of embryos destined to become females; i.e., female-mortality (FM) type CI. Both MD-type and FM-type CI produce male-biased broods of offspring. However, they can be distinguished because the latter also results in fewer offspring relative to the number produced in compatible crosses. Whereas MD-type CI probably reflects the complete loss of paternal chromosomes, FM-type CI may be due to their incomplete loss causing lethality (Vavre et al. 2000).

Effects on host physiology

In addition to influencing the sex and survival of the host's offspring, *Wolbachia* can affect the host directly. These effects may be beneficial or detrimental, and are confounded by genetic and environmental factors.

Several studies document benefits of *Wolbachia* infection to the host. Infections may be essential for host survival (Foster et al. 2005 and refs. therein) or increase host fecundity (Girin & Bouletreau 1995; Poinsot & Mercot 1997; Fry et al. 2004) and survival (Dobson et al. 2004; Fry et al. 2004). In multiply inseminated females of the flour beetle *Tribolium confusum* (Coleoptera: Tenebrionidae), sperm of infected males appear to outcompete sperm of uninfected males (Wade & Chang 1995). Infections also may suppress the actions of otherwise deleterious genes in the host's genome. Chi^2 is an allele for a gene affecting growth regulation in *D. melanogaster*. Completely lethal in uninfected flies, chi^2 does not cause lethality in *Wolbachia*-infected flies (Clark et al. 2005; see Starr & Cline 2002 for a second example). This ability to shelter their hosts from deleterious mutations may be another factor promoting the widespread prevalence of *Wolbachia* infections.

Detrimental effects of infections are exemplified with reference to the *Wolbachia* '*popcorn*' strain. First reported in a laboratory colony of *D. melanogaster*, *popcorn* over-replicates in the adult host to disrupt cells and hasten mortality (Min & Benzer 1997; McGraw et al. 2002; Reynolds et al. 2003). Infections of non-*popcorn* strains in *Leptopilina heterotoma* also can reduce locomotory performance by about 30%, and lower both adult survival and fecundity (Fleury et al. 2000).

There also are cases where infections of *Wolbachia* appear to have no effect on individual host parameters. Antibiotic treatments used to reduce bacterial densities of *Wolbachia* in the wasp *Muscidifurax uniraptor* increased production of male offspring,

but did not affect the longevity or fecundity of female hosts nor numbers of F_1 offspring surviving to become adults (Zchori-Fein et al. 2000). Similarly, infections of *Wolbachia* in *D. melanogaster* did not affect larval developmental time, heat resistance, or the size of adults reared on a nutritionally poor diet (Harcombe & Hoffmann 2004).

Confounding factors

Numerous factors affect the outcome of *Wolbachia*-host interactions (see review by Weeks et al. 2002). Cytoplasmic incompatibility (CI) can be nearly absolute when infected male *Drosophila melanogaster* aged 24-h post eclosion are mated with uninfected females, but may be undetectable when these crosses are repeated using infected males aged 4–5 days post-eclosion (Hoffmann et al. 1990; Reynolds & Hoffmann 2002). This difference is attributed to declines in *Wolbachia* density associated with male age. Similarly, bacterial density can affect the expression of *Wolbachia*-induced parthenogenesis in the wasp *Muscidifurax uniraptor* (Zchori-Fein et al. 2000). Higher temperatures enhance the ability of the *popcorn* strain of *Wolbachia* to shorten the lifespan of *D. melanogaster* (Min & Benzer 1997; McGraw et al. 2002, Reynolds et al. 2003), which probably reflects different propagation rates of *Wolbachia* in host cells. Host genotype also can affect the outcome of infections (Olsen et al. 2001; Reynolds et al. 2003) and is the key factor determining whether CI in the wasp genus *Nasonia* is expressed as male-development (MD) or female-mortality (FM) type CI (Bordenstein et al. 2003).

Additional complications may arise when two or more strains co-occur in the same individual (superinfections) or when different strains occur in different individuals of the same population (multiple infections). Superinfections that are stable in populations of *Drosophila simulans* reared in optimal conditions can become multiple infections in crowded rearing conditions with the loss of one strain from some individuals (Sinkins et al. 1995). The wasp, *Leptopilina heterotoma*, carries superinfections of three *Wolbachia* strains. Each strain independently induces a type of CI intermediate between MD and FM type CI, which increasingly resembles MD type CI as the number of strains increases within the host (Mouton et al. 2005). Singly-infected female *L. heterotoma* can mate successfully only with singly-infected males carrying the same strain, whereas females carrying all three strains can mate successfully with any male in the population (Mouton et al. 2005).

