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Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease

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Abstract

The bacterial endosymbiont *Wolbachia* manipulates arthropod host biology in numerous ways including sex ratio distortion and differential offspring survival. These bacteria infect a vast array of arthropods, some of which pose serious agricultural and human health threats. *Wolbachia*-mediated phenotypes such as cytoplasmic incompatibility and/or pathogen interference can be utilized for vector and disease control. However, many medically important vectors and important agricultural species are uninfected or are infected with strains of *Wolbachia* that do not elicit phenotypes desirable for disease or pest control. The ability to transfer strains of *Wolbachia* into new hosts (transinfection) can create novel *Wolbachia*-host associations. Transinfection has two primary benefits. First, *Wolbachia*-host interactions can be examined to tease apart the influence of the host and bacteria on phenotypes. Secondly, desirable phenotypes induced by *Wolbachia* in a particular insect can be transferred to another recipient host. This can allow for manipulation of insect populations that transmit pathogens or detrimentally affect agriculture. As such, transinfection is a valuable tool to explore *Wolbachia* biology and control arthropod-borne disease. This review summarizes what is currently known about *Wolbachia* transinfection methods and applications. We also provide a comprehensive list of published successful and unsuccessful *Wolbachia* transinfection attempts.

Keywords

Transinfection; *Wolbachia*; symbionts; horizontal transmission; pathogen interference; insects; arthropods; arthropod-borne disease; reproductive manipulation; microinjection

Introduction

Wolbachia are obligate intracellular bacteria that infect arthropods and nematodes. Within arthropods, *Wolbachia* infect a wide spectrum of insects, and the bacteria's success has been attributed to their ability to manipulate host reproduction to favor its own maternal transmission. It is evident that over evolutionary time, horizontal transfer between species has occurred. Incongruent phylogenies of the host and the bacteria suggest that these horizontal transfers are commonplace (O'Neill et al. 1992; Vavre et al. 1999; Werren et al. 1995). The permissive nature of *Wolbachia* to transfer horizontally between individuals has been exploited in the laboratory to artificially infect new insect species.

Wolbachia strains have been artificially transferred, both interspecifically and intraspecifically, in many arthropod species. Recipient hosts include naturally infected species that have previously had their *Wolbachia* infection cleared, infected species that consequentially became superinfected with a new strain of *Wolbachia*, and species that were naturally uninfected. Artificial transfer of symbionts is not limited to *Wolbachia*, with other examples including *Sodalis* symbionts in tsetse fly (Weiss et al. 2006), diverse symbionts of aphids (Russell and Moran 2005; Tsuchida et al. 2005) and *Spiroplasma* in flies and beetles

(Hutchence et al. 2011; Tinsley and Majerus 2007). However, in terms of numbers of studies and diversity of artificial interactions created, transfer of *Wolbachia* is by far the most developed (see Table 1). This review covers the current knowledge of transfer of *Wolbachia* between hosts, evaluates different transfer techniques, explores the biology of novel associations and discusses prospects for transinfecting new insect species.

What is transinfection?

Transinfection is the mechanical transfer of symbionts into a novel host. As opposed to introgression, whereby a *Wolbachia*-infected line is repeatedly backcrossed with males to homogenize the host background, transinfection allows the creation of diverse novel *Wolbachia*-host associations and is not constricted by species mating barriers. Two procedures have been successfully used to create arthropods transinfected with *Wolbachia*: embryo microinjection and adult microinjection. By far the most widespread technique used to develop transinfected lines is embryo microinjection, whereby *Wolbachia* are injected into the posterior pole of pre-blastoderm embryos using a fine needle and micromanipulator. Embryos are left to develop to adulthood, and the subsequent progeny are screened to determine if germline infection and transmission occurred. As the name suggests, adult microinjection differs in that the recipient is at the adult stage rather than embryo. For this technique to be successful, *Wolbachia* must traverse through tissues and cross membranes to reach the germline where it can then be transmitted to the next generation. In some instances, *Wolbachia* has been injected into the pupal or pre-pupal life-stages (Grenier et al. 1998; Kageyama et al. 2008; Kubota et al. 2005), however similar constraints apply in that ultimately, *Wolbachia* need to reach the germline. Other techniques such as co-rearing of the recipient and donor species (Huigens et al. 2004) and hemolymph transfer (Rigaud and Juchault 1995) have achieved transfer of *Wolbachia* to a new host to some degree but these approaches are only suitable for a limited number of arthropods and are generally not considered viable or efficient options for transinfection in many insect species.

