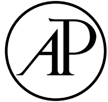

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Note

Presence of *Wolbachia* endosymbionts in different silkworm species and races and in their uzi fly parasites

The genus *Wolbachia* is composed of endosymbiotic bacteria that occur in the cytoplasm of reproductive tissues and other tissues in many arthropod species. These bacteria are capable of causing reproductive alterations in their hosts such as cytoplasmic incompatibility (Barr, 1980; Breeuwer et al., 1992; O'Neill et al., 1992), parthenogenesis in wasps (Stouthamer et al., 1993), and feminization of males in isopods (Rousset et al., 1992). In addition, these bacteria may cause rapid speciation in some arthropods (Werren, 1997). Extensive horizontal transmission of *Wolbachia* has occurred among insect taxa, including between different orders of insects (Werren et al., 1995b).

Sericulture is an industry based on the rearing of silkworms (Lepidoptera) on host plants for the raw silk production. Among the parasites that attack silkworms, the most important is the uzi fly (Diptera: Tachinidae), an endoparasite that causes considerable mortality (Siddappaji and Channabasavanna, 1990). The initimate association of the uzi fly and silkworm provided an opportunity to determine whether there was horizontal transmission of Wolbachia endosymbionts between these two insects.

In previous studies, systematic surveys for *Wolbachia* have been carried out using the polymerase chain reaction (PCR) to detect 16S rDNA, *ftsZ*, and *wsp* genes (Werren et al., 1995a). Using this technology, horizontal transmission has been shown to occur in some insect host-parasitoid systems (Werren et al., 1995b). In the present study, we used PCR to detect the presence of *wsp* gene in different silkworm races and in its uzi fly parasites. We further compared the PCR products to determine whether there was horizontal transmission between the uzi fly and silkworms.

The different silkworm species and races and their parasitoid uzi flies screened for *Wolbachia* are listed in Tables 1 and 2. Total genomic DNA from the silkworms and flies was prepared for PCR amplification following the standard protocol (Sambrook et al., 1989). PCR was done in 25 µl reaction volume containing 1 µl of sample DNA, 2.5 µl 10× buffer (Genei), 0.5 µl dNTPs (10 mM each), 1 µl 26 µM forward primer and 1 µl 35 µM reverse primer, and 0.5 µl *Taq* DNA polymerase (Genei). Distilled, deionized water was added to a final volume of 25 µl. The reaction mixture was prepared in one batch and then added to each sample. PCR amplification was done under the following thermal profile: 92 °C 30 s, 52 °C 30 s, and 75 °C 30 s per cycle for 30 cycles (MJ Research PTC-200). PCR products were separated by electrophoresis in a 1.5% agarose gel to determine the presence and size of amplified DNA, afterwhich the gels were photographed on a UV transilluminator.

The general *wsp* primers used for PCR amplification were as follows:

wsp 81F (5′ TGG TCC AAT AAG TGA TGA AGA AAC) and *wsp* 691R (5′ AAA AAT TAA ACG CTA CTC CA).

By use of these *wsp* primers, a *wsp* gene fragment was detected in different uzi fly populations of *E. sorbillans* (Diptera: Tachinidae) and *Blepharipa zebina* (Diptera: Tachinidae). The mobilities of DNA fragments that were amplified using DNA from different fly populations are shown in Figs. 1 and 2. The prominent reaction products were represented by single bands of the expected size of approximately 600 bp. Using the same primers, however, we obtained no PCR products from the silkworm samples (Figs. 1 and 2).

Braig et al. (1998) found that the primer combination of *wsp* 81F and *wsp* 691R was able to amplify *wsp* gene fragments from different strains of *Wolbachia*. By using the same general *wsp* primers, Zhou et al. (1998) amplified fragment of the *wsp* gene from 28 *Wolbachia* strains. They reported that these primers amplify a DNA fragment ranging from 590 to 632, the size depending on the individual *Wolbachia* strain. These primers only amplified fragments from infected insects. Thus, the results we obtained in the present study using *wsp* primers demonstrate that *Wolbachia* was present in *E. sorbillans* (see also, Madhu and Puttaraju, 2001) and *B. zebina*. It is also evident from this study that *Wolbachia* was not present in any of the silkworm species or races.

Several routes of horizontal transfer have been discussed (Hurst et al., 1992) and there is evidence of cross-infection in predator–prey (Jolianowicz and Hoy, 1996) and host–parasitoid systems (Werren et al., 1995b). O'Neill et al. (1992) and Rousset et al. (1992)

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Table 1
Silkworm species and races and their *Wolbachia* infection status determined by the PCR using *wsp* primers

Silkworm species/race	Voltinism	Place of origin	PCR results (+/−)
Mulberry silkworm (*Bombyx mori*) (Lepidoptera: Bombycidae)			
Race			
Pure Mysore	Multivoltine	Karnataka	−
Nistari	Multivoltine	West Bengal	−
Sarupat	Multivoltine	Assam	−
Moria	Multivoltine	Assam	−
Guangnong	Multivoltine	China	−
NB-1	Bivoltine	Karnataka	−
NB-7	Bivoltine	Karnataka	−
NB4D2	Bivoltine	Karnataka	−
KA	Bivoitine	West Bengal	−
HU-204	Bivoltine	China	−
Non-mulberry silkworms (species) (Lepidoptera: Bombycidae)			
Antheraea mylitta (Temperate Tasar)	Bivoltine & Trivoltine	India	−
Antheraea proylei (Temperate Tasar)	Univoltine	India	−
Antheraea roylei (Temperate Tasar)	Univoltine	India	−
Antheraea pernyi (Temperate Tasar)	Univoltine	China	−
Samia cynthia ricini (Eri)	Mu1tivoltine	India	−
Antheraea assamensis (Muga)	Multivoltine	India	−