Evolutionary time may add further variability to the *Wolbachia*–host relationship. Intuitively, infections of *Wolbachia* that favor reproduction by infected females are best suited to establish and persist in host populations. Hence, there should be selective pressure for newly acquired infections to shift from being parasitic to benign or mutualistic. Support for this prediction comes from observations of the *popcorn Wolbachia* strain after experimental transfer from *Drosophila melanogaster* into *D. simulans*. In the generations following the initial transfer, densities of *Wolbachia* in the ovaries of the novel host were high and associated with reduced fecundity and egg hatch (McGraw et al. 2002). However, bacterial densities subsequently declined as did the associated negative effects on host reproduction (McGraw et al. 2002). Similarly, the obligate symbiosis between *Wolbachia* and filarial nematodes may reflect a long-term evolutionary relationship in this parasite–host system (Foster et al. 2005).

Prevalence of *Wolbachia* in research programs

To illustrate the relevance of *Wolbachia* research in biological control, we screened populations of laboratory-reared and field-collected arthropods for infection. Taxa primarily included pest species and species being sold and (or) studied as biocontrol agents, but also species being maintained in culture for other reasons. We tried to obtain a broad spectrum of taxa, but ultimately were limited to testing the specimens that were received in response to requests. Material was received from more than 20 sources, primarily Agriculture and Agri-Food Canada research centers from across Canada. Additional material was received from commercial suppliers and from colleagues in Argentina, Australia, Denmark, France, Peru, Switzerland, and the United States. General surveys for *Wolbachia* in arthropods have been performed previously (see Table 1 in Stevens et al. 2001). To our knowledge, however, the current survey is the first to target diverse taxa of specific interest to the biocontrol community.

Screening was performed using the general methods described in Kyei-Poku et al. (2003). In brief, DNA was extracted from samples containing one or more whole insects depending upon their size and availability. Extracted DNA was amplified using polymerase chain reaction (PCR) with *Wolbachia*-specific *wsp* primers (Braig et al. 1998; Zhou et al. 1998). These primers amplify *Wolbachia* DNA (if present) to detectable levels that can be visualized as band of characteristic size (ca. 600 bp) on agarose gels. To confirm amplification of *Wolbachia* in infected insects, we used positive controls of DNA extracted from horn fly, *Haematobia irritans* (Diptera: Muscidae) that our preliminary studies showed to be infected with *Wolbachia*. Negative controls of HPLC grade distilled deionized water added to the PCR reaction mix were used to test for foreign DNA contamination. The primer set 28S rDNA, which amplifies the mitochondrial DNA of most eukaryotes, was used as a positive control to confirm the extraction of insect DNA, template quality and amplifiability.

A total of 177 populations were screened, representing 105 species in 10 orders. Infections were detected in 83 (47%) of these populations, representing 48 (46%) of the species tested (Table 1). Previous reports of *Wolbachia* prevalence in arthropods vary, depending upon the range of taxa being surveyed; e.g., within families or across orders. Previous surveys across orders have detected *Wolbachia* in 17–30% of tested species, although one survey reported a prevalence of 76% (see Table 1 in Stevens et al. 2001).

The incidence of *Wolbachia* in species of interest to biocontrol research is likely to be higher than occurs among arthropod taxa in general for at least two reasons. First, biocontrol programs often rely upon laboratory colonies of arthropods that are small and genetically-closed populations reared under homogeneous conditions. Hence, a low level incidence of *Wolbachia* in starting populations can reach fixation in relatively few generations. Kyei-Poku et al. (2003) tested for *Wolbachia* in populations of the wasp *Urolepis rufipes* from three field sites and in a lab colony started several generations earlier with material from these same sites. Whereas the incidence of infection was 23% ($n = 30$ males, 30 females) combined across the field populations, infection was 100% ($n = 40$ males, 40 females) in the lab colony. Similarly, Reynolds and Hoffmann (2002) showed the incidence of *Wolbachia* infection to increase from 50 to 100% for populations of *Drosophila melanogaster* caged in the field.

Second, *Wolbachia* is linked with parthenogenesis, and parthenogenic species are favored as biocontrol agents. Parthenogenic species of wasps can have higher intrinsic

Table I. Arthropod species tested for *Wolbachia* in the current study. Species that tested positive for *Wolbachia* are identified in bold font.