Comparison of embryonic and adult microinjection

For the majority of cases where the successful establishment of stable transinfected lines has occurred, the embryonic microinjection technique was employed, however both this technique and microinjection of other life stages have their advantages and disadvantages. Embryonic microinjection localizes *Wolbachia* directly within the developing embryo, compared to adult microinjection where *Wolbachia* are intrathoracically injected. This direct access to the pole cells means that cells that differentiate into germline or soma are already infected, rather than *Wolbachia* needing to gain entry to the germline when injected into more developed life stages. *Wolbachia* have a natural propensity to infect the germline, and in adult *Drosophila melanogaster*, intrathoracically injected *Wolbachia* are seen to localize in the stem cell niche within the germline (Frydman et al. 2006). As the soma can be infected, embryonic microinjection can often lead to somatic infection in the transinfected line, which can be important for *Wolbachia* phenotypes such as pathogen interference (Bian et al. 2010; Moreira et al. 2009; Walker et al. 2011).

When *Wolbachia* are microinjected into adult insects the bacteria must evade the insect immune response. With naturally occurring associations, *Wolbachia* seem to neither elicit nor suppress the immune response (Bourtzis et al. 2000; Xi et al. 2008), but when analyzing artificial associations, *Wolbachia* have been shown to affect immunity (Bian et al. 2010; Hughes et al. 2011b; Kambris et al. 2009; Moreira et al. 2009). After injection of *Wolbachia* into an arthropod host, there is evidence that the bacteria may manipulate host immunity, perhaps to enhance their own proliferation (Braquart-Varnier et al. 2008; Hughes et al. 2011a). In crustaceans, *Wolbachia* is present within hemocytes and in hematopoietic organs,

and these have been suggested to act as a reservoir to discharge the bacteria into the hemolymph (Chevalier et al. 2011). But whether hemocytes can act as a source of *Wolbachia* in insects is still to be resolved. Microinjected *Anopheles* mosquitoes were also seen to contain *Wolbachia* within circulating hemocytes, however it is uncertain if the bacteria were viable (Hughes et al. 2011a). To overcome diminishing *Wolbachia* levels due to the host immune response, more brute force approaches can be adopted by injecting more bacteria, a luxury that can be more easily afforded in adult microinjection. Alternatively, host immunity can be suppressed in adults using molecular techniques such as RNA interference (RNAi).

Innate differences between adult and embryo immunity may aid *Wolbachia* establishment in embryos. In *Drosophila melanogaster* early embryogenesis, hemocytes are restricted to a region within the head mesoderm and have highly divergent roles in development (Holz et al. 2003; Wood and Jacinto 2007). Another study using *Drosophila* embryos showed that specialist insect pathogens employ methods to avoid phagocytosis, whereas *Escherichia coli* were engulfed (Vlisidou et al. 2009). Given the evidence that *Wolbachia* can avoid or manipulate host immunity, it could be predicted that similar mechanisms are employed by the bacteria to avoid detection in injected embryos. While little is known regarding the interaction of *Wolbachia* with the host immune system in developing embryos, it is feasible that the host may treat microinjected *Wolbachia* in a similar fashion to maternally transmitted bacteria, however further work is required to examine *Wolbachia*-host interaction in embryos to validate this theory.

Another difference between the transinfection techniques is the number of viable G_0 (injected generation) females produced, presumably due to the sensitivity of each life stage to injection. Using adult microinjection, a high number of G_0 can be attained, compared to embryo microinjection where only a small proportion of reproducing adults will arise from injected embryos. These differences can either be viewed in a positive or negative light, and preference for a particular technique may lie where resources allocation is preferred. A larger number of G_0 obtained from adult injection means more time needs to be spent on screening progeny for successful transfers, while the lower number of G_0 from embryonic microinjection means greater effort needs to be allocated in the injection process. For either technique, the progeny of the G_0 need to be screened, as both can lead to somatic infection (which is not necessarily indicative of germline infection). As *Wolbachia* are maternally inherited, infected males are a dead-end for the bacteria. Obviously for adult injections, females will be injected, but prior to embryo microinjection sex is unknown, therefore approximately half of surviving G_0 insects are of no use for establishment of infected lines. Lastly, embryonic microinjection is a highly specialized technique that requires a substantial initial outlay of funds for equipment and trained personnel, whereas adult microinjection is less technical and cheaper to undertake.

