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Superinfection of *Laodelphax striatellus* with *Wolbachia* from *Drosophila simulans*

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Wolbachia are maternally inherited, intracellular α-proteobacteria that infect a wide range of arthropods. They manipulate the reproduction of hosts to facilitate their spread into host populations, through ways such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing. The influence of *Wolbachia* infection on host populations has attracted considerable interest in their possible role in speciation and as a potential agent of biological control. In this study, we used both microinjection and nested PCR to show that the *Wolbachia* naturally infecting *Drosophila simulans* can be transferred into a naturally *Wolbachia*-infected strain of the small brown planthopper *Laodelphax striatellus*, with up to 30% superinfection frequency in the F_{12} generation. The superinfected males of *L. striatellus* showed unidirectional CI when mated with the original single-infected females, while superinfected females of *L. striatellus* were compatible with superinfected or single-infected males. These results are, to our knowledge, the first to establish a superinfected horizontal transfer route for *Wolbachia* between phylogenetically distant insects. The segregation of *Wolbachia* from superinfected *L. striatellus* was observed during the spreading process, which suggests that *Wolbachia* could adapt to a phylogenetically distant host with increased infection frequency in the new host population; however, it would take a long time to establish a high-frequency superinfection line. This study implies a novel way to generate insect lines capable of driving desired genes into *Wolbachia*-infected populations to start population replacement.
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Introduction

Wolbachia are striking and intriguing maternally inherited, intracellular endosymbionts, which are probably the most widespread bacteria in invertebrates, infecting 20–76% of insect species (Jeyaprakash and Hoy, 2000; Werren and Windsor, 2000), some mites (Johanowicz and Hoy, 1995), numerous terrestrial isopods (Bouchon *et al*, 1998) and filarial worms (Bandi *et al*, 1998). Instead of increasing their hosts' reproduction or survival (Fine, 1975; Yamamura, 1993), *Wolbachia* have evolved toward 'reproductive parasitism', altering hosts' reproduction to facilitate their spread in the host population (O'Neill *et al*, 1997). Examples are the feminization of genetic males in terrestrial isopods (Martin *et al*, 1973; Bouchon *et al*, 1998), parthenogenesis in *Trichogramma* (Stouthamer *et al*, 1990), male killing in *Drosophila* (Hurst *et al*, 2000) and cytoplasmic incompatibility (CI) in numerous host taxa (O'Neill *et al*, 1997).

The diversity of *Wolbachia* strains across hosts and the range of their effects cannot be explained by strict vertical transmission alone. Horizontal infectious transmission between different host species or taxa is required to explain the overall lack of congruence between host and symbiont phylogenies (Rousset *et al*, 1992; Rigaud and Rousset, 1996; Vavre *et al*, 1999).

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Several studies using experimental infection have revealed that *Wolbachia* can infect foreign hosts. However, stable transovarial transmission of the infection in lineages after transfer has been more difficult to achieve. Failures often occurred when *Wolbachia* have been transfected into a host phylogenetically distant from their native host (Rigaud and Juchault, 1995; Van Meer and Stouthamer, 1999; Heath *et al*, 1999; Pintureau *et al*, 2000), whereas successes were generally found between closely related species (Boyle *et al*, 1993; Braig *et al*, 1994; Clancy and Hoffmann, 1997). Only a few attempts at horizontal transfer of *Wolbachia* have led to permanent establishment in a new host species (Breeuwer and Werren, 1990; Clancy and Hoffmann, 1997; Pintureau *et al*, 2000). The common feature of most of these experimental transfers was that either the recipients were *Wolbachia* negative or *Wolbachia* positive, but the recipients and the donors were closely related species.

Planthoppers (Homoptera: Delphacidae) are the major insect pests of rice in the world, and transmit various viral rice diseases and cause a decrease in rice yield. Among planthopper species, the small brown planthopper (*Laodelphax striatellus*) is one of the major vectors of rice stripe virus (RSV). In nature, some *L. striatellus* populations are infected with *Wolbachia*, and some are not (Hoshizaki, 1997; Noda *et al*, 2001). In this study, we reported the results of experiments that aimed to transfer the natural *Wolbachia* from *Drosophila* into *L. striatellus* by microinjection. Our aim was to determine if a stable superinfection could be generated and if it would result

in the expression of CI. These experiments provide insights into the potential use of *Wolbachia* to spread RSV transmission blocking genes into natural populations of this insect disease vector.