Species	Common name[1], Relevance (Result[2])	Species	Common name[1], Relevance (Result[2])
ORDER ACARI		ORDER HYMENOPTERA (cont.)	
Family Phytoseiidae		Family Encyrtidae	
Amblyseius cucumeris	NCN, biocontrol agent for thrips (0 of 1)	***Tachinophaegus zealandicus***	NCN, potential biocontrol agent of pest flies (1 of 2)
Phytoseiulus persimilis	NCN, biocontrol agent of spider mite (0 of 1)	Family Eucoilidae	
Family Tetranychidae		***Kleidotoma* sp.**	NCN, parasitoid of flies (1 of 1)
Tetranychus urticae	Twospotted spider mite, pest of greenhouse crops (0 of 1)	Family Ichneumonidae	
		Phygadeuon prob. fumator	NCN, parasitoid of pest flies (0 of 4)
ORDER COLEOPTERA		Family Megachilidae	
Family Chrysomelidae		*Megachile rotundata*	Alfalfa leafcutting bee, commercialized pollinator of legume crops (0 of 1)
Aphthona lacertosa	NCN, imported biocontrol agent for leafy spurge (weed) (0 of 1)	Family Pteromalidae	
Aphthona nigriscutis	NCN, imported biocontrol agent for leafy spurge (weed) (1 of 1)	*Muscidifurax raptor*	NCN, commercialized biocontrol agent for pest flies (0 of 5)
Family Coccinellidae		*Muscidifurax raptorellus*	NCN, commercialized biocontrol agent for pest flies (0 of 2)
Cryptolaemus montrouzieri	Mealybug destroyer, biocontrol agent for mealybugs (0 of 1)	***Muscidifurax uniraptor***	NCN, commercialized biocontrol agent for pest flies (1 of 1)
Galerucella calmariensis	NCN, imported biocontrol agent for purple loosestrife (weed) (0 of 1)	*Muscidifurax zaraptor*	NCN, commercialized biocontrol agent for pest flies (0 of 7)
Family Curculionidae		***Nasonia vitripennis***	NCN, commercialized biocontrol agent for pest flies (4 of 4)
Ceutorhynchus assimilis	Cabbage seedpod weevil, pest of canola (7 of 7)	***Pachycrepoideus vindemiae***	NCN, parasitoid of pest flies (1 of 1)
Mecinus janthinus	NCN, imported biocontrol agent for toadflax spp. (weed) (0 of 2)	*Pteromalus venustus*	NCN, economically important parasitoid of Alfalfa leafcutting bee (0 of 1)
Mogulones cruciger	NCN, imported biocontrol agent for houndstongue (weed) (0 of 1)	***Spalangia cameroni***	NCN, commercialized biocontrol agent for pest flies (14 of 15)
Sitona lineatus	Pea leaf weevil, pest of legume crops (0 of 1)	***Spalangia endius***	NCN, commercialized biocontrol agent for pest flies (2 of 4)

Table I (*Continued*)

Species	Common name[1], Relevance (Result[2])	Species	Common name[1], Relevance (Result[2])
Family Scolytidae		***Spalangia gemina***	NCN, potential biocontrol agent of pest flies (1 of 1)
Dendroctonus ponderosae	Mountain pine beetle, forest pest (0 of 1)	***Spalangia nigra***	NCN, parasitoid of pest flies (1 of 2)
Family Tenebrionidae		***Spalangia nigroaenea***	NCN, commercialized biocontrol agent for pest flies (3 of 3)
Tenebrio molitor	Yellow mealworm, pest of stored grain products (0 of 1)	***Trichomalopsis sarcophagae***	NCN, commercialized biocontrol agent for pest flies (2 of 2)
Tribolium castaneum	Red flour beetle, pest of stored grain products (0 of 1)	*Trichomalopsis viridescens*	NCN, parasitoid of pest flies (0 of 1)
		Urolepsis rufipes	NCN, potential biocontrol agent of pest flies (2 of 4)
ORDER DICTYOPTERA		Family Trichogrammatidae	
Family Blaberidae		***Trichogramma brassicae***	NCN, commercialized biocontrol agent of pest Lepidoptera (1 of 1)
Blaberus giganteus	*Giant cave roach*, non-pest (0 of 1)	***Trichogramma cacoeciae***	NCN, commercialized? biocontrol agent of pest Lepidoptera (1 of 1)
Gromphadorhina portentosa	*Madagascar giant hissing roach*, non-pest (0 of 1)	***Trichogramma chilonis***	NCN, commercialized biocontrol agent of pest Lepidoptera (1 of 1)
		Trichogramma leptoparameron	NCN, parasitoid of pest Lepidoptera (1 of 1)
ORDER DIPTERA		***Trichogramma minutum***	Minute egg parasite, commercialized biocontrol agent of pest Lepidoptera (1 of 1)
Family Calliphoridae		***Trichogramma pintoi***	NCN, parasitoid of pest Lepidoptera (1 of 1)
Pollenia rudis	Cluster fly, nuisance pest (1 of 1)	***Trichogramma platneri***	NCN, commercialized biocontrol agent of pest Lepidoptera (1 of 1)
Protocalliphora sialia	*Birdnest blowfly*, non-pest (1 of 1)	***Trichogramma sibericum***	NCN, parasitoid of pest Lepidoptera (1 of 1)
Protophormia terraenovae	NCN, non-pest (0 of 1)		
Family Muscidae		ORDER LEPIDOPTERA	
Haematobia irritans	Horn fly, pest of livestock (3 of 3)	Family Lasiocampidae	
Hydrotaea aenescens	*Black garbage fly*, biocontrol agent for pest flies (0 of 1)	*Malacosoma disstria*	Forest tent caterpillar, forest pest (0 of 1)
Musca autumnalis	Face fly, pest of cattle (0 of 2)	Family Lymantriidae	