Source of donor *Wolbachia*

There are several sources where *Wolbachia* can be obtained for microinjection. When using the embryo microinjection technique, *Wolbachia* is predominantly extracted from the egg cytoplasm of an infected species then transferred to the recipient species. *Wolbachia* can be extracted from either the anterior or posterior regions of the donor egg (Xi and Dobson 2005), while homogenized ovaries or eggs can also be used as a source of *Wolbachia*. Alternatively *Wolbachia* can be extracted from cell culture systems. Cell culture is advantageous in that a high density of homogeneous bacteria can be obtained for microinjection and cell lines can be used to adapt strains of bacteria to the desired host background. The adaptation of the wMelPop strain of *Wolbachia* by serial passage in a RML-12 *Aedes aegypti* cell line was seen as critical for successful transinfection of this

strain into the mosquito, *Ae. aegypti* (McMeniman et al. 2009). After transfer of *Aedes*–adapted wMelPop back into the native *D. melanogaster* host, phenotypic shifts were interpreted as genetic adaptation of *Wolbachia* to the mosquito intracellular environment (McMeniman et al. 2008), however it is unclear if these effects were specifically due to adaptation in *Aedes* cells or changes to the *Wolbachia* phenotype resulting from serial passage in cell lines. In contrast, the wAlbB strain was transferred from *Ae. albopictus* to *An. stephensi* without adaptation in cell lines (Bian et al. 2013). Although more laborious, adaptation of *Wolbachia* to the novel host could also be achieved by subsequent microinjection into the recipient species followed by extraction after *Wolbachia* has replicated within the insect (Frydman 2006). However, this may preferentially select for *Wolbachia* capable of infecting somatic tissue. The most optimal adaptation system would be a cell line derived from the germline of the recipient species or genus.

Phenotype modification upon transinfection

Phenotypic shifts are common upon transfer of *Wolbachia* to a novel host, probably due to the maladaptation of the new association. Theory predicts that novel host-parasite relationships are likely to be maladapted, compared to old associations that are more mutualistic (Levin 1996; Turelli 1994). There are several examples where transinfected insects, representing a novel association, display fitness costs. Transfer of wVul from *Armadillidium vulgare* to *Porcellio dilatatus* males was lethal (Bouchon et al. 1998), while wMelPop from *D. melanogaster* had pathogenic effects in *Ae. albopictus* (Suh et al. 2009). *Anopheles stephensi* mosquitoes infected with wAlbB have severe reductions in fecundity (Bian et al. 2013). Furthermore, fitness costs were incurred in *Drosophila* species transinfected with *Wolbachia* (Clancy and Hoffmann 1997; McGraw et al. 2002). However, in a relatively short time period, shifts from pathogenic to mutualistic associations can occur, demonstrated in both transinfected (McGraw et al. 2002) and natural infections (Weeks et al. 2007).

Selection of transinfected lines

Due to the maladaptation of the novel *Wolbachia*-host associations, the infection frequency in early generations can fluctuate stochastically. This may be due to incomplete cytoplasmic incompatibility (CI; a form of reproductive manipulation induced by *Wolbachia* on the host), inefficient vertical transmission and/or pathogenic association between the *Wolbachia* and host. Therefore, selection plays a critical role in establishing stable transinfected lines. In transinfected *D. melanogaster* lines, an initial selection period saw infection frequencies rise before a period where relaxed selection led to a drop in infection frequency (McMeniman et al. 2008). A second selection period after approximately G₁₅ led to 100% infection in subsequent generations (McMeniman et al. 2008). After transinfection of a life-shortening strain of *Wolbachia* from *D. melanogaster* to *Ae. albopictus*, selection and outcrossing to wild type males was crucial in preventing the loss of infection (Suh et al. 2009). In contrast, Xi and Dobson (2005) suggest that after extensive screening at G₀ and G₁ stages, it was unusual to lose an infection from a *Drosophila* transinfected line. However, this may be the case in intraspecific transfers, particularly into *Drosophila* backgrounds, which are likely to be more amenable to infection. As associations tend to shift towards mutualism over time, selection in the initial phase after transinfection may avoid loss of infection due to instability in infection frequencies and temporary fitness costs due to novel interactions between the host and bacteria.

Applications of transinfected arthropods

Transinfection can be used to examine novel associations and to disentangle the role of the bacteria or the host genotype on the type of reproductive alterations induced. Such examples are prominent within the order Lepidoptera. When the feminizing strain *wSca* from the moth *Ostrinia scapularis* was transinfected into *Ephesia kuehniella*, it induced male killing, demonstrating the influence of host factors determining reproductive phenotype (Fujii et al. 2001). The reciprocal transfer of *wKue*, which induces CI in *E. kuehniella*, also induced CI when transferred into *O. scapularis*, suggesting this phenotype is derived from the bacteria (Sakamoto et al. 2005). Transfer of *wCauA* from *Cadra cautella* induced male killing in *E. kuehniella*, while transfer of *wCauB* induced incomplete CI (Sasaki et al. 2002). Host suppression of *Wolbachia*-mediated reproductive manipulations and hidden *Wolbachia* phenotypes have been observed in other natural associations (Hornett et al. 2006; Hornett et al. 2008).