Materials and methods

Wolbachia and their hosts

The following strains of *Drosophila* species which harbor *Wolbachia* infections were maintained in the laboratory: *D. simulans* Riverside (DSR), *D. melanogaster* yw67c23 (YW), *D. sechellia* (S9) and *D. simulans* Noumea (R3A). The corresponding *Wolbachia* were named as *w*Ri, *w*Mel, *w*Ha and *w*No, respectively (provided by Prof. Scott O'Neill, Queensland University, Australia). Flies were grown at 25°C on corn flour/sugar/yeast medium (7.9 g agar, 110 g sucrose, 27.5 g yeast, 52 g cornmeal, 2.38 g Nipagin made up to 1 l with water).

The small brown planthoppers (*Laodelphax striatellus*) were collected from Chuxiong (China) and maintained with rice seedlings, at 25°C in an insectary with a light cycle of 12 h light/12 h dark.

Wolbachia preparation and microinjection

The *Drosophila* that were to serve as *Wolbachia* donors were washed with 70% ethanol for 5 min, followed by three sterile water washes, 5 min each time. The legs of about 200 flies were removed with sterile forceps under the microscope. The flies were placed into a 0.2 ml tube with a small hole punched in the bottom and loosely plugged with glass wool. The tube was put inside a 1.5 ml Eppendorf tube and spun at 750 g for 3 min. The hemolymph obtained was kept chilled on ice, and was then microinjected into the abdomen of *L. striatellus* within 24 h post-eclosion.

PCR assay

Four sets of primers were used (Zhou *et al*, 1998):

1. *wsp* 81F/691R universal for all *Wolbachia* strains (81F 5′-TGG TCC AAT AAG TGA TGA AGA AAC; 691R 5′-AAA AAT TAA ACG CTA CTC CA); these primers were used to amplify a DNA fragment about 610 bp.
2. *wsp* 202F/691R specific for *L. striatellus Wolbachia* (*w*Stri) (202F 5′-AAA AGG ATA GTC CCT TAA C; 691R 5′-AAA AAT TAA ACG CTA CTC CA); these primers were used to amplify a DNA fragment of 489 bp.
3. *Wsp* 169F/569R specific for *Wolbachia* from *D. simulans* Riverside (*w*Ri) (169F 5′-ATT GAA TAT AAA AAG GCC ACA GAC A; 569R 5′-CCC CCT TGT CTT TGC TTG CTG CAG), *wsp* 183F/570R specific for *Wolbachia* from *D. simulans* Noumea (*w*No) (183F 5′-AAG GAA CCG AAG TTC ATG; 570R 5′-GAT CTC TTT AGT AGC TGA TAC), *wsp* 308F/YW-R specific for *Wolbachia* from *D. melanogaster* yw67c23 (*w*Mel) (308F 5′-TTA AAG ATG TAA CAT TTG; YW-R 5′-CCG GTT GAA TTT TTA GGA TC), *wsp* 178F/S9-R specific for *Wolbachia* from *D. sechellia* (*w*Ha) (178F 5′-AAA GAA GAC TGC GGA TAC; S9-R 5′-CCC CCT TGT CTT TGC TTG C); these primers were used to amplify a DNA fragment about 400 bp.
4. 12SAI/12SBI universal for insect mtDNA which serve as a positive control for DNA extraction (12SAI 5′-CTA GGA TTA GAT ACC CTA TT; 12SBI 5′-AAG AGC GAC GGG GCG ATG), these primers were used to amplify a DNA fragment of approximately 400 bp.

The whole adult of *Drosophila* or *L. striatellus* minus its head was homogenized in 100 µl STE (100 mmol/l NaCl, 10 mmol/l Tris-HCl pH 8.0, 1 mmol/l EDTA pH 8.0) and incubated in the solution with 0.5 µg/ml proteinase K and 1% SDS at 55°C for 1 h. DNA was isolated by the phenol/chloroform extraction method and was finally resuspended in 30 µl of ddH_2O. The PCR amplification was performed at 94, 55 and 72°C, 1 min each, repeated for 35 cycles in a buffer containing 2.5 mmol/l $MgCl_2$, 0.25 mmol/l dNTP and 500 nmol/l of each primer. The PCR products were analyzed by 1% agarose gel electrophoresis, while the mitochondrial primers 12SAI/12SBI were used as a positive control in a separate reaction.

