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SHORT COMMUNICATION

Wolbachia infection shared among planthoppers (Homoptera: Delphacidae) and their endoparasite (Strepsiptera: Elenchidae): a probable case of interspecies transmission

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Abstract

***Wolbachia*, a group of parasitic bacteria of arthropods, are believed to be horizontally transmitted among arthropod taxa. We present a new probable example of interspecies horizontal transmission of *Wolbachia* by way of an endoparasite based on the conformity of *Wolbachia* gene sequences. Field samples of two rice planthoppers, *Laodelphax striatellus* and *Sogatella furcifera* possessed identical *Wolbachia*. Among three major endoparasites of planthoppers, a strepsipteran, *Elenchus japonicus*, harboured the identical *Wolbachia* strain, suggesting strepsipteran transmission of *Wolbachia* from one planthopper to the other. No *Wolbachia* was detected in a mermithid nematode *Agamermis unka*, and dryinid wasps possessed different types of *Wolbachia*.**

Keywords: dryinid wasp, horizontal transmission, planthopper, Strepsiptera, *Wolbachia*.

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Introduction

Wolbachia are a group of rickettsial symbiotes distributed in various groups of arthropods (Werren *et al*. 1995a). These endosymbiotes cause reproductive alterations in arthropod hosts (reviewed by O'Neill *et al*. 1997). *Wolbachia* are primarily transmitted vertically from female parent to offspring. However, horizontal transmission has also occurred among arthropod lineages: closely related *Wolbachia* strains are often found in distantly related arthropod species, and different *Wolbachia* strains occasionally infect closely related or even a single host species (e.g. Moran & Baumann 1994; Schilthuizen & Stouthamer 1997; Vavre *et al*. 1999). A most likely path of horizontal transmission of *Wolbachia* is that by way of endoparasites (Werren *et al*. 1995b; van Meer *et al*. 1999). Heath *et al*. (1999) reported spontaneous transmission of *Wolbachia* from *Drosophila simulans* to its parasitoid, *Leptopilina boulardi*, in the laboratory. Intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* frequently occurs among larvae of *Trichogramma kaykai* in laboratory conditions (Huigens *et al*. 2000). Attempts to find the horizontal transfer of *Wolbachia* in host–parasitoid associations in the field were not fruitful (Schilthuizen & Stouthamer 1998; West *et al*. 1998).

The whitebacked planthopper *Sogatella furcifera* is distributed throughout southeast Asia, migrates from China to Japan every early summer, and cannot winter in Japan (Kisimoto 1987). The small brown planthopper *Laodelphax striatellus* shows a wider geographical distribution and winters in Japan (Noda 1992). *Wolbachia* in the two rice planthoppers cause cytoplasmic incompatibility (CI) (Noda 1984a, 1984b; Noda *et al*. 2001). *Wolbachia* strains found in single laboratory colonies of *L. striatellus* and *S. furcifera* were identical to each other in terms of nucleotide sequences in four *Wolbachia* genes (16S rDNA, *ftsZ*, *groEs* and *wsp*), strongly suggesting that the same *Wolbachia* strain is shared between the two colonies of planthoppers (Noda *et al*. 2001).

We have studied nucleotide sequences of *Wolbachia* genes in the field populations of the two planthoppers and their endoparasites to evaluate the horizontal transmission of *Wolbachia* in these hosts/parasite associations. The major parasites of the rice planthoppers are

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a mermithid nematode, *Agamermis unka* (Nematoda: Mermithidae) (Choo & Kaya 1990), a strepsipteran *Elenchus japonicus* (Strepsiptera: Elenchidae) (Kifune & Maeta 1986; Kathirithamby 1998) and dryinid wasps (Hymenoptera: Dryinidae). At least six species of Dryinidae are known in Japan (Kitamura 1982).

Materials and methods

Animals

The locations for sample collection are shown in Table 1.

Electronmicroscopy

Strepsipterans were cut into several pieces in 0.1 M cacodylate buffer (pH 7.3). The samples were fixed with 2.5% glutaraldehyde in the buffer, postfixed with 1% OsO_4, and embedded in Epon 812. Thin sections were stained with 2% uranyl acetate and 0.4% lead citrate and observed with a JEM-1010 electron microscope.