The *Drosophila* species complex harbor many strains of *Wolbachia*, with variable levels of CI (McGraw and O'Neill 1999; Merçot and Charlat 2004). The variability within these strains lies with the ability of the bacteria to induce and/or rescue CI, which has led to classification of strains based on their modification (mod) and rescue (resc) propensity (Werren and O'Neill 2004). Four possible variants are possible: mod+/resc+, mod+/resc-, mod-/resc+, and mod-/resc-. The strains *wYak*, *wTei* and *wSan* do not cause CI in their natural hosts (*Drosophila yakuba*, *Drosophila teissieri*, and *Drosophila santomea*, respectively), but when these strains were transferred into *D. simulans*, they all induced CI (Zabalou et al. 2008). Conversely, when the *wRi* strain from *D. simulans* was transferred into *D. yakuba*, *D. teissieri*, or *D. santomea*, this strain induced CI in all three species (Zabalou et al. 2004a). Unexpectedly, the natural infections (*wYak*, *wTei* and *wSan*) rescued the modification of *wRi*-transfected flies (Zabalou et al. 2004a). Interestingly, when the *wTei* strain was transfected into *D. simulans*, it was unable to rescue its own CI modification, suggesting that this association changes *wTei* into a "suicide strain" (mod+/resc-) (Zabalou et al. 2008). Taken together, these novel associations demonstrate the unique interactions between *Wolbachia* and the host that influence reproductive phenotypes.

Wolbachia has been suggested as an agent for vector and disease control given its ability to spread into insect populations and inhibit pathogens (for reviews see (Bourtzis 2008; Brownlie and Johnson 2009; Cook and McGraw 2010)). However, many important vector species are uninfected. These uninfected insects can be considered an open niche for *Wolbachia* modification. Transinfected disease vectors that transmit human or agricultural pathogens with a stable *Wolbachia* infection include, *An. stephensi* (Bian et al. 2013), *Ae. aegypti* (McMeniman et al. 2009; Ruang-Areerate and Kittayapong 2006; Walker et al. 2011; Xi et al. 2005b), *Ae. albopictus* (Blagrove et al. 2011; Calvitti et al. 2010; Fu et al. 2010; Suh et al. 2009; Xi et al. 2006), *Ae. polynesiensis* (Andrews et al. 2012) *Culex pipiens* (Walker et al. 2009) *Ceratitis capitata* (Zabalou et al. 2009; Zabalou et al. 2004b), *Nilaparvata lugens* (Kawai et al. 2009), and *Laodelphax striatellus* (Kang et al. 2003). Some of these transinfected insects have been evaluated in semi-field and field conditions and are being implemented as control strategies (Hoffmann et al. 2011; Rasgon 2011; Walker et al. 2011), which is encouraging for the use of *Wolbachia* in an applied setting. Still, despite many transinfection attempts, *Wolbachia* infection remains illusive in some important vector species.

Insects recalcitrant to infection

Some hosts are seemingly impervious to *Wolbachia* infection. The reasons for refractoriness are unknown, but could relate to either bacterial or host factors. It is likely that the number

of hosts recalcitrant to infection is under-estimated as predominantly transinfection attempts are made on either model organisms or insects of economical and medical importance. Furthermore (and unfortunately for the scientific community) unsuccessful transinfection attempts are rarely published. Despite *Tribolium confusum* being naturally infected and amenable to transinfection (Chang and Wade 1994; Chang and Wade 1996), hundreds of attempts to infect *T. castaneum* with *Wolbachia* from *T. confusum* have not yielded an infected line despite using the same methodology (pers com. M.J. Wade). Transinfection attempts by injecting *Wolbachia* into *Bombyx mori* larvae lead to somatic infection in G₀, but no stable infection (Kageyama et al. 2008).

For many years transinfection was attempted on mosquitoes within the *Anopheles* genus given their importance in human health. Due to many failed attempts, and the lack of natural infection in native Anophelines (Kittayapong et al. 2000; Rasgon and Scott 2004; Ricci et al. 2002), there were suggestions that this genus was impervious to *Wolbachia* infection. However infection of *Anopheles* cells (Rasgon et al. 2006) and somatic infection of adults (Hughes et al. 2011a; Hughes et al. 2012b; Jin et al. 2009; Kambris et al. 2010) indicated these mosquitoes are capable of harboring *Wolbachia*, suggesting the inability to create a stable line was due to other barriers. Recently, Bian and colleagues (Bian et al. 2013) developed a stable line using embryonic microinjection in the Indian malaria vector, *Anopheles stephensi* demonstrating *Wolbachia* can stably associate in this genus of mosquitoes. However, a stable infection in the major African malaria vector, *An. gambiae*, which is the main vector of *Plasmodium* parasites in sub-Saharan Africa, still remains elusive. Promisingly, the tissue distribution of *Wolbachia* and pathogen inhibitory effects between stably infected *An. stephensi* (Bian et al. 2013) and somatically infected *An. gambiae* (Hughes et al. 2011a; Hughes et al. 2012b; Kambris et al. 2010) are similar, suggesting stable infection in the African malaria mosquito is feasible.