Nested PCR was used to check for the existence of introduced *Wolbachia*. First, 81F/691R were used in PCR to check for the existence of general *Wolbachia*, then the PCR product was diluted 1000-fold. The specific primers for introduced *Wolbachia* were used for the second round of PCR. A total volume of 1 µl of final PCR product was directly ligated into the vector pGEM-T (Promega) without further purification in a 10 µl-reaction overnight at 16°C. At least three independent clones were sequenced to exclude errors introduced by *Taq* polymerase.

Establishment of a superinfected isofemale line of *L. striatellus*

After microinjection, natural male insects (1–2 individuals) were put into a culture bottle containing an injected female for mating. After about 2 weeks, larvae could be seen and the number of larvae was counted. Adult *L. striatellus* would emerge after 1 month. Then, one superinfected *L. striatellus* female and one or two males of the F_1 generation were placed into one culture bottle for mating. When the larvae of the F_2 generation appeared, the F_1 generation *L. striatellus* were used for PCR assay to verify the existence of introduced *Wolbachia*. The same method was used to select the *L. striatellus* isofemale lines containing *Drosophila*-specific *Wolbachia* for the following generations.

Determination of CI

Once the *L. striatellus* isofemale lines with a high level of introduced *Wolbachia* were established, mating tests were performed to determine the presence of CI. For each species tested, the following four types of crosses were set up: superinfected female × superinfected male, superinfected female × single-infected male, single-infected female × superinfected male and single-infected female × single-infected male. All matings were set up with one female and one male (both virgins). After the offspring appeared, the parents of each cross were used to perform PCR assay for the determination of the presence of introduced *Wolbachia*. CI was assessed by the number of hatched larvae in each cross. In the case of multiple comparisons, one-way ANOVA and Tukey's method were used. All the related statistical analysis was done by SAS JMP software.

Results

Detection of *Wolbachia* in microinjected *L. striatellus* lines

Table 1 shows the viability rate of *L. striatellus*, the proportion that could produce offspring after being microinjected with different *Wolbachia* strains. The data indicates that the average viability of superinfected *L. striatellus* is 15.5–25.8% with 95% confidence interval. Furthermore, by Pearson's test, the probability from χ^2 is 0.8841, which means there was no significant difference among the different strains of *Wolbachia*. However, up to now we have only obtained superinfected isofemale lines of *L. striatellus* using *w*Ri. The other three *Wolbachia* strains superinfected into *L. striatellus* were lost at the F_1 generation.

The *wsp* gene, which is evolving at a much faster rate than any other previously reported *Wolbachia* genes, that is, 16S rRNA and the cell-cycle gene *fts*Z (Zhou *et al*, 1998), was used as a probe to detect the presence of *Wolbachia*. To improve the sensitivity of the test, a nested PCR strategy was developed to check for the existence of introduced *Wolbachia*. Figure 1 shows that the injected F_0 females of *L. striatellus* and some individual superinfected *L. striatellus* of the F_1 generation were *w*Ri positive. The sequencing result of nested PCR product (ligated into Promega T-vector) confirmed that it was identical to the sequence of *wsp* fragment from *w*Ri, and not to the sequence of the *wsp* fragment from *w*Stri that naturally infects *L. striatellus*.

Transmission of supertransfecting *Wolbachia* from *D. simulans* into *L. striatellus*

Superinfected *L. striatellus* isofemale lines were successfully established by a one female–one male selection strategy. Table 2 shows the average frequency dynamics of *w*Ri from 11 isofemale lines of superinfected *L. striatellus*. Figure 2 is the corresponding curve. The superinfection frequency is stable at 25.7–35.0% (with 95% confidence intervals) in the F_{12} generation.

Table 1 Viability rate of *L. striatellus* microinjected with different *Wolbachia* strains

Wolbachia strain	*Number of insects injected*	*Number of injected insects able to produce offspring*
*w*Ri	112	22 (19.6%)
*w*No	46	9 (19.6%)
*w*Mel	54	13 (24.1%)
*w*Ha	39	7 (17.9%)
Total	251	51 [15.5–25.8%] (with 95% confidence interval)

Prob(χ^2)=0.8841(Pearson test).