Diagnostic polymerase chain reaction

Samples were homogenized with 30–50 μL of STE [100 mM NaCl, 1 mM EDTA (pH 8.0), 10 mM Tris-HCl (pH 8.0)] and digested with proteinase K (O'Neill *et al*. 1992). Specific primers for 16S rDNA, 99F/994R (O'Neill *et al*. 1992), were used to detect *Wolbachia*. DNA was amplified in 20 μL of a standard reaction buffer with 0.15 mM each dNTP, 10 pmol primers and 1.5–2.0 U *Taq* DNA polymerase. The polymerase chain reaction (PCR) thermal cycle was 95 °C for 30 s, followed by 30 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 2 min, and 72 °C for 5 min as a final extension after the last cycle. PCR negative samples of planthoppers, strepsipterans, and dryinid wasps were further tested

Table 1 Samples used in this study and *Wolbachia* type in each sample

Host species	Parasitic host	Collection site/Year	*Wolbachia*	Sequence analysis
Laodelphax striatellus		Izumo, Japan/1987	a	f, g, w, 16
		Shanghai, China/1995	a	f, g, w
		Yunnan, China/1995	a	f, g, w, 16
		Kumamoto, Japan/1998	a	f, g, w
Sogatella furcifera		Izumo, Japan/1987	a	f, g, w, 16
		Omagari, Japan/1996	a	f, g, w
		Kumamoto, Japan/1998	a	f, g, w
		Hangzhou, China/1998	a	f, g, w
Agamermis unka	*S. furcifera*	Hangzhou, China/1998	— (0/1)	
	Nilaparvata lugens	Hangzhou, China/1998	— (0/10)	
Elenchus japonicus	*L. striatellus*	Gohyakugawa*, Japan/1996	— (0/2)	
	L. striatellus	Furukawa*, Japan/1996	— (0/2)	
	L. striatellus	Natori*, Japan/1996	a (1/1)	f, g, w
	L. striatellus	Furukawa*, Japan/1997	a (1/12)	f, g, w
	S. furcifera	Kumamoto, Japan/1998	a (4/10)	f, g, w
	L. striatellus	Kumamoto, Japan/1998	a (4/4)	f, g, w
Dryinid wasp	*L. striatellus*	Shiga, Japan/1995	c,e (1/2)	w
	L. striatellus	Yaita*, Japan/1995	— (0/2)	
	L. striatellus	Koriyama*, Japan/1996	b,d (6/6)	f, g, w
	L. striatellus	Fukushima*, Japan/1996	— (0/1)	
	L. striatellus	Kunimi*, Japan/1996	b (1/1)	
	L. striatellus	Gohyakugawa*, Japan/1996	b (2/2)	
	L. striatellus	Furukawa*, Japan/1996	b (1/1)	
	L. striatellus	Yahaba*, Japan/1997	b (2/3)	f, g, w

Izumo strains of *L. striatellus* and *S. furcifera* were previously studied (Noda H *et al*. 2001). *represents locations in Tohoku district shown in Fig. 1.

Letters, 'a' — 'e', indicate *Wolbachia* strains based on gene types, and identical sequences are indicated by the same letter. Types 'a', 'b', 'd' and 'e' are members of group B, and type 'c' is one of group A. The gene types of the samples whose gene sequences were not examined were determined by endonuclease digestion of PCR products (see text). The numbers in parentheses indicate the number of *Wolbachia*-infected samples and the number examined.

'f', 'g', 'w' and '16' mean *ftsZ* gene, *groE* genes, *wsp* gene and 16S rDNA, respectively. Sequence sizes of the *ftsZ* gene, *groE* genes, and 16S rDNA were 1060, 783 and 852 bp. The sizes of the *wsp* gene in types 'a', 'b' and 'd' were 555 bp, and those of 'c' and 'e' were 561 and 557 bp, respectively. At least three clones were analysed in each gene. Types 'd' and 'e' were different from major type 'b' and 'c', respectively, in nucleotide sequences of the *wsp* gene and were found only one clone each. Type 'd' showed a three-nucleotide difference from 'a' and nine from 'b'. Type 'e' showed much greater difference in nucleotide sequence from other types and included one nucleotide deletion.

for validity of template preparation with primers for mitochondrial genes: 12S rDNA (Simon *et al*. 1991), cytochrome *b* (Cyt*b*; Muraji *et al*. 2000), and cytochrome oxidase I (COI; Navajas *et al*. 1994), respectively.

Nucleotide sequence analysis

The *ftsZ* gene (Holden *et al*. 1993; Werren *et al*. 1995b), *groE* genes (*groES* and *groEL*) (Masui *et al*. 1997) and *wsp* gene (Zhou *et al*. 1998) of *Wolbachia* were amplified. The PCR products were cloned into pBluescript II (Stratagene) or pGEM-T (Promega) vectors. The sequences were determined with a DNA Sequence System (model 373 A, 377, Perkin Elmer) or the DNA sequencer ALF Express (Pharmacia Biotech).