Table I (*Continued*)

Species	Common name[1], Relevance (Result[2])	Species	Common name[1], Relevance (Result[2])
Musca domestica	House fly, nuisance pest (0 of 7)	*Lymantria dispar*	Gypsy moth, forest pest (0 of 1)
Stomoxys calcitrans	Stable fly, pest of livestock (0 of 7)	*Orgyia antiqua*	Rusty tussock moth, forest pest (0 of 1)
Family Oestridae		*Orgyia leucostigma*	Whitemarked tussock moth, forest pest (0 of 1)
Hypoderma lineatum	Common cattle grub, pest of cattle (0 of 1)	*Orgyia pseudotsugata*	Douglas-fir tussock moth, forest pest (0 of 1)
Family Sarcophagidae		Family Noctuidae	
Ravinia querula	NCN, non-pest (1 of 1)	*Lambdina fiscellaria fiscellaria*	Hemlock looper, forest pest (0 of 1)
Sarcophaga bullata	NCN, non-pest (0 of 1)	*Trichoplusia ni*	Cabbage looper, pest of cole crops (0 of 1)
Family Scathophagidae		Family Pyralidae	
Scatophaga stercoraria	*Yellow dung fly*, potential biocontrol agent of pest flies (0 of 2)	*Dioryctria abietivorella*	*Fir coneworm*, forest pest (0 of 1)
Scatophaga furcata	NCN, non-pest (0 of 1)	Family Saturniidae	
Family Sepsidae		*Bombyx mori*	Silkworm, commercialized for silk production (0 of 1)
Sepsis sp.	NCN, non-pest (0 of 1)	Family Tortricidae	
		Choristoneura conflictana	Large aspen tortrix, forest pest (0 of 1)
ORDER HEMIPTERA		*Choristoneura fumiferana*	Spruce budworm, forest pest (0 of 1)
Family Cimicidae		*Choristoneura occidentalis*	Western spruce budworm, forest pest (0 of 1)
Cimex lectularius	Bedbug, pest of humans (1 of 1)	*Choristoneura pinus*	Jack pine budworm, forest pest (0 of 1)
Family Miridae		*Choristoneura rosaceana*	Obliquebanded leafroller, forest pest (0 of 1)
Lygus borealis	NCN, pest of legume crops (0 of 1)		
Lygus elisus	Pale legume bug, pest of legume crops (0 of 1)	ORDER ORTHOPTERA	
Lygus keltoni	NCN, pest of legume crops (0 of 1)	Family Gryllidae	
Lygus lineolaris	Tarnished plant bug, pest of legume crops (0 of 1)	***Gryllus bimaculatus***	*Two-spotted cricket*, non-pest (1 of 1)
Family Pemphigidae		***Gryllus pennsylvanicus***	*Fall field cricket*, non-pest (1 of 1)
Pemphigus betae	*Sugarbeet root aphid*, pest of sugar beet (0 of 1)	***Gryllus rubens***	*Southeastern field cricket*, non-pest (1 of 1)
Pemphigus populicaulis	*Poplar leaf-petiole gall aphid*, non-pest (0 of 1)	***Teleogryllus oceanicus***	Oceanic field cricket, non-pest (1 of 1)
Pemphigus populiglobuli	*Poplar bullet gall aphid*, non-pest (0 of 1)		
Pemphigus populiramorulum	Poplar twig gall aphid, non-pest (0 of 1)	ORDER PHTHIRAPTERA	
		Family Haematopinidae	

Table I (*Continued*)