Table 2
Populations of the uzi flies *E. sorbillans* and *B. zebina* and their *Wolbachia* infection status as determined by PCR using *wsp* primers

Species	Locality	PCR results (+/−)
E. sorbillans	Ramanagar (Karnataka)	+
E. sorbillans	Sira (Karnataka)	+
E. sorbillons	Madlaugiri (Karnataka)	+
E. sorbillans	Mandya (Karnataka)	+
E. sorbillans	Chitradurga (Karnataka)	+
E. sorbillans	Ananthpur (Andhrapradesh)	+
B. zebina	Bastar (Madhyapradesh)	+

suggested that *Wolbachia* strains could be transmitted horizontally between insect taxa, because closely related bacteria have been found in distantly related hosts. Werren et al. (1995b) proposed that transmission between parasitic insects and their hosts is one vehicle for intertaxon transmission of *Wolbachia*. An obvious test of this hypothesis is to show that parasitoids and their host insects share *Wolbachia* strains that are more closely related. For example, *Nasonia giraulti* and *N. longicornis* are parasitoid wasps harbour B group *Wolbachia* that are closely related to those of their preferred Protocalliplioridfly hosts. A second possible exchange between parasitoid and host involves *Drosophila melanogaster* and the drosophilid larval parasitoid *Asobara tabida*. These two species share nearly identical *Wolbachia*. However, using a PCR assay, West et al. (1998) surveyed for *Wolbachia* infection in 82 insect species from two host–parasitoid communities. One host–parasitoid community was based around leaf-mining Lepidoptera, and the other around aphids. They found that phylogenetic analyses of the sequence

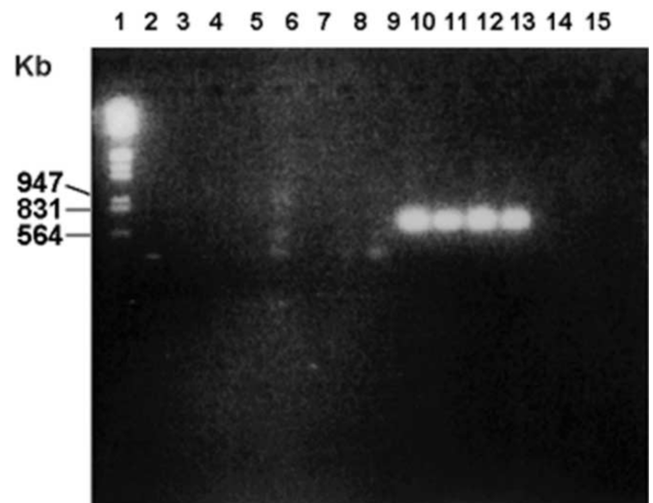

Fig. 1. PCR screening for the presence of *Wolbachia* in races of the mulberry silkworm, *B. mori*, and its parasite the uzi fly, *E. sorbillans* using *wsp* primers. Lane 1, *Hin*dIII–*Eco*R1 digest as markers. Lanes 2–9, *B. mori* samples from the following races, respectively: Lane 2, Pure Mysore; 3, Nistari; 4, Sarupat; 5, Moria; 6, Guangnong; 7, NB-1; 8, NB-7; 9, NB4D2. Lanes 10–13, *E. sorbillans* samples from the following sites, respectively, Lane 10, Ramanagar; 11, Sira; 12, Madhugiri; and 13, Mandya. Lanes 14 and 15, *B. mori* samples from races KA and HU-204, respectively.

data suggested host–parasitoid transfer of *Wolbachia* was not the major route through which the species they examined became infected. Since in the present study we found *Wolbachia* in the uzi flies, but not in any of the silkworm species or races, we conclude that the *Wolbachia* organisms in the flies are not transmitted to the silkworms.

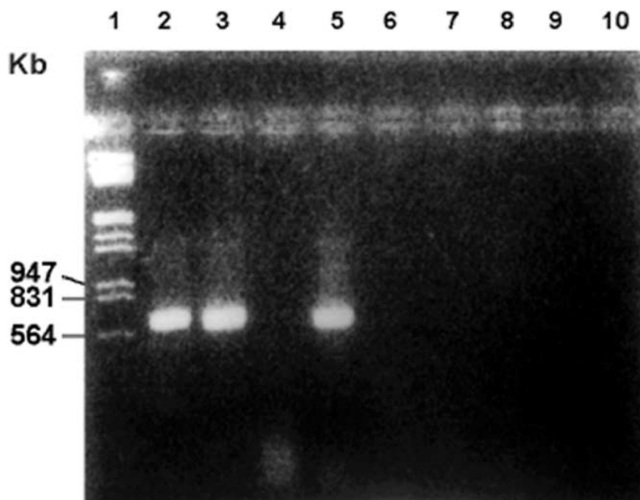

Fig. 2. PCR screening for the presence of *Wolbachia* in species of non-mulberry silkworms and their uzi fly parasites, *E. sorbillans* and *B. zebina* using *wsp* primers. Lane 1, *Hin*dIII–*Eco*R1 digest as markers. Lanes 2 and 3, respectively, samples from *E. sorbillans* from Chitradurga and Anathpur. Lane 4, sample from the silkworm, *A. mylitta*. Lane 5, sample from the uzi fly, *B. zebrina*. Lanes 6–10, samples from non-mulberry silkworms: 6, *A. proylei*; 7, *A. roylei*; 8, *A. pernyi*; 9, *S. cynthia ricini*; and 10, *A. assamensis*.

Keywords: PCR; Silkworm species; *Bombyx mori*; *Uzi flies*; *Exorista sorbillans*; *Blepharipa zebina*; *Wolbachia*; *wsp gene*; Cytoplasmic incompatibility; Horizontal transmission

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