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Wolbachia Screening in Spiders and Assessment of Horizontal Transmission between Predator and Prey

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Abstract

Recent studies have revealed that the prevalence of *Wolbachia* in arthropods is attributable not only to its vertical transmission, but also to its horizontal transfer. In order to assess the horizontal transmission of *Wolbachia* between predator and prey, arthropods belonging to 11 spider families and six insect families were collected in the same field of rice. The distribution of *Wolbachia* in these arthropods was detected by diagnostic PCR amplification of the *wsp* (Wolbachia outer surface protein gene) and *16S* rDNA genes. *Nurscia albofasciata* Strand (Araneae: Titanoecidae), *Propylea japonica* Thunberg (Coleoptera: Coccinellidae), *Paederus fuscipes* Curtis (Coleoptera: Staphylinidae), and *Nilaparvata lugens* Stal (Homoptera: Delphacidae) were infected with *Wolbachia*. This is the first report of infection of *N. albofasciata* and *P. fuscipes* by *Wolbachia*. No direct evidence indicated the existence of horizontal transmission of *Wolbachia* between predator and prey.

Introduction

Wolbachia are alpha-proteobacteria that infect a wide range of arthropods (O'Neill *et al* 1992, Rousset *et al* 1992, Werren & O'Neill 1997) and nematodes (Bandi *et al* 1998) throughout the world. The effects of these bacteria on the reproduction of their hosts (Werren 1997) include cytoplasmic incompatibility, parthenogenesis, male killing, and feminization. Cytoplasmic incompatibility has been reported in insects, mites, and isopods (Yen & Barr 1971, Hoffmann *et al* 1986, Breeuwer & Werren 1990, O'Neill & Karr 1990). Thelytokous parthenogenesis has been found in haplodiploid wasps (Stouthamer *et al* 1990), male killing in insects (Hurst *et al* 1999), and feminization of genetic males in isopods and insects (Rousset *et al* 1992, Hiroki *et al* 2002, Negri *et al* 2008).

Recently, because of the prevalence of *Wolbachia* in arthropods, an increasing number of studies have examined the modes of transmission of *Wolbachia* among their arthropod hosts (West *et al* 1998, Vavre *et al* 1999, Huigens *et al* 2000, 2004, Sintupachee *et al* 2006, Vaishampayan *et al* 2007). Vertical transfer of *Wolbachia* is not the only transmission mode, and other modes of transmission, including the horizontal transmission, are known to occur in different hosts of *Wolbachia* (West *et al* 1998, Huigens *et al* 2004, Kittayapong *et al* 2003, Sintupachee *et al* 2006, Raychoudhury *et al* 2009). Although many of these studies proposed that horizontal transmission of *Wolbachia* occurs between different hosts, most of these inferences are based on molecular phylogenetic methods, and additional ecological proofs for *Wolbachia* horizontal transmission are needed.

In order to assess the possibility of horizontal transfer of *Wolbachia* between predator and prey, we evaluated the possible acquisition of *Wolbachia* by spiders (belonging to 11 spider families) from their possible prey

insects (belonging to six insect families) In a field of rice. *Wolbachia* infection in the individuals collected in this community was detected by PCR amplification of *wsp* and *16S* rDNA fragments. Because *Wolbachia* transfers from prey to predator have not yet been verified, in this study, the results of molecular phylogenetic and the potential ecological relationship of predation between spiders and insects were expected to provide valuable proof of whether *Wolbachia* is able to transfer horizontally from prey to predator.

Material and Methods

Arthropod collection and DNA extraction

A total of 317 individuals of arthropods belonging to five orders, 17 families, and 25 species were collected from a 50 × 10 m plots from a field of rice at the Huazhong Agricultural University, Wuhan, Hubei Province, China, from November 2007 to October 2008. All individuals were identified using specific morphological keys (Table 1).

All spiders were placed in labeled vials, taken alive to the laboratory, and kept under controlled conditions (25°C, 70% RH) without any food for three months in order to avoid false positive results of *Wolbachia* from prey present in the spider's digestive system, before DNA extraction.

The insects were placed in 100% ethanol and stored in -20°C. DNA was extracted from the head tissues of carnivorous species and from the abdomen of phytophagous species. Genomic DNA was obtained by standard phenol-chloroform extraction (Kocher *et al* 1989). In order to avoid cross-contamination, each individual spider or insect was first dipped in 75%

Table 1 *Wolbachia* infection in a ricefield arthropod community.