Species	Common name[1], Relevance (Result[2])	Species	Common name[1], Relevance (Result[2])
ORDER HYMENOPTERA		***Haematopinus suis***	Hog louse, pest of swine (1 of 1)
Family Braconidae		Family Linognathidae	
Aphaereta pallipes	NCN, parasitoid agent of pest flies (1 of 1)	***Linognathus africanus***	*Goat louse*, pest of goats (1 of 1)
***Glyptapanteles* sp.**	NCN, parasitoid of pest Lepidoptera (1 of 1)	***Linognathus setosus***	Dog sucking louse, pest of dogs (2 of 2)
Microplitis mediator	NCN, imported biocontrol agent for bertha armyworm, *Mamestra configurata* (Noctuidae) (0 of 1)	***Linognathus vituli***	Longnosed cattle louse, pest of cattle (1 of 1)
Macrocentrus linearis	NCN, imported biocontrol agent for pest Lepidoptera (0 of 1)	Family Pediculidae	
Peristenus digoneutus	NCN, imported biocontrol agent for pest *Lygus* spp. (1 of 1)	***Pediculus humanus capitis***	Head louse, pest of humans (3 of 3)
Peristenus stygicus	NCN, imported biocontrol agent for pest *Lygus* spp. (1 of 1)	***Pediculus humanus humanus***	Body louse, pest of humans (2 of 2)
Family Cephidae		***Pthirus pubis***	Crab louse, pest of humans (1 of 1)
Cephus cinctus	Wheat stem sawfly, pest of wheat (0 of 1)	Family Polyplacidae	
Family Chalcididae		***Polyplax serrata***	*Mouse louse*, pest of rodents (1 of 1)
Brachymeria podagrica	NCN, parasitoid of pest flies (1 of 2)	Family Trichodectidae	
Dirhinus himalayanus	NCN, parasitoid of pest flies (1 of 1)	***Bovicola bovis***	Cattle biting louse, pest of cattle (1 of 1)
Family Diapriidae			
Trichopria nigra	NCN, potential biocontrol agent for pest flies (2 of 2)	ORDER SIPHONAPTERA	
Family Diprionidae		Family Pulicidae	
Gilpinia hercyniae	European spruce sawfly, pest of spruce (0 of 1)	***Ctenocephalides felis***	Cat flea, pest of cats (2 of 2)

[1] 'NCN', no common name; italics indicate unofficial common name; non-italicized common names as listed in '*Common Names of Insects and Related Organisms*', Entomological Society of America.

[2] Number of populations testing positive for *Wolbachia* out of the total number of populations tested.

rates of increase and, being all female, every individual in the population has the ability to kill the target host species via oviposition. In contrast, non-parthenogenic populations produce males that consume resources, but which do not kill the target species. For example, parthenogenic species of trichogrammatid wasps are common biocontrol agents. *Wolbachia* was detected in each of the eight species tested in our survey (Table I). Almeida (2004) lists 16 species of *Trichogramma* in which *Wolbachia* causes parthenogenesis.

Our findings also suggest that certain taxa may be particularly prone to, and (or) retaining, infections. We detected *Wolbachia* in nine of nine species of lice (Anoplura, Mallophaga) (Table I). These species were a subset from a total of 19 lice species tested in a larger study and which all were infected with one or more strains of *Wolbachia* (Kyei-Poku et al. 2005). Unlike the findings for *Trichogramma*, this high incidence of infection cannot be attributed to a sampling bias for parthenogenic biocontrol agents. Similarly, we detected infections in 10 of 15 species of pteromalid wasps of which only one is parthenogenic; i.e., *M. uniraptor* (Table I).

We note also that our results underestimate the incidence of infected species. For example, we did not detect *Wolbachia* in the mite *Tetranychus urticae* (Tetranychidae) for which infections previously have been reported (see Hoy and Jeyaprakash 2005 and refs. therein). This discrepancy may be due to one of several causes. Not all populations of the same species may be infected, nor all individuals in the same population. Hence, a negative result in the tested material does not preclude infections in other individuals of the same species. In addition, *Wolbachia* may go undetected even when present; i.e., 'false' negatives. The DNA obtained from whole arthropods may be of insufficient quantity or quality for testing. This may arise from the extraction method, the age or preservation method of the specimen, and (or) factors inhibiting DNA amplification during PCR. Further, detection may be confounded when *Wolbachia* are present at very low densities or, conversely, when concentrations of *Wolbachia* DNA are too high in the PCR amplification mixture (e.g., see Werren et al. 1995). Infections also may be undetectable with some *Wolbachia*-specific primers due to sequence variation in primer binding areas of the target DNA. The primer pairs *ftsZ*A and *ftsZB* are specific for *Wolbachia*, but only for isolates in Supergroups A and B, respectively (Werren et al. 1995).

Application of *Wolbachia* to biocontrol

Methods of manipulation

Ease of manipulation will affect the use of *Wolbachia* in biocontrol research. Manipulations include elimination, transfer, or genetic modification. Infections can be eliminated by adding antibiotics (e.g., rifampicin, tetracycline) to the diet of larval or adult feeding stages of the arthropod host (e.g., see Kyei-Poku et al. 2003 and refs. therein). In a modified approach, *Wolbachia* were eliminated in a parasitoid wasp by applying rifampicin in the diet of the wasp's *Drosophila* host (Mouton et al. 2003). Elimination also can be accomplished by rearing hosts at elevated temperatures (see Kyei-Poku et al. 2003 and refs. therein) or by rearing hosts under crowded conditions (Sinkins et al. 1995). However, treatments to eliminate *Wolbachia* also may eliminate non-*Wolbachia* bacteria. The loss of these latter organisms may or may not be of concern, depending upon their role in the host arthropod; e.g., nutritional symbiont versus pathogen.