While speculative, there are several strategies that could be implemented to achieve infection in this important mosquito species. Although more convenient, the use of laboratory mosquito lines may decrease genetic heterogeneity in the host background. Greater genotypic divergence in both the host and the bacteria may provide a wider base to select for compatible associations. Furthermore, pathogenic *Wolbachia* strains such as wMelPop, despite having desirable phenotypes for vector control, may not be best suited to infection. Recently, we developed an *ex vivo* germline assay to determine the infectivity of *Wolbachia* into ovaries (Hughes et al. 2012a). *Wolbachia* strains infected native hosts or close relatives at higher titers compared to more divergent hosts, with the wAlbB strain (from *Aedes albopictus*) more adapted to the *Anopheles* germline than *Wolbachia* from flies. This technique may be used to assess the suitability of bacterial strains in a particular host and to determine if adaptation to a new species is occurring. Specifically adapting a host to infection may be achieved by genetic modification of the insect, yielding the hosts more susceptible to *Wolbachia*. In *Aedes* mosquitoes, host factors induced by *Wolbachia* manipulation include the host microRNA profile which subsequently increased bacterial titre (Hussain et al. 2011). The development of new transgenic techniques that allows manipulation of host gene expression at early life stages provide tools that could be used to enhance embryonic microinjection in species where transinfection attempts have previously been unsuccessful (Peng et al. 2011). Further research that identifies barriers to horizontal transfer of *Wolbachia* between species and novel transinfection techniques would be advantageous to the scientific community and accelerate the creation of new *Wolbachia*-host associations.

Summary

Diverse *Wolbachia*-host combinations can be created through transinfection. This allows a tractable method to study *Wolbachia*-host interactions. Embryonic microinjection is predominantly used to create stable *Wolbachia* infected lines, however the number of examples that use other techniques is increasing. Novel transinfection techniques may be the key to transfer *Wolbachia* into hosts that have so far resisted infection. Further developments in this field may allow for the biological control of arthropod-borne diseases.

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Figure 1.

Comparison of adult and embryonic microinjection for stable line development. When microinjected into adults (A), *Wolbachia* needs to (1) avoid the immune response, (2) compete with the native microbiota, and (3) infect the germline to be vertically transferred to the next generations. To infect the germline (B), *Wolbachia* must traverse through both the muscular epithelium (4) and the peritoneal sheath (5), then the follicular epithelium of the ovary to enter the ovarian follicles. The bacterium may enter the germarium infecting the germline stem cell niche (I) or the somatic stem cell niche (II) or directly infect the ovarian follicle (III). Embryonic microinjection localizes *Wolbachia* directly within the developing embryo before pole cell development (C). The germline develops and becomes infected with *Wolbachia*. This process bypasses the barriers to germline infection that the bacterium encounters during adult microinjection. The possible outcomes of infection are compared for adult and embryonic microinjection (below). For both processes, germline infection is critical for vertical transmission of *Wolbachia*, and both approaches require selection for development of a stable line.

Table 1

Wolbachia transinfection attempts in arthropods.