Order, family	Species screened	Number screened (♀/♂)	*Wolbachia* infection (+, -)*
Araneae, Agelenidae	*Agelena labyrinthica* (Clerck)	10 (9/1)	-
Araneae, Araneidae	*Araneus cornutus* (Clerck)	15 (12/3)	-
Araneae, Araneidae	*Araneus ventricosus* (L. Koch)	2 (2/0)	-
Araneae, Araneidae	*Argiope bruennichii* (Scopoli)	15 (12/3)	-
Araneae, Araneidae	*Larinia argiopiformis* (Boes. et Str.)	1 (1/0)	-
Araneae, Araneidae	*Neoscona doenitzi* (Boes. et Str.)	11 (11/0)	-
Araneae, Clubionidae	*Clubiona hummedi* (Schenkel)	2 (2/0)	-
Araneae, Linyphiidae	*Eigone prominens* (Westring)	2 (2/0)	-
Araneae, Linyphiidae	*Ummeliata insecticeps* (Boes. et Str.)	20 (12/8)	-
Araneae, Lycosidae	*Pardosa laura* (Karsch)	23 (18/5)	-
Araneae, Lycosidae	*Pardosa pseudoannulata* (Boes. et Str.)	1 (1/0)	-
Araneae, Lycosidae	*Priata tenuisetaceus* (Chai)	2 (2/0)	-
Araneae, Oxyopidae	*Oxyopes sertalus* (L. Koch)	4 (4/0)	-
Araneae, Salticidae	*Marpissa magister* (Karsch)	10 (10/0)	-
Araneae, Tetragnathidae	*Tetragnatha vermiformis* (Emerton)	20 (11/9)	-
Araneae, Theridiidae	*Coleosoma octomaculatum* (Boes. et Str.)	25 (20/5)	-
Araneae, Thomisidae	*Misumenops tricuspidatus* (Fabricius)	41 (25/16)	-
Araneae, Titanoecidae	*Nurscia albofasciata* (Strand)	8 (8/0)	+A (2)
Coleoptera, Coccinellidae	*Harmonia axyridis* (Pallas)	22	-
Coleoptera, Coccinellidae	*Propylea japonica* (Thunberg)	44	+B (1)
Coleoptera, Lariidae	*Bruchus rufimanus* (Boheman)	6	-
Coleoptera, Staphylinidae	*Paederus fuscipes* (Curtis)	7	+B (1)
Hemiptera, Pentatomidae	*Nezara viridula* (L.)	8	-
Homoptera, Delphacidae	*Nilaparvata lugens* (Stal)	10	+B (1)
Neuropteran, Chrysopidae	*Chrysopa sinica* (Tieder)	8	-

*"A" means *Wolbachia* supergroup A, and "B" means *Wolbachia* supergroup B. The number of infected individuals is in parentheses.

ethanol for 2 min and then washed with distilled water.

PCR amplification and sequencing

Wolbachia infection was tested by carrying out PCR with two primer sets separately: 1) for the outer surface protein (*wsp*)- *wsp*81F (5'-TGG TCC AAT AAG TGA TGA AGA AAC-3') and *wsp*691R (5'-AAA AAT TAA ACG CTA CTC CA-3') (Braig *et al* 1998); 2) for the *16S* rDNA of *Wolbachia*- *16wol* F (5'-TTG TAG CCT GCT ATG GTA TAA CT-3') and *16wol* R (5'-GAA TAG GTA TGA TTT TCA TGT-3') (O'Neill *et al* 1992). PCR reactions using *wsp*136F (5'-TG AAA TTT TAC CTC TTT TC-3') and *wsp*691R , or *wsp*81F and *wsp*522R (5'-ACC AGC TTT TGC TTG ATA-3') were also used to check if *Wolbachia* belonged to supergroup A or B, respectively (Zhou *et al* 1998). PCR was conducted in 30 µl reaction mixtures consisting of 1 µl DNA template, 1.5u Taq, 3.0 µl of 10 × PCR buffer, 1µM of each primer, 0.2 mM dNTPs, and with a final $MgCl_2$ concentration of 1.5 mM. The thermal cycling profile consisted of 94°C for 4 min, 35 cycles (30 s at 94°C, 30 s at 55°C, 30 s at 72°C), 72°C for 10 min, and then held at 4°C, and PCR product amplification was verified by gel electrophoresis on a 1.0% agarose gel.