CI phenotype

Once the *L. striatellus* isofemale lines with a high level of introduced *Wolbachia* were established, mating tests were performed to determine the presence of CI. Table 3 shows the results of test crosses for CI. Since the eggs of

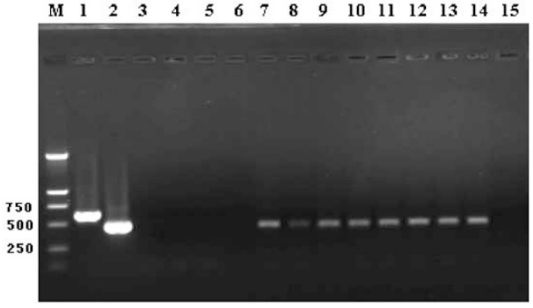

Figure 1 PCR assays of *w*Ri infection in *L. striatellus* of the F_0 and the F_1 generation. The results were obtained by nested PCR, amplified with 81F/691R and 169F/569R. This gel shows results of second round of amplification. M: molecular weight marker (DL2000); 1: DSR amplified by universal primer 81F/691R; 2: positive control, DSR; 3–6: negative control, *L. striatellus* (Chuxiong, China); 7–10: superinfected female of the F_0 generation of *L. striatellus*; 11–14: individuals of the F_1 generation from superinfected *L. striatellus*; 15: blank.

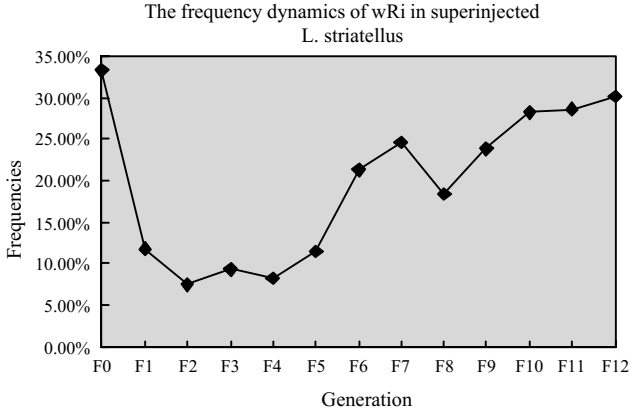

Figure 2 Frequency dynamics of *w*Ri in superinfected *L. striatellus*.

Table 2 Average superinfection frequency of different generations from 11 isofemale lines

	F_0	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
T	18	111	215	194	206	289	375	219	239	327	421	380	394
+	6	13	16	18	17	33	80	54	44	78	119	109	119
%	33.3	11.7	7.4	9.3	8.3	11.4	21.3	24.7	18.4	23.8	28.3	28.7	30.2
95% UCL (%)	59.0	19.2	11.8	14.3	12.9	15.7	25.8	30.9	23.9	28.9	32.8	33.5	35.0
95% LCL (%)	13.5	6.4	4.3	5.6	4.9	8.0	17.3	19.1	13.7	19.3	24.0	24.2	25.7

F_0–F_{12}: generation, T: the total insects detected, +: the superinfected insects, %: the superinfection frequency; UCL: upper confidence limit; LCL: lower confidence limit.

Table 3 CI test

	B ♀++ × ♂++	*C* ♀++ × ♂+	*D* ♀+ × ♂++	*E* ♀+ × ♂+
Average number of offspring	55.6	56.5	3.1	60
(No. of crosses)	(10)	(10)	(10)	(10)
SD	11.0	8.7	5.4	14.0
95% UCL	63.5	61.5	5.8	70.0
95% LCL	47.7	49.4	0	50.0

UCL: upper confidence limit, LCL: lower confidence limit; ++: superinfection; +: single infection.

Table 4 Average numbers of offspring in each generation from superinfected *L.striatellus*

	Average number of offspring	*SD*	*95% confidence value*
F_1(10)	25.2	24.7	15.3
F_2(35)	26.4	18.5	6.1
F_3(60)	15.6	18.2	4.6
F_4(74)	17.5	18.6	4.3
F_5(29)	17.6	18.0	6.5
F_6(55)	22.5	18.1	4.8
F_7(25)	20.6	17.0	6.5
F_8(27)	35.8	24.1	9.1
F_9(30)	30.0	21.2	7.6
F_{10}(65)	35.8	23.6	5.7
F_{11}(33)	50.1	19.8	6.7

The number in brackets means the number of crosses analyzed.