Order/class	Recipient species	donor <i>Wolbachia</i> λ	Microinjection technique and <i>Wolbachia</i> source	Transfer result	Phenotype in recipient host $\dagger$	Reference
Diptera, Drosophilidae	<i>D. melanogaster</i>	wRi	Embryonic, egg cytoplasm	Stable - G9	CI	(Boyle et al. 1993)
	<i>D. simulans</i>	wRi	Embryonic, egg cytoplasm	Stable - G4	CI	
	<i>D. simulans</i>	wMel	Embryonic, egg cytoplasm	Stable - G4	CI	
<i>D. simulans</i>		wAlbA, wAlbB	Embryonic homogenized eggs	Stable -G6	CI	(Braig et al. 1994)
	<i>D. simulans</i>	wRi	Embryonic, egg cytoplasm	Stable - G5	CI	(Rousset and de Stordeur 1994)
	<i>D. simulans</i>	wHA	Embryonic, egg cytoplasm	Stable - G5	CI	
<i>D. simulans</i> [∞]		wHa	Embryonic, egg cytoplasm	90% at G12	CI	(Sinkins et al. 1995)
<i>D. simulans</i>		wRi	Embryonic, egg cytoplasm	Stable - G11	CI	(Giordano et al. 1995)
<i>D. simulans</i>		wMau	Embryonic, egg cytoplasm	Stable - G11	none	
<i>D. mauritiana</i>		wRi	Embryonic, egg cytoplasm	Stable - G11	CI	
<i>D. serrata</i>		wRi	Embryonic, egg cytoplasm	Stable - G45	CI	(Clancy and Hoffmann 1997)
<i>D. simulans</i>		wMel	Embryonic, egg cytoplasm	Stable - 10 years ϕ	CI	(Poinot et al. 1998)
<i>D. simulans</i> [¶]		wRi, wHa	Embryonic, egg cytoplasm	Stable	CI	(Rousset et al. 1999)
<i>D. simulans</i>		wUni	Embryonic, egg cytoplasm	Lost after G7	T $\rightarrow$ none	(Van Meer and Stouthamer 1999)
<i>D. simulans</i>		wHa	Embryonic, egg cytoplasm	Stable - G3	CI	(Poinot and Mercot 2001)
<i>D. simulans</i>		wNo	Embryonic, egg cytoplasm	Stable - G40	CI	
<i>D. simulans</i>		wHa, wNo	Embryonic, egg cytoplasm	Stable - G40	CI	
<i>D. simulans</i>		wHa	Embryonic, egg cytoplasm	Stable - G40	CI	
<i>D. simulans</i>		wNo	Embryonic, egg cytoplasm	Stable - G40	CI	
<i>D. simulans</i>		wHa, wNo	Embryonic, egg cytoplasm	Stable - G40	CI	
<i>D. simulans</i>		wMelPop	Embryonic, egg cytoplasm	Stable - G20	CI	(McGraw et al. 2001)
<i>D. simulans</i>		wMelPop	Embryonic, egg cytoplasm	Stable - G40	CI	(McGraw et al. 2002)

Order/class	Recipient species	donor <i>Wolbachia</i> λ	Microinjection technique and <i>Wolbachia</i> source	Transfer result	Phenotype in recipient host [†]	Reference
	<i>D. yakuba</i>	wRi	Embryonic, egg cytoplasm	Stable >G200	CI	(Zabalou et al. 2004a)
	<i>D. teissieri</i>	wRi	Embryonic, egg cytoplasm	Stable >G100	CI	
	<i>D. santomea</i>	wRi	Embryonic, egg cytoplasm	Stable >G70	CI	
	<i>D. simulans</i>	wCer1, wCer2 β	Embryonic, egg cytoplasm	Stable - G20	CI	(Riegler et al. 2004)
	<i>D. simulans</i>	wRi	Embryonic, egg cytoplasm/ homogenized eggs	Stable - G19	CI	(Xi and Dobson 2005)
	<i>D. melanogaster</i>	wMel	Adult, homogenized tissue	Stable - G29	NT	(Frydman et al. 2006)
	<i>D. simulans</i>	wHa	Embryonic, egg cytoplasm	Stable - G10	CI NT	(Dean 2006)
	<i>D. simulans</i>	wHa	Embryonic, egg cytoplasm	Stable - G10	NA, NT	
	<i>D. simulans</i>	wHa	Embryonic, egg cytoplasm	Stable - G10	N/A, NT	
	<i>D. simulans</i>	wYak	Embryonic, egg cytoplasm	Stable >G200	None $\rightarrow$ CI	(Zabalou et al. 2008)
	<i>D. simulans</i>	wTei	Embryonic, egg cytoplasm	Stable >G200	None $\rightarrow$ CI	
	<i>D. simulans</i>	wSan	Embryonic, egg cytoplasm	Stable >G200	None $\rightarrow$ CI	
	<i>D. melanogaster</i>	wMelPop	Embryonic, cell culture	Stable - G46	CI	(McMeniman et al. 2008)
Diptera, Tephritidae	<i>C. capitata</i>	wCer1, wCer2 β	Embryonic, egg cytoplasm	Stable - G26	CI	(Zabalou et al. 2004b)
	<i>C. capitata</i>	wCer4	Embryonic, egg cytoplasm	Stable - G26	CI	
	<i>C. capitata</i>	wCer2	Embryonic, egg cytoplasm	Stable - G60	CI	(Zabalou et al. 2009)
	<i>B. oleae</i>	wCer2	Embryonic, egg cytoplasm	Stable - G8	CI	(Apostolaki et al. 2011)
Diptera, Culicidae	<i>Ae. albopictus</i>	wAlbA, wAlbB α	Embryonic, egg cytoplasm	Stable - G8	CI	(Xi et al. 2005a)
	<i>Ae. aegypti</i>	wAlbA, wAlbB α	Embryonic, egg cytoplasm	Stable - G13	CI ^{ψ}	(Xi et al. 2005b)
	<i>Ae. aegypti</i>	wAlbA, wAlbB	Adult, homogenized tissue	40% at G12	Partial CI	(Ruang-Areerate and Kittayapong 2006)
	<i>Ae. albopictus</i>	wRi	Embryonic, egg cytoplasm	90% at G11	CI	(Xi et al. 2006)