The quality of genomic DNA was tested by PCR amplification of *28s* rDNA (Werren et al 1995, West *et al* 1998). Only DNA samples yielding amplification products were used for further analysis. PCR products of *16S* rDNA and *wsp* were purified using a DNA Purification Kit (Promega) before direct sequencing. If direct sequencing failed three times, each PCR product was inserted into the vector pMD18-T according to the manufacturer's protocol (Takara), and transferred into competent cells of *Escherichia coli*. Positive insert-containing colonies were selected, and at least three clones per individual were sequenced.

Sequence assemblage and phylogenetic analyses

The sequences obtained were aligned with homologous sequences that were deposited at the GenBank by using the CluxtalX 1.83 algorithm (Thompson *et al* 1997). DNAsp4 (Rozas *et al* 2003), MEGA3.1 (Kumar *et al* 2004) and PAUP 4.0b10 (Swofford 1999) were used to analyze all data and to construct the phylogenetic trees. Phylogenies were constructed using both maximum likelihood (ML) and Bayesian inference (BI) approaches. MrModeltest version 2 (Nylander 2002) was used to construct the appropriate models.

The selected models via the standard AIC using MrModeltest 2 were as follows: HKY+I+G for the 876 bp fragment of *16S* rDNA, and GTR+G for the 606 bp fragment of *wsp* genes. ML trees were constructed using PAUP 4.0b10 with 100 replicates of random stepwise addition sequences and tree-bisection-reconnection branch swapping. For the Bayesian analyses, the analysis for each gene consisted of 3,000,000 generations and four chains using MrBayes version 3.0 (Ronquist & Huelsenbeck 2003). Trees were sampled every 100 generations, resulting in 30,000 total trees. The first 3,000 trees (10%) were discarded as "burnin". Bayesian posterior probabilities were calculated using a 50% majority rule consensus. Three independent runs were performed for each dataset.

Results

Infection of Wolbachia

One species of spider, *Nurscia albofasciata* Strand (Araneae: Titanoecidae), and three species of insects from three families, *Propylea japonica* Thunberg (Coleoptera: Coccinellidae), *Paederus fuscipes* Curtis (Coleoptera: Staphylinidae), and *Nilaparvata lugens* Stal (Homoptera: Delphacidae) were infected with *Wolbachia*. This is the first report of infection of *P. fuscipes* and *N. albofasciata* with *Wolbachia*. Two individuals of *N. albofasciata* were infected with supergroup A of *Wolbachia*, and one individual each of *P. japonica*, *N. lugens*, and *P. fuscipes* were infected with supergroup B (Table 1).

Wolbachia *phylogenies*

According to the phylogenetic analyses of *Wolbachia 16S* rDNA and *wsp* genes (Figs 1, 2), *Wolbachia* infecting *N. lugens*, *P. fuscipes,* and *P. japonica* all belonged to supergroup B; while *Wolbachia* infecting *N. albofasciata* belonged to supergroup A. *Wolbachia 16S* rDNA sequences from *N. lugens* showed high nucleotide sequence similarity to those from *P. fuscipes* (99.7% homology) and *P. japonica* (98.8% homology). For the *wsp* sequences from *Wolbachia*, the homology was 92.2% between *N. lugens* and *P. fuscipes*. Furthermore, the *Wolbachia wsp* sequences in *P. fuscipes* indicated high nucleotide sequence similarity (99.7% homology) to that from *Tetranychus urticae* (Acari: Tetranychidae) deposited in GenBank (accession number: AY763428).

Discussion

Wolbachia was not found in any spiders except *N. albofasciata*, and *Wolbachia* from this species proved to be distantly related to insect endosymbionts, involving *Wolbachia* of *P. japonica, P. fuscipes,* and *N. lugens*. Thus, our study provided no phylogenetic evidence of horizontal transmission of *Wolbachia* between spiders and insects.