L. striatellus were wrapped in the leaves of seedlings, the growth of superinfected *L. striatellus* would obviously be affected if the eggs were cultured outside the seedlings. So the number of nymphs of each cross was used as the parameter for the CI test instead. Our original hypothesis is: H_o: $\mu_B = \mu_C = \mu_D = \mu_E$; the alternative hypothesis is H_a: not all μ_i are equal (i = B, C, D, E). Prob. based on the statistic = 0.0001 was obtained by one-way ANOVA, which means that there is a significant difference among the four types of the crosses. Further by using Tukey's method, Cross D is significantly different from the other three crosses, while there are no significant differences among Crosses B, C and E.

Results from the statistical analysis showed that the superinfected *L. striatellus* males displayed high percentages of unidirectional CI when crossed with the naturally single-infected females, while the superinfected females were compatible with both superinfected males and single-infected males.

Wolbachia segregation from superinfected *L. striatellus*

According to the results of nested PCR analysis, the products of the first round of PCR from some offsprings of superinfected *L. striatellus* were positive, whereas those of the final round of PCR were positive or negative. Some superinfected isofemale lines even lost their superinfection characteristics during the selection process. It indicated the existence of heterogeneity of offspring of the superinfected *L. striatellus*.

Relationship between the introduced *Wolbachia* and superinfected *L. striatellus*

Considering that the average fecundity of superinfected *L. striatellus* increased with each successive generation (Table 4), up to the F_{11} generation, the average fecundity is approximately normal (results from CI test). This suggests that the local adaptation between introduced *Wolbachia* and the new host has been established, from a low level of 'disruption' to 'compatibility'.

Discussion

Establishment of superinfected *L. striatellus* isofemale lines

This study has shown that *Wolbachia* can be transferred between phylogenetically distant insects, and that the superinfection is inherited to the F_{12} generation. The spread of superinfected *Wolbachia* within a new host population requires stable vertical transmission. However, the establishment of stable vertical transmission within new hosts requires the following conditions.

The first concerns the *Wolbachia* strains. To address the potential effects of *Wolbachia* strains on superinfection, a comparison was made by superinfecting different *Wolbachia* strains into the same host background. As shown by experimental data, only *w*Ri were successfully superinfected into the naturally single-infected *L. striatellus*. This is supported by the fact that *w*Ri are more phylogenetically distant from *w*Stri than are *w*Ha and *w*No (Zhou *et al*, 1998). On the another hand, although *w*Mel are also more phylogenetically distant from *w*Stri than *w*Ri, the CI phenotype of *w*Mel is weaker than that of *w*Ri. Moreover, compared to the infection of different *Wolbachia* strains, *w*Ri infection may be much more recent, because they are associated with a specific mitochondrial DNA subtype (Hale and Hoffmann, 1990; Turelli *et al*, 1992), and it is easier for *w*Ri to adapt to the physiological environment of the novel host. All the above indicates that *w*Ri, phylogenetically distant from the naturally existing *Wolbachia*, with the ability to induce strong CI, will play an important role in the route of superinfection.

The second point concerns the relationship between introduced *Wolbachia* and their new hosts. In order to be established in a new host population after infection, *Wolbachia* symbionts have three requirements: (i) compatibility; (ii) transmission, the ability for the infection to be transmitted in the novel host; and (iii) disruption, the ability to turn the host's reproduction to their own advantage, thereby creating a mechanism for spreading

in the new host population. The results from Table 4 are consistent with the progression from a small amount of 'disruption' to 'compatibility', demonstrating that it takes a long time for introduced *Wolbachia* to establish a higher infection frequency in the *L. striatellus* population. However, knowledge of the detailed mechanisms of interaction between *Wolbachia* and the new host requires further study.

Our third point concerns the vertical transmission of the introduced *Wolbachia*. For vertical transmission, *Wolbachia* must be present in mature and viable eggs, which requires that they undergo replication and segregation synchronous with their host during oogenesis. Our results are compatible with the lack of congruence found between molecular phylogenies of parasitoid guilds and their hosts (Plantard *et al*, 1998; West *et al*, 1998). This raises the possibility that it is the vertical component of transmission, not the horizontal one, that is one of the rate-limiting steps in the spread and maintenance of *Wolbachia* in new host populations. In our study, to address this problem, hemolymph of *Drosophila* was microinjected into the abdomen of *L. striatellus*. The aim of this method is to strengthen the vertical transmission of *Wolbachia*. One reason is that hemolymph offers a similar physiological environment between the recipients and donors. Secondly, it would benefit the vertical transmission of *Wolbachia* since the abdomen contains the reproductive tissues.