PCR–restriction fragment length polymorphism (RFLP) analysis was applied to the parasite samples not subjected to the sequence determination. Three major species of *wsp* genes ('a', 'b' and 'c') were distinguished by digesting the PCR products with the endonucleases *Bgl*II, *Pvu*II and *Taq*I.

Phylogenetic analyses

The phylogenetic relationship among *Wolbachia* strains was analysed using nucleotide sequences of *wsp* gene. The sequences were aligned with CLUSTAL_X (Thompson *et al*. 1997). All gaps were deleted, and 452 bases were used for analyses. Neighbour-joining analysis was performed with a PHYLIP software package v.3.5c (Felsenstein 1995) based on Kimura 2-parameter distances with the transition/transversion ratio of two. Maximum parsimony analysis, as implemented in PAUP vs. 4.0b4a (Swofford 2000), was based on a heuristic search. Bootstrap analysis was carried out with 1000 replications in both analyses.

Results

Nucleotide sequences of three *Wolbachia* genes, *ftsZ*, *groEs* and *wsp*, were determined for the field-collected *Laodelphax striatellus* and *Sogatella furcifera* (Table 1). All the examined planthopper samples showed identical sequences in each of the three *Wolbachia* genes (hereafter referred to as type 'a' *Wolbachia*; Table 1). The type 'a' *Wolbachia* was also found in laboratory colonies of *L. striatellus* and *S. furcifera* both of which were collected at Izumo (Fig. 1; Table 1) (Noda *et al*. 2001; accession nos AB039038–AB039043). The *Wolbachia* 16S rDNA sequence of *L. striatellus* from Yunnan was also identical with those of laboratory-cultured Izumo strains of *L. striatellus* and *S. furcifera* (Table 1; accession nos AB039036 and AB039037).

Eleven individuals of a parasitic nematode, *Agamermis unka*, infecting *Nilaparvata lugens* or *S. furcifera* were tested by PCR. No infection was found in the nematode samples (Table 1).

Ten of 31 *Elenchus japonicus* samples were infected with *Wolbachia* (Table 1). Under the electron microscopy, *Wolbachia*-like microorganisms were found in the ovary, muscle and trachea of *E. japonicus* (data not shown). Nucleotide sequences of *ftsZ*, *groE* and *wsp* genes were determined for four *Wolbachia* strains infecting in *E. japonicus* collected from Natori, Furukawa and Kumamoto (Table 1). The sequences of all the examined clones were identical to those of the type 'a' *Wolbachia* in each of the three genes (AB039279, AB039281 and AB039283).

Thirteen of 18 dryinid wasps possessed *Wolbachia* (Table 1). Species of the examined wasps could not be identified because they were in larval stages, but *Haplogonatopus atratus* seems to be the dominant dryinid species parasitizing *L. striatellus* in Japan. The three *Wolbachia* gene sequences in two dryinid wasps collected from Koriyama

Fig. 1 Collection sites of planthoppers and their endoparasites. Some of *Elenchus japonicus* and dryinid wasps were collected at Tohoku district (see Table 1).

and Yahaba were determined (Table 1). All examined sequences were identical among clones for both *ftsZ* and *groEs* ($n = 6$ each) (type 'b' *Wolbachia*; AB0390280 and AB039282). For the *wsp* gene, 14 of 15 examined clones were identical (type 'b' *Wolbachia*; AB039284), and one clone from the Koriyama sample was distinct (type 'd' *Wolbachia*; AB046720). The *wsp* gene of *Wolbachia* in the Shiga sample was also examined, which showed two unique sequences (types 'c' and 'e' *Wolbachia*; AB039372 and AB046721). Type 'e' *wsp* sequence was found in one of 10 clones examined, and had one nucleotide deletion.

PCR–RFLP analysis of *wsp* sequences was performed for *Wolbachia* strains in the *E. japonicus* and dryinid samples that were not subjected to the sequence determination. Type 'c' (605 bp) is digested into two fragments by *Taq*I (517 and 88 bp). Type 'b' (599 bp) has the restriction site of *Bgl*II (133 and 466 bp) and *Pvu*II (227 and 372 bp). Type 'a' (599 bp) is cut into two fragments by *Bgl*II (133 and 466 bp), and not by *Pvu*II or *Taq*I. Type 'e' is indigestible by the three enzymes. Type 'd' should show the same RFLP pattern as type 'a'. All the *Wolbachia* strains in *E. japo*nicus samples were type 'a', and the dryinid wasps were infected with type 'b' bacteria (Table 1).