In this study, 212 individuals of spiders belonging to 11 families were screened for *Wolbachia*, but only two individuals were positive. The possible causes for the low

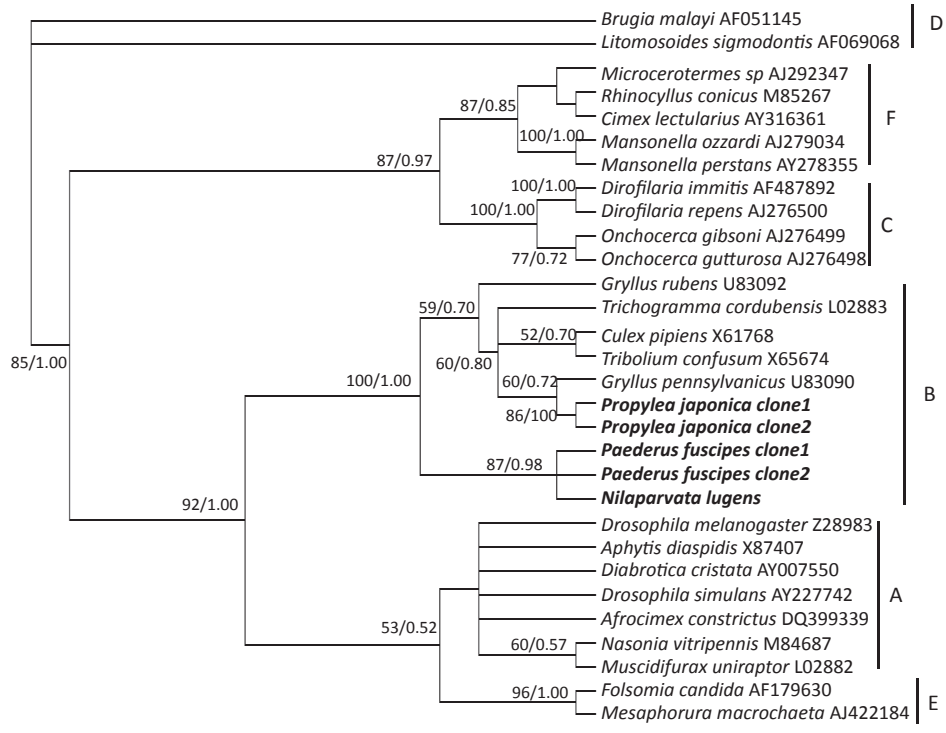

Fig 1 Unrooted phylogeny of *16S* rDNA of *Wolbachia* reconstructed using the maximum likelihood (ML) method. The names of taxa are those of the hosts. Levels of confidence for each node are shown as bootstrap values. Trees inferred from Bayesian analyses were similar, and the posterior probabilities are shown following the bootstrap values from ML analyses. Sequences from this study are indicated in bold. *Wolbachia* supergroups (A-F) are indicated.