Moreover, theoretical arguments suggest that the initial infection frequencies may influence whether or not the infection ultimately becomes evolutionarily stable (Rigaud *et al*, 2001). The existence of *Wolbachia* segregation from superinfected *L. striatellus* indicated that low densities of introduced *Wolbachia* in the host insect would appear to produce a proportion of uninfected cystoblasts as a result of the stochastic loss of bacteria during mitosis, thus jeopardizing successful vertical transmission of the superinfection. A very high level of introduced *Wolbachia* infection in the donor hosts, specific aspects of the introduced *Wolbachia's* development, or a combination of the two factors may have important influences on the initial titer of *Wolbachia*.

In conclusion, successful horizontal transmission of *Wolbachia* between phylogenetically distantly related species would probably require strong selection in a new *Wolbachia* variant. This does not mean that such transfers are impossible, as suggested by phylogenetic analyses (Bouchon *et al*, 1998; Cordaux *et al*, 2001), but these events will be rare. From the point of view of evolution, maybe several million years ago *Wolbachia* had more potential (plasticity) to infect several host species than at present, and they may have lost this following selection and coevolution with particular hosts. There is a possibility, however, that some *Wolbachia* lineages are less specialized and could infect other host species more easily, that is, *w*Ri in our study.

Superinfected *L. striatellus* could take advantage of unidirectional CI to spread in their natural population

The superinfected males of *L. striatellus* showed strong unidirectional CI with the naturally single-infected female of *L. striatellus*. As a result , superinfected females have a reproductive advantage in a mixed population of infected and uninfected individuals (Caspari and Watson, 1959). Since *Wolbachia* are maternally inherited, this process of unidirectional incompatibility will lead to a rapid increase in the proportion of infected hosts in an interbreeding population (Turelli and Hoffmann, 1991). The results from our experiments indicate that a higher density of introduced *Wolbachia* could cause stronger CI, while at the same time CI itself strengthens the spread of introduced *Wolbachia* in the whole population. These two factors interact with each other to spread the introduced *Wolbachia* in the new host.

It should be noted that the induction of CI by *w*Ri in superinfected *L. striatellus* was even stronger than in *D. simulans* (Turelli and Hoffmann, 1995). The reason behind this is still unknown. The effects of host, the naturally existing *Wolbachia*, or a combination of the two factors, might be responsible for it.

Conclusion

Our study provides the possibility of supertransfecting CI-causing *Wolbachia* into *L. striatellus* from a phylogenetically distant host by adult female microinjection. The introduced *Wolbachia* also induce the same CI phenotype as in the former hosts. The superinfected *L. striatellus* show unidirectional CI when mating with the original naturally infected species, which means the superinfected *L. striatellus* have a reproductive advantage in the natural population. Our results provide insights into the potential use of *Wolbachia* to spread RSV transmission blocking genes into natural populations of this insect disease vector.

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References

Bandi C, Anderson TJC, Genshi C, Blaxter ML (1998). Phylogeny of *Wolbachia* in filarial nematodes. *Proc R Soc London B* **265**: 2407–2413.

Bouchon D, Rigaud T, Juchault P (1998). Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. *Proc R Soc London B* **265**: 1081–1090.

Boyle L, O'Neill SL, Robertson HM, Karr TL (1993). Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science* **260**: 1796–1799.

Braig HR, Guzman H, Tesh RB, O' Neill SL (1994). Replacement of the natural Wolbachia symbiont of *Drosophila simulans* with a mosquito counterpart. *Nature* **367**: 453–455.

Breeuwer JA, Werren JH (1990). Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* **346**: 558–560.

Caspari E, Watson GS (1959). On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution* **13**: 568–570.

Clancy DJ, Hoffmann AA (1997). Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *D. serrata*, a novel host. *Am Nat* **149**: 975–988.

Cordaux R, Michel-Salzat A, Bouchon D. (2001). *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission. *J Evol Biol* **14**: 237–243.

Fine PEM (1975). Vectors and vertical transmission: an epidemiologic perspective. *Ann N Y Acad Sci* **266**: 173–194.