The neighbour-joining and maximum parsimony analyses of *wsp* sequences showed similar results. Type 'c' *Wolbachia* was phylogenetically classified in A-group *Wolbachia*, while the other four types were in B-group. The type 'd' found in a dryinid wasp and the type 'a' found in planthoppers and *E. japonicus* were very closely related to each other. In the A-group *Wolbachia* (data not shown), type 'c' formed a monophyletic group with *Wolbachia* strains from *Leptopilina heterotoma* (AF124860), *Ephestia kuehniella* (AF071911), *Trichogramma kaykai* (AF071912) and *T. bourachae* (AF071913).

Discussion

The sequence identity found in the present results indicates that the three *Wolbachia* strains from two planthopper and one strepsipteran species are identical or at least very closely related, strongly supporting a hypothesis that *Wolbachia* infections occasionally undergo horizontal transmission between distantly related insect lineages by way of parasitoids or parasites. *Wolbachia* in either *Laodelphax striatellus* or *Sogatella furcifera* may have been transmitted to the other planthopper by way of *Elenchus japonicus*. *E. japonicus* may have acquired *Wolbachia* from other sources and have transmitted it to *L. striatellus* and *S. furcifera*. The other possibility is that the infection in *E. japonicus* may have originated in an ancestral species of *E. japonicus*. Kathirithamby (1998) reported that three other species of Strepsiptera harbour rickettsia-like microorganisms, and therefore it would be interesting to examine *Wolbachia* infections in strepsipteran insects. The successful inoculation of *Wolbachia* from *E. japonicus* to host planthoppers would be achieved when an infected *E. japonicus* dies within the body of its host (West *et al*. 1998).

The dryinid samples showed a complex pattern of infection with *Wolbachia*. Four *Wolbachia* strains in total were found in spite of a limited number of samples. Type 'd' *Wolbachia* is also likely to have undergone horizontal transmission among planthoppers and dryinid wasps in the past based on the close relationship between types 'a' and 'd' (Fig. 2).

Type 'a' *Wolbachia* in the present planthopper samples was also found on Ishigaki Island (Japan) (16S rDNA, X65672; Rousset *et al*. 1992) and in Yunnan (China) (*wsp* gene, AF020080; Zhou *et al*. 1998) in *L. striatellus*. The infection

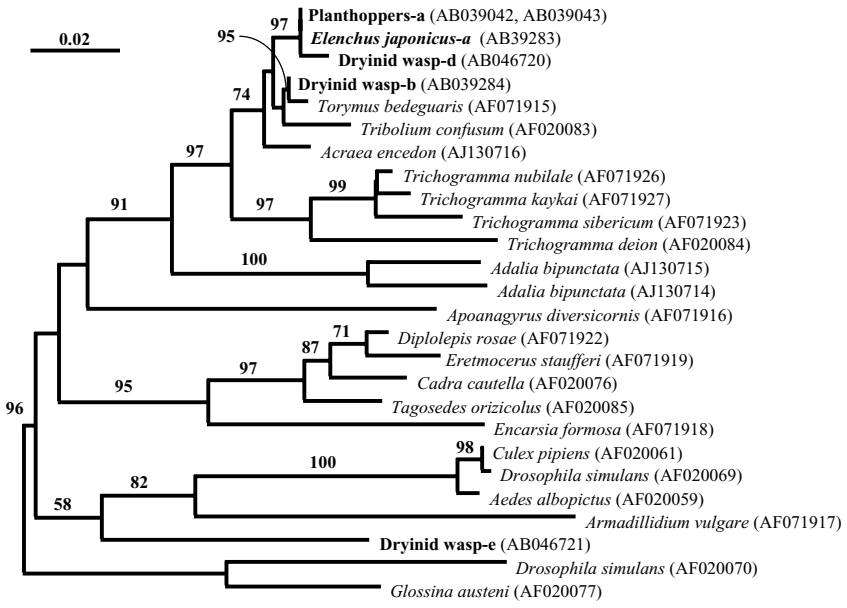

Fig. 2 Neighbour-joining tree of *wsp* sequences of B-group *Wolbachia*, with A-group *Wolbachia* of *Glossina austeni* and *Drosophila simulans* as outgroups. *Wolbachia* strains are represented by names of their hosts. Accession numbers are shown in parentheses. The numbers show bootstrap values greater than 55.

of a single strain of *Wolbachia* over a wide geographical area in each planthopper species seems to be explained by the following reasons: (i) the long distance migration of the two planthopper species; (ii) CI due to *Wolbachia* infection in the two planthopper species, which was first argued by Laven (1959) and shown in *L. striatellus* (Hoshizaki & Shimada 1995); and (iii) horizontal transmission of *Wolbachia* by way of *E. japonicus*.

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