Transfer of strains into novel hosts ('transfection') has been experimentally achieved by microinjection with mixed results. A CI-inducing strain from *Drosophila simulans* and transferred into several genetic lines of *D. melanogaster*, produced lower levels of CI in the novel host and eventually was lost from some lines (Boyle et al. 1993). A parthenogenesis-inducing (PI) strain from the wasp *Trichogramma pretiosum* was transferred into *T. dendrolimi* where the transfection was retained for at least 26 generations, but only a slight parthenogenic effect was observed (Grenier et al. 1998). A transfection of PI *Wolbachia* from *Muscidifurax uniraptor* into *D. simulans* had no detectable effect and was lost in the novel host after six generations (Van Meer & Stouthamer 1999). Riegler et al. (2004) established transfections of two *Wolbachia* strains from the fruit fly *Rhagoletis cerasi* (Tephritidae) in *D. simulans*. One strain was eliminated in the novel host by the second generation. The second strain was retained for at least 20 generations, but was transmitted between generations at low levels and seemed to reduce female fecundity. Xi et al. (2005) established in the mosquito *Aedes aegypti*, an infection of *Wolbachia* obtained from *Aedes albopictus*. The infection was stable, caused a high level of CI, and reached fixation in a laboratory colony of originally uninfected hosts to which infected mosquitoes had been introduced. Inconsistent results among these and other studies may reflect variation of *Wolbachia* densities in the novel host, infection transmission across generations, and (or) the role of the recipient host genetic background on the establishment of the transferred *Wolbachia* in its new environment. Xi and Dobson (2005) provide a useful summary of methods used in transfection.

Most difficult, and yet to be achieved, is the genetic modification of *Wolbachia* strains.

Effects on non-target species

Interspecific horizontal transfer (HT) of *Wolbachia* does occur and potentially could affect non-target species, but the barriers to HT are formidable. *Wolbachia* are restricted to vacuoles within the cells of their host. Infections normally are transferred vertically from infected mother to offspring via egg cytoplasm. HT can occur only when *Wolbachia* in cells of the infected host come into contact with, are incorporated by, and survive in, cells of the novel host. Furthermore, the infection only can persist in the novel host population if the newly-infected individual is female, if *Wolbachia* colonize germ cells, and if densities of the bacteria attain sufficient densities in eggs to permit vertical transmission. Experimentally, HT has been achieved with variable success by mechanically injecting *Wolbachia* into novel host species (see examples discussed above). We know of only one report providing direct evidence for the natural occurrence of HT. Huigens et al. (2004) studied HT from infected to uninfected *Trichogramma* larvae developing in the same lepidopteran egg. HT only was observed between two of the four *Trichogramma* species examined, and newly acquired infections were not maintained beyond two generations.

Phylogenetic studies provide indirect evidence that HT has occurred frequently over evolutionary time. Genetically similar strains of *Wolbachia* appear to occur in very different host taxa, and genetically-distinct strains co-occur in the same host (e.g., Dedeine et al. 2005; Haine & Cook 2005 and refs. therein). Such discrepancies suggest that strains have been vectored between host species that may, for example, share a common parasitoid. However, this interpretation may be confounded by

recombination events between genes within *Wolbachia* strains (Jiggins et al. 2001; Werren & Bartos 2001) or the transfer of genetic material between strains (Bordenstein & Wernegreen 2004 and refs. therein). Further study is needed for clarification.

Some examples

The following three subsections illustrate the potential use of *Wolbachia* in biocontrol research. The first two subsections relate to *Wolbachia* in classical and inundative biocontrol, respectively. The third subsection relates to *Wolbachia* in the biocontrol of arthropod-vectored diseases. The potential use of *Wolbachia* and other sex-distorting bacteria in biocontrol research is reviewed in more detail in Stouthamer (2004).

Wolbachia *in classical biocontrol. Aphthona nigriscutis* (Chrysomelidae) is one of five species of *Aphthona* beetles introduced from Europe into North America to control the weed, leafy spurge (*Euphorbia esula* – Euphorbiaceae) (Gassmann et al. 1996). Recent studies indicate that infections of *Wolbachia* in *A. nigriscutis* may be affecting sex ratios, genetic population structure and, possibly the success of the biocontrol program.

First released into North America in 1988, subsequent variation in establishment success among populations of *A. nigriscutis* stimulated research to identify causative factors. Surveys revealed sex ratios of 80–100% female in all populations examined ($n = 121$ populations) and infections of *Wolbachia* in 85% of 68 populations tested (Kazmer 2001). In contrast, sex ratios of 40–60% female were detected in surveys of the congeneric species *A. flava*, and *A. lacertosa* ($n = 16$ populations combined) for which no infections of *Wolbachia* were detected ($n = 29$ populations) (Kazmer 2001). This circumstantial evidence suggests that *Wolbachia* infections may be skewing sex ratios in populations of *A. nigriscutis*. If the relative rarity of males lowers female mating success, then *Wolbachia* infection indirectly may be reducing population growth in *A. nigriscutis*.