Hale LR, Hoffmann AA (1990). Mitochondrial DNA polymorphism and cytoplasmic incompatibility in natural population of *Drosophila simulans*. *Evolution* **44**: 1383–1386.

Heath BD, Butcher RDJ, Whitfield WGF, Hubbard SF (1999). Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a natural occurring mechanism. *Curr Biol* **9**: 313–316.

Hoshizaki S (1997). Allozyme polymorphism and geographic variation in the small brown planthopper, *Laodelphax striatellus* (Homoptera: Delphacidae). *Biochem Genet* **35**: 383–393.

Hurst GD, Johnson AP, Schulenburg JH, Fuyama Y (2000). Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. *Genetics* **156**: 699–709.

Jeyaprakash A, Hoy MA (2000). Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. *Insect Mol Biol* **9**: 393–405.

Johanowicz DL, Hoy MA (1995). Molecular evidence for A-*Wolbachia* endosymbiont in the predatory mite *Metaseiulus accidentalis*. *J Cell Biochem* **21A**: 198.

Martin G, Juchault P, Legrand JJ (1973). Mise en e' videnced'un micro-organisme intracytoplasmique symbiote de l'oniscoide *Armadillidium vulgare* (L.), dont la pre' sence accompagne-l'intersexualite'ou la fe' minisation totale des mâ les ge'ne' tiques de la ligne' e the' lyge' ne. *C R Acad Sci Paris III* **276**: 2313–2316.

Noda H, Koizumi Y, Zhang Q, Deng K (2001). Infection density of *Wolbachia* and incompatibility level in two planthopper species, *Laodelphax striatellus* and *Sogatella furcifera*. *Insect Biochem Mol Biol* **31**: 727–737.

O'Neill SL, Hoffmann AA, Werren JH (1997). *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford University Press, Oxford.

Pintureau B, Grenier S, Bole' at B, Lassablie're F, Heddi A, Khatchadourian C (2000). Dynamics of *Wolbachia* populations in transfected lines of *Trichogramma*. *J Invertebr Pathol* **76**: 20–25.

Plantard O, Rasplus JY, Mondor G, LeClaainche I, Solignac M (1998). Wolbachia-induced thelytoky in the rose gallwasp *Diplolepis spinosissimae*(Giraud) (Hymenoptera: Cynipidae), and its consequences on the genetic structure if its host. *Proc R Soc London B* **265**: 1075–1080.

Rigaud T, Pennings PS, Juchault P (2001). Wolbachia bacteria effects after experimental interspecific transfers in terrestrial isopods. *J Invertebr Pathol* **77**: 251–257.

Rigaud T, Juchault P (1995). Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. *J Evol Biol* **8**: 249–255.

Rigaud T, Rousset F (1996). What generates the diversity of *Wolbachi*a-arthropod interactions? *Biodivers Conserv* **5**: 999–1013.

Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M (1992). *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc R Soc London B* **250**: 91–98.

Stouthamer R, Luck RF, Hamilton WD (1990). Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc Natl Acad Sci USA* **87**: 2424–2427.

Turelli M, Hoffmann AA (1991). Rapid spread of an inherited incompatibility factor in California *Drosophila*. *Nature* **353**: 440–442.

Turelli M, Hoffmann AA (1995). Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural population. *Genetics* **140**: 1319–1338.

Turelli M, Hoffmannn AA, Mckechnie SW (1992). Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* **132**: 713–723.

Van Meer MM, Stouthamer R (1999). Cross-order transfer of *Wolbachia* from *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae) to *Drosophila simulans* (Diptera: Drosophilidae). *Heredity* **82**:163–169.

Vavre F, Fleury F, Lepetit D, Fouillet P, Boule' treau M (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host–parasitoid associations. *Mol Biol Evol* **16**: 1711–1723.

Werren JH, Windsor D (2000). *Wolbachia* infection frequencies in insects: evidence of a global equilibrium. *Proc R Soc London B* **267**: 1277–1285.

West SA, Cook JM, Werren JH, Godfray HCJ (1998). *Wolbachia* in the two insect host-parasitoid communities. *Mol Ecol* **7**: 1457–1465.

Yamamura N (1993). Vertical transmission and evolution of mutualism for parasitism. *Theor Pop Biol* **44**: 95–109.

Zhou WG, Rousset F, O'Neill SL (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. *Proc R Soc London* **265**: 509–515.