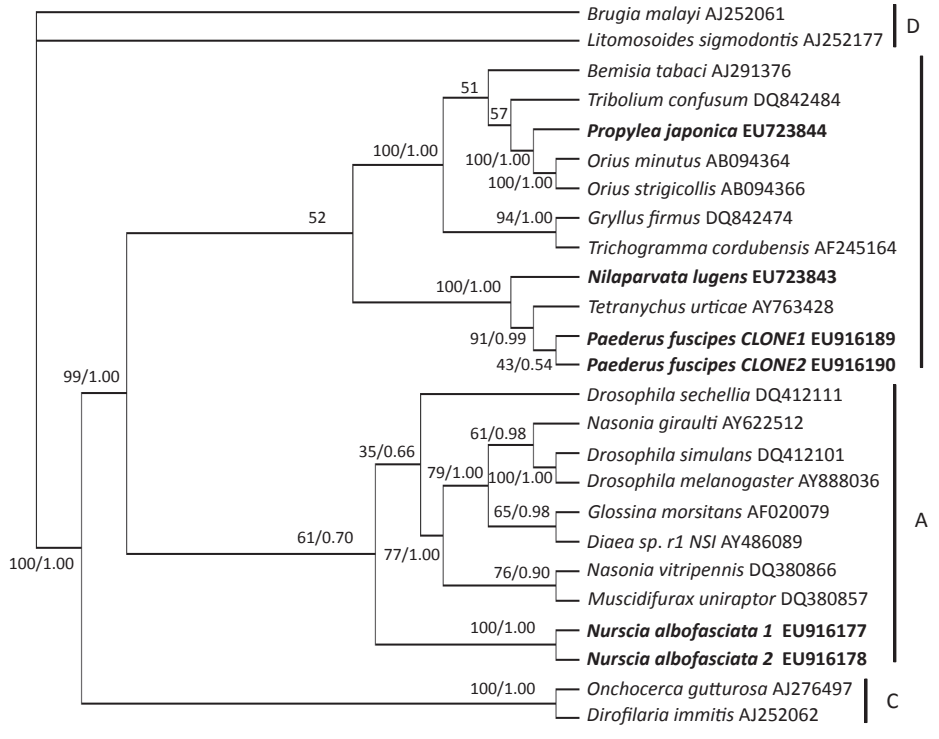

Fig 2 Unrooted phylogeny of *wsp* gene of *Wolbachia* reconstructed using the maximum likelihood (ML) method. The names of taxa are those of the hosts. Levels of confidence for each node are shown as bootstrap values. Trees inferred from Bayesian analyses were similar, and the posterior probabilities are shown following the bootstrap values from ML analysis. Sequences from this study are indicated in bold. *Wolbachia* supergroups (A-D) are indicated.

infection rates of spiders by *Wolbachia* could be: 1) the sample was not large enough to show the natural infection rate; 2) it is unlikely that *Wolbachia* shifted from prey to spiders. In this study, samples including both spiders and insects were collected in the same habitat.

Spiders can predate upon many insects. Only three of the seven insect species (belonging to six families) tested in this study were infected with *Wolbachia*. If *Wolbachia* can be transferred from infected insects to uninfected spiders by predation, then the incidence of *Wolbachia* among spiders should be much higher. Concerning *Wolbachia* horizontal transmission between spiders and insects, Cordaux *et al* (2001) detected *Wolbachia* infection in a woodlouse-eating spider *Dysdera erythrina* (Araneae: Dysderidae), and proposed that the predator-prey route cannot transfer *Wolbachia* because the symbionts seemed unlikely to survive in the predators' digestive tract. Their suggestion is congruent with the results in this study.

We also argue that the specialized food-intake mechanisms in spiders impede the transmission of *Wolbachia*. In spiders, digestion is initiated outside the body. After the prey is subdued, spiders regurgitate their digestive fluids from the intestinal tract into the victim, and then suck in a drop of the predigested liquid prey, repeating this process many times (Foelix 1996). *Wolbachia* is an endosymbiont that cannot live outside its host's cells (Werren 1997). Therefore, the extra-oral digestion system of spiders may be able to destroy the cell structure of the victim.

The phylogenetic analysis of the *16S* rDNA and *wsp* gene fragments indicated a close similarity in nucleotide sequence between *Wolbachia* in *N. lugens* and *P. fuscipes*. *Paederus fuscipes* and *N. lugens* have a potential predator-prey relationship. However, *wsp* or *16S* rDNA sequences obtained from them were dissimilar. Previous studies indicated that high rates of recombination have occurred in the *wsp* gene (Baldo *et al* 2005, Roy & Harry 2007, Verne *et al* 2007), and therefore phylogenetic reconstruction according to *wsp* fragments is not completely reliable. Recently, Multilocus Sequence Typing (MLST) has been an effective means of detecting diversity among strains within a single host, as well as for identifying closely related strains found in different hosts (Baldo *et al* 2006, Baldo & Werren 2007, Baldo *et al* 2008). In addition to spiders, other natural enemies of insects are suitable subjects to test the possibility of horizontal transfer of *Wolbachia* through predation by means of the MLST method, in future studies.

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References

Baldo L, Ayoub NA, Hayashi CY, Russell JA, Stahlhut JK, Werren JH (2008) Insight into the routes of *Wolbachia* invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. Mol Ecol 17: 557-569.

Baldo L, Bordenstein S, Wernegreen JJ, Werren JH (2005) Widespread recombination throughout *Wolbachia* genomes. Mol Biol Evol 23: 437-449.

Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MCJ, Tettelin H, Werren JH (2006) Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol 72: 7098-7110.

Baldo L, Werren JH (2007) Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. Curr Microbiol 55: 81-87.

Bandi C, Anderson TJC, Genchi C, Blaxter ML (1998) Phylogeny of *Wolbachia* in filarial nematodes. Proc R Soc Lond B Biol Sci 265: 1-7.