More recent work implicates *Wolbachia* as a factor affecting the genetic structure of *A. nigriscutis* (Roehrdanz et al. 2006). North American populations of this species have a majority of individuals with little or no mtDNA diversity and infections of *Wolbachia*. A minority of specimens have high mtDNA diversity and test negative for *Wolbachia*. Mitochondria and *Wolbachia* both are maternally inherited in egg cytoplasm. Because *Wolbachia* may favour reproduction by infected females, individuals with high mtDNA diversity eventually may be excluded. If both mitochondrial types are similarly effective as biocontrol agents, then the effect of *Wolbachia* in eliminating this latter mtDNA category may have no consequence. Until this comparison is performed, however, the effect of this reduced mtDNA diversity remains unknown.

The example of *A. nigriscutis* illustrates the potential value of testing for *Wolbachia*, classical biocontrol agents prior to their release into new geographic areas. Studies to assess the beetle's potential as a biocontrol agent, prior to release into NA, may have been performed on *Wolbachia*-infected individuals, uninfected individuals, or a mixture of both. Screening for *Wolbachia* during this pre-release phase would have identified the presence of infection and provided the opportunity to test the effect of

Wolbachia on agent fitness. Depending on the results of such tests, researchers could then have released only infected or only uninfected beetles.

Wolbachia *in inundative biocontrol.* House fly, *Musca domestica*, and stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) are cosmopolitan pests that are parasitized in North America by more than two dozen species of wasps in Family Pteromalidae (Floate & Gibson 2004). Several species of these wasps have been commercialized, or are being studied for commercialization, as biocontrol agents of the pest flies. The wasps have lower fecundities and longer developmental times than the flies, and can suffer high levels of winter mortality (Floate & Skovgård 2004). Hence, inundative use of the wasp is recommended each year with mass-releases every 2–4 weeks during the fly season.

Infections of *Wolbachia* are common in this guild of parasitoids and can profoundly affect host reproduction (Table I; and see Kyei-Poku et al. in press). In *Muscidifurax uniraptor*, infections induce parthenogenesis such that male individuals are rare (Zchori-Fein et al. 2000). In *Nasonia vitripennis*, infections cause male-development (MD) type CI; i.e., eggs of uninfected females fertilized by infected male partners, remain haploid and develop into males (Breeuwer & Werren 1990). In *Spalangia cameroni*, crosses between infected partners may produce fewer offspring than crosses between uninfected partners, with the fewest offspring being produced from incompatible crosses (Kyei-Poku et al. in press). Infections in *Urolepis rufipes* induce aspects of both female-mortality (FM) and MD-type CI (Kyei-Poku et al. 2003). Incompatible crosses produce fewer offspring of which all are male (FM-type CI), but the total number of males is greater than that produced in compatible crosses (MD-type CI).

The presence or absence of *Wolbachia* can influence the outcome of both classical and inundative biocontrol programs, but with a key difference. In classical biocontrol, potential action is possible only for the one or few generations that the agent is held prior to its release into the new environment. In inundative biocontrol, the opportunity to study and manipulate infections is ongoing. Hence, tests to establish the status of *Wolbachia* in colonies of arthropods used in inundative biocontrol seem particularly useful. Consider the results of our survey as they pertain to the wasp *Spalangia cameroni*. *Wolbachia* was detected in all tested field populations from diverse geographic regions including Canada, Denmark, France, Israel, Kazakhstan, Peru, Russia, and the United States (Kyei-Poku et al. in press). The only uninfected population was maintained, unknowingly, by a commercial insectary. Now knowing its infection status, the insectary can choose to retain the uninfected colony, introduce infections into the existing colony by adding field-collected material, or to start the colony anew using infected, field-collected material. Because the available data suggest that infections in *S. cameroni* can reduce fecundity (Kyei-Poku et al. in press), eliminating infections may be the preferred course of action.

The prescreening of agents for *Wolbachia* has been advocated by other authors who have examined the effect of infections in biocontrol programs. *Wolbachia* induces parthenogenesis in species of *Trichogramma* wasps, which commonly are used as biocontrol agents of lepidopteran pests. However, infected individuals have reduced fecundity and dispersal in laboratory studies (Silva et al. 2000). Nevertheless, because of the greater production of female progeny by parthenogenesis, infected females are predicted to be more efficacious in pest control (Silva et al. 2000). *Cotesia sesamiae* is a

braconid wasp widely distributed in Africa where it attacks several species of pestiferous stem-boring lepidopterans. Some populations of the wasp are infected with *Wolbachia* that causes CI. Other populations are uninfected. Releases of *C. sesamiae* are being considered to augment local populations, but mixing of infected and uninfected populations may retard population growth rates due to CI effects (Mochiah et al. 2002). Prescreening to identify the infection status of individuals can avoid such unintended consequences.