Order/class	Recipient species	donor <i>Wolbachia</i> λ	Microinjection technique and <i>Wolbachia</i> source	Transfer result	Phenotype in recipient host $\ddagger$	Reference
	<i>C. pipiens</i>	wPipMol π	Embryonic, egg cytoplasm	Stable - G11	CI	(Walker et al. 2009)
	<i>Ae. aegypti</i>	wMelPop	Embryonic, egg cytoplasm	Stable - G17	CI ψ	(McMeniman et al. 2009)
	<i>Ae. albopictus</i>	wMelPop	Embryonic, egg cytoplasm	Stable - G10	LS $\ddagger\ddagger$	(Suh et al. 2009)
	<i>Ae. albopictus</i>	wPip	Embryonic, egg cytoplasm	Stable - G11	CI	(Calvitti et al. 2010)
	<i>Ae. albopictus</i>	wRi	Embryonic, egg cytoplasm	Stable - G13	CI	(Fu et al. 2010)
	<i>Ae. aegypti</i>	wMel	Embryonic, cell culture	Stable - G8	CI ψ	(Walker et al. 2011)
	<i>Ae. albopictus</i>	wMel	Embryonic, egg cytoplasm	Stable - G8	CI	(Blagrove et al. 2011)
	<i>An. gambiae</i>	wMelPop,	Adult, cell culture	Infection in G0	BMM ψ	(Hughes et al. 2011a)
	<i>An. gambiae</i>	wAlbB	Adult, cell culture	Infection in G0	None ψ	
	<i>Ae. polynesiensis</i>	wAlbB	Embryonic, egg cytoplasm	Stable - G8	CI	(Andrews et al. 2012)
	<i>Ae. albopictus</i>	wAlbA	Embryonic, egg cytoplasm	Stable - G8	CI	(Calvitti et al. 2012)
		wAlbB	Embryonic, egg cytoplasm	Stable - G8	CI	
	<i>An. stephensi</i>	wAlbB	Embryonic, egg cytoplasm	Stable - G34	CI	(Bian et al. 2013)
Lepidoptera	<i>E. kuehniella</i>	wKue	Embryonic, egg cytoplasm	Stable - G3	CI	(Sasaki and Ishikawa 2000)
	<i>E. kuehniella</i>	wSca	Embryonic, egg cytoplasm	Stable - G21	F $\rightarrow$ MK	(Fujii et al. 2001)
	<i>E. kuehniella</i>	wCauA, wCauB	Embryonic, egg cytoplasm	Stable - G15	CI $\rightarrow$ many δ	(Sasaki et al. 2002)
	<i>O. scapularis</i>	wKue	Embryonic, egg cytoplasm	Stable - G7	MK $\rightarrow$ CI	(Sakamoto et al. 2005)
	<i>B. mori</i>	wHecFem	Prepupae and pupae, cell culture	Infection in G0		(Kageyama et al. 2008)
	<i>B. mori</i>	wHecCI	Prepupae and pupae, cell culture	Infection in G0		
Hymenoptera	<i>T. dendrolimi</i>	<i>T. pretiosum</i>	Prepupae and pupae, insect	Stable - G26 ε		(Grenier et al. 1998)