Braig HR, Zhou W, Dobson SL, O'Neill SL (1998) Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont. J Bacteriol 180: 2373-2378.

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

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.

Foelix RF (1996) Biology of spiders. Second edition, Oxford, Oxford University Press, 38p.

Hiroki M, KatoY, Kamito T, Miura K (2002) Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hecabe* (Lepidoptera: Pieridae). Naturwissenschaften 89: 167-170.

Hoffmann AA, Turelli M, Simmons GM (1986) Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution 40: 692-701.

Huigens ME, Luck RF, Klaasen RHG, Maas MFPM, Timmermans MJTN, Stouthamer R (2000) Infectious parthenogenesis. Nature 405: 178-179.

Huigens ME, Almeida RP de, Boons PAH, Luck RF, Stouthamer R (2004) Natural interspecific and intraspecific horizontal *Wolbachia* transfer of parthenogenesis-inducing in *Trichogramma* wasps. Proc R Soc Lond B Biol Sci 271: 509-515.

Hurst GD, Bandi C, Sacchi L, Cochrane AG, Bertrand D, Karaca I, Majerus ME (1999) *Adonia variegata* (Coleoptera: Coccinellidae) bears maternally inherited flavobacteria that kill males only.

Parasitology 118: 125-134.

Kittayapong P, Jamnongluk W, Thipaksorn A, Milne JR, Sindhusake C (2003) *Wolbachia* infection complexity among insects in the tropical rice-field community. Mol Ecol 12: 1049-1060.

Kocher TD, Thomas WK, Meyer A, Edwards SV, PaÈaÈbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A 86: 6196-6200.

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5: 150-163.

Negri I, Pellecchia M, Mazzoglio PJ, Patetta A, Alma A (2008) Feminizing *Wolbachia* in *Zyginidia pullula* (Insecta, Hemiptera), a leafhopper with an XX/XO sex determination system. Proc R Soc Lond B Biol Sci 273: 2409-2416.

Nylander JAA (2002) MrModeltest 2.2, Evolutionary Biology Centre, Uppsala University.

O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM (1992) *16S* rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc Natl Acad Sci U S A 89: 2699-2702.

O'Neill SL, Karr TL (1990) Bidirectional cytoplasmic incompatibility between conspecific populations of *Drosophila simulans*. Nature 348: 178-180.

Raychoudhury R, Baldo L, Oliveira DC, Werren JH (2009) Modes of acquisition of *Wolbachia*: horizontal transfer, hybrid introgression, and codivergence in the *Nasonia* species complex. Evolution 63: 165-83.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxf) 19: 1572-1574.

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

Roy V, Harry M (2007) Diversity of *Wolbachia* isolated from the *Cubitermes* sp. *affinis subarquatus* complex of species (Termitidae), revealed by multigene phylogenies. FEMS Microbiol Lett 274: 102-111.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics (Oxf) 19: 2496-2497.

Sintupachee S, Milne JR, Poonchaisri S, Baimai V, Kittayapong P (2006) Closely related *Wolbachia* strains within the pumpkin arthropod community and the potential for horizontal transmission via the plant. Microb Ecol 51: 294-301.

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

Swofford DL (1999) PAUP: phylogenetic analysis using parsimony. Centre for Agriculture and Bioscience International.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876-4882.

Vaishampayan PA, Dhotre DP, Gupta RP, Lalwani P, Ghate H, Patole MS, Shouche YS (2007) Molecular evidence and phylogenetic affiliations of *Wolbachia* in cockroaches. Mol Phylogenet Evol 44: 1346-1351.

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

Verne S, Johnson M, Bouchon D, Grendjean F (2007) Evidence for recombination between feminizing *Wolbachia* in the isopod genus *Armadillidium*. Gene 397: 58-66.

Werren JH (1997) Biology of *Wolbachia*. Annu Rev Entomol 42: 587-609.

Werren JH, O'Neill SL (1997) The Evolution of Heritable Symbionts. Oxford, Oxford University Press, 1-41p.

Werren JH, Windsor D, Guo L (1995) Distribution of *Wolbachia* among neotropical arthropods. Proc R Soc Lond B 262: 197-204.

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

Yen JH, Barr AR (1971). The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. J Invertebr Pathol 22: 242-250.

Zhou W, Rousset F, O'Neil S (1998) Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. Proc R Soc Lond B Biol Sci 265: 509-515.