Wolbachia *in the control of arthropod-vectored diseases. Wolbachia* has been identified as having potential application in the biocontrol of arthropod-vectored diseases via paratransgenesis, transgenesis, or via the transfection of *Wolbachia* strains to reduce host longevity.

With application to disease control, paratransgenesis involves the transfer of novel genes ('transgenes') into the genome of a benign micro-organism residing within the arthropod vector of a plant or animal disease-causing pathogen. Products of these transgenes are deleterious to the pathogen, which reduces the arthropod's vector competence. This approach has been demonstrated for a non-*Wolbachia* symbiont of a reduviid bug (reviewed by Beard et al. 2002). *Rhodnius prolixus* (Heteroptera: Reduviidae) is a vector of the protozoan, *Trypanosoma cruzi*. *T.* cruzi is the causative agent for Chagas disease, which affects humans in Central and South America. *Rhodococcus rhodnii* is an extracellular actinomycete symbiont living in the hindgut of *R. prolixus* in close proximity to *T. cruzi*. Genes coding for molecules with antitrypanosomal activity have been inserted into *R. rhodnii*. In turn, the genetically-modified (GM) symbiont has been reintroduced into its arthropod host. In laboratory studies, hosts containing the GM-symbiont exhibited marked declines or elimination of *T. cruzi*. The GM-symbiont does not affect host fitness, which facilitates the symbiont's spread in the host population. In similar fashion, paratransgenesis of *Wolbachia* is being considered for use in mosquitoes to reduce the spread of malaria (e.g., see review by Sinkins 2004), and in tsetse fly to reduce the spread of sleeping sickness (e.g., Aksoy & Rio 2005).

Nuclear rescue construct (NRC) has been suggested as a transgenic approach to avoid some of the difficulties associated with the use of *Wolbachia* in a paratransgenic approach (Sinkins & Godfray 2004). To date, there have been no successful attempts to genetically modify *Wolbachia*, the isolation of *Wolbachia* within vacuoles inside host cells complicates movement of transgene products to sites of action against target pathogens, and modified *Wolbachia* would need to have little or no fitness costs to the arthropod host. Rather than modify *Wolbachia* to spread an 'anti-pathogen' gene in an arthropod population, the NRC links the anti-pathogen gene to a 'rescue' gene. The construct is then inserted into the host's nuclear genome. For a *Wolbachia*-infected population of arthropods, the insertion would allow females to produce offspring with the NRC, regardless of the *Wolbachia*-infection status of their male partner. The net effect would be to increase in the arthropod population, the proportion of individuals whose genomes carry the anti-pathogen gene (Sinkins & Godfray 2004).

A third approach involves the transfection of life-shortening *Wolbachia* strains into arthropod vector populations (Sinkins & O'Neill 2000; Rasgon et al. 2003). Dengue virus is vectored by mosquitoes. Virus titer increases with mosquito age. Hence, older mosquitoes are most likely to transmit dengue to humans. The *popcorn* strain of *Wolbachia* reduces the longevity of its *Drosophila* hosts, but still allows them to survive

long enough to lay eggs and perpetuate infections in the population (Min & Benzer 1997). Introducing this *Wolbachia* isolate into a mosquito population could theoretically reduce the average age and titre levels of individuals and, therefore, reduce the risk of dengue transmission.

Summary

Wolbachia infect diverse arthropod taxa. Their hosts include pest species of economic importance and beneficial species being studied to control these pests. Our haphazard survey suggests that at least 46% of these pest and beneficial species are infected with one or more strains of *Wolbachia*. Hence, many biocontrol research programs include *Wolbachia* as a component even though their presence may be unsuspected. Testing arthropods for infection readily can be achieved using PCR techniques and *Wolbachia*-specific primers.

The presence of *Wolbachia* in arthropods of biocontrol interest may or may not have implications for program objectives. Infections may alter host reproduction via the feminization of genetic males, the induction of parthenogenesis, male embryonic mortality or cytoplasmic incompatibility. Infections also may influence the host directly; e.g., by affecting fecundity, activity, or longevity. Alternatively, infections may have no discernable effect. The consequences of infections on host fitness will need to be assessed on a case-by-case basis. Assessments can be performed by comparing fitness parameters of *Wolbachia*-infected individuals versus individuals from the same genetic source population, but for which infections have been eliminated using antibiotic or heat treatments.

Incorporation of *Wolbachia* in biocontrol research strategies may be prohibited by technical challenges. For example, infections can be manipulated by elimination, transfection or genetic modification. The former has been achieved in many cases, transfection has been reported less often, and genetic modification has yet to be achieved. However, given advances in recent years, we are optimistic that results of ongoing and future research will expand opportunities to use *Wolbachia* and similar endosymbiotic bacteria in biocontrol programs.

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