Order/class	Recipient species	donor <i>Wolbachia</i> λ	Microinjection technique and <i>Wolbachia</i> source	Transfer result	Phenotype in recipient host [†]	Reference
Hemiptera	<i>T. kaykai</i>	wKay	Co-rearing, live insects	Infection in G0	PI	(Huigens et al. 2004)
	<i>T. deion</i>	wDei	Co-rearing, live insects	Infection in G0	PI	
	<i>Tr. atopovirilia</i>	<i>T. atopovirilia</i>	Co-rearing, live insects	No infection		
	<i>T. deion</i>	wKay	Co-rearing, live insects	Infection at G0		
	<i>T. kaykai</i>	wDei	Co-rearing, live insects	Lost at G3	PI	
	<i>T. atopovirilia</i>	<i>T. pretiosum</i>	Co-rearing, live insects	No infection		
	<i>T. pretiosum</i>	<i>T. atopovirilia</i>	Co-rearing, live insects	No infection		
Hemiptera	<i>T. kaykai</i>	wKay	Pupae, cell culture	Transmission to G1		(Kubota et al. 2005)
	<i>L. striatellus</i>	wRi χ	Adult, hemolymph	30% at F12	CI	(Kang et al. 2003)
	<i>L. striatellus</i>	wMel χ	Adult, hemolymph	Lost at G1		
	<i>L. striatellus</i>	wHa χ	Adult, hemolymph	Lost at G1		
	<i>L. striatellus</i>	wNo χ	Adult, hemolymph	Lost at G1		
Coleoptera	<i>N. lugens</i>	wStri	Nymphal, cell culture	<10% at G40–60	CI	(Kawai et al. 2009)
	<i>T. confusum</i>	<i>Tribolium</i> sp.	Embryonic, egg cytoplasm	Stable - G2	CI	(Chang and Wade 1994)
	<i>T. confusum</i>	<i>Tribolium</i> sp.	Embryonic, egg cytoplasm	Stable - G2	NA	(Chang and Wade 1996)
Isopoda	<i>A. nasatum</i>	wVul	Adult, homogenized ovary	Infected G1 females	F	(Rigaud and Juchault 1995)
	<i>A. vulgare</i>	wElo	Adult, homogenized ovary	Somatic infection in G0		
	<i>A. vulgare</i>	wVul	hemolymph transfer, hemolymph	Infection in G0	F	
Isopoda	Isopods $\S$	Isopod <i>Wolbachia</i> *	Adult, homogenized tissue	Infected ~ 400 dpi	F	(Bouchon et al. 1998)
	<i>O. asellus</i>	wAse	Adult, homogenized ovary	Stable - G3	FBB	(Rigaud et al. 1999)
	<i>A. vulgare</i>	wCc	Adult, homogenized tissue	Infection in G0	CI	(Moret et al. 2001)
Coleoptera	<i>P. scaber</i>	wVul	Adult, homogenized ovary	No infection (N=19)		(Rigaud 2001)
	<i>P. scaber</i>	wNas	Adult, homogenized ovary t	9% at G1	None	
	<i>O. asellus</i>	wVul	Adult, homogenized ovary	7% at G1	None	

Order/class	Recipient species	donor <i>Wolbachia</i> λ	Microinjection technique and <i>Wolbachia</i> source	Transfer result	Phenotype in recipient host $\ddagger$	Reference
	<i>O. asellus</i>	wNas	Adult, homogenized ovary	No infection (N=12)		
	<i>A. vulgare</i>	wVul	Adult, homogenized ovary	95% at G1	F	
	<i>A. vulgare</i>	wNas	Adult, homogenized ovary	88% at G1	F	
	<i>A. nasatum</i>	wVul	Adult, homogenized ovary	84% at G1	F	
	<i>A. nasatum</i>	wNas	Adult, homogenized ovary	95% at G1	F	
	<i>A. vulgare</i>	wVulC	Adult, homogenized ovaries	Stable - G2	NT	(Braquart-Varnier et al. 2008)

Abbreviations: NT- not tested, CI – cytoplasmic incompatibility, F – feminization, PI – parthenogenesis inducing, MK – male killing, FBB – female bias broods, BMM – blood meal mortality, dpi – days post injection;

λ Two *Wolbachia* strains listed indicates both strains were microinjected, if no *Wolbachia* strain is listed, the arthropod host is named;

$\ddagger$ affect of transinfection of *Wolbachia* on reproduction in recipient host

* Five *Wolbachia* strains from *Armadillidium nasatum*, *Armadillidium vulgare*, *Cylisticus convexus*, *Chaetophiloscia elongata*, *Porcellionides pruinosus* were used;

$\S$ Up to ten isopods recipients: *Armadillidium nasatum*, *Armadillo officinalisa*, *Porcellio scaber*, *Porcellio dilatatus dilatatus*, *Porcellio dilatatus pruinosa*, *Porcellionides pruinosus*, *Cylisticus convexus*;

$\P$ recipient host was infected with wHa and wNo;

∞ recipient host was infected with wRi,

π recipient host was infected with wPipCol;

α only wAlbB infected the host;

β only wCer2 infected the host;

χ recipient host was infected with wStri;

ε details about infection from (Pintureau et al. 2000);

ϕ details about infection from (Zabalou et al. 2008);

δ wCauA - MK, wCauB - CI, wCauA and wCauB – MK.

Arrow indicates a change in phenotype from natural (left of arrow) to novel (right of arrow) host;

$\ddagger\ddagger$ high embryonic mortality in transinfected line masked potential CI effects;

$\P\P$ insect also display pathogen interference.