

Wolbachia and nuclear–nuclear interactions contribute to reproductive incompatibility in the spider mite *Panonychus mori* (Acari: Tetranychidae)

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Maternally transmitted bacteria of the genus *Wolbachia* are obligate, intracellular symbionts that are responsible for cytoplasmic incompatibility in a wide range of arthropods such as insects and mites. Spider mites often show uni- and bidirectional incompatibilities among populations with and without *Wolbachia*. Therefore, we surveyed the presence of *Wolbachia* by PCR and then conducted crossing experiments among 25 populations of *Panonychus mori* to determine how *Wolbachia* are related to the incompatibility in this species. Five out of the 25 populations were infected with *Wolbachia*. These five populations were treated with an antibiotic (rifampicin) to eliminate *Wolbachia*. We carried out round-robin crosses among 20 *Wolbachia*-uninfected populations, five infected populations and five rifampicin-treated populations (30 × 30 = 900 crosses in total). Incompatibility among *P. mori* populations was caused by *Wolbachia*

infection, nuclear–cytoplasmic interactions or nuclear–nuclear interactions. *Wolbachia*-mediated incompatibility was observed in crosses between uninfected females and infected males or between females and males harboring different *Wolbachia* strains. Nuclear–cytoplasmic interactions may be responsible for the unidirectional incompatibility in crosses between the two northern populations and one of the southern populations. Bidirectional incompatibility caused by nuclear–nuclear interactions was observed in 99 combinations of interpopulation crosses (99/300 = 0.33). Although no geographical trends were detected in the distribution of bidirectionally compatible populations, the results reveal a genetic divergence among *P. mori* populations.

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Introduction

Intracellular bacteria of the genus *Wolbachia* are the causative agents of cytoplasmic incompatibility (CI) found in many arthropod species such as insects (Werren and O'Neill, 1997) and mites (Breeuwer, 1997). *Wolbachia*-induced CI results in embryonic lethality and/or a male-biased sex ratio in crosses between males that are infected with *Wolbachia* and females that are uninfected or infected with a different *Wolbachia* strain (O'Neill *et al.*, 1997). CI results in the production of few or no offspring in diploidiploids (Hoffmann *et al.*, 1986) or in the production of only male offspring in haploidiploids (Breeuwer and Werren, 1990). The CI mechanism has been explained by the modification/rescue model: *Wolbachia* modify sperm or male chromosomes in the host (mod +; modification capable) and this modification, which is normally detrimental to the host, is rescued by the same kind of *Wolbachia* in the females (resc +; rescue capable) (O'Neill *et al.*, 1997; Bourtzis *et al.*, 1998). Some *Wolbachia* strains (mod– resc +) do not

modify the sperm but rescue the detrimental modification caused by a mod + resc + strain (Bourtzis *et al.*, 1998; Mercot and Poinso, 1998).

Antibiotics, such as tetracycline and rifampicin, eliminate *Wolbachia* from the host (O'Neill, 1989; Stouthamer *et al.*, 1990; Noda *et al.*, 2001). When insects are infected with CI-inducing *Wolbachia*, the crosses between antibiotic-treated females and untreated males are incompatible, but the reciprocal crosses are compatible. Antibiotics can also eliminate *Wolbachia* from spider mites (Breeuwer, 1997; Gomi *et al.*, 1997; Gotoh *et al.*, 2003).

The CI of the spider mites *Tetranychus turkestanii* and *T. urticae* (red-form) was found through crossing experiments between antibiotic-treated females and untreated males. The CI observed in these spider mite species was shown by the death of fertilized eggs (Breeuwer, 1997), although incompatibility in haploidiploids was expected to produce all male offspring or a male-biased sex ratio without any death of eggs. On the other hand, *Wolbachia* infection does not induce reproductive incompatibility in *T. kanzawai* (Gomi *et al.*, 1997; Gotoh *et al.*, 1999a) or *T. urticae* (green-form) (Gotoh *et al.*, 1999b), although many populations collected from a wide range of Japanese islands were tested from these species. The present study is of another spider mite species, *Panonychus mori*, which showed bidirectional reproductive incompatibility in crosses between the southwestern

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population at Tottori (35°N) and the northern population at Sapporo (43°N) and resulted in an abnormal male-biased sex ratio (ca. 97–99% males) (Gotoh and Higo, 1997). Bidirectional incompatibility is assumed to be caused by either nuclear–nuclear interactions, which are the result of lethal combinations of genes, or infection by different *Wolbachia* strains. Our preliminary crossing experiments for this species also indicated that the crosses between females of some populations and males of other populations are incompatible, but the reciprocal crosses are compatible and give a female-biased sex ratio. This unidirectional incompatibility may be due to infection by *Wolbachia*.

In the present study, we surveyed for the presence of *Wolbachia* by PCR and conducted crossing experiments to determine the relationship between *Wolbachia* infection and reproductive incompatibility observed among the populations of *P. mori*. We also analyzed the possible causes of the reproductive incompatibility observed among *Wolbachia*-free populations that were prepared by treating the *Wolbachia*-infected populations with an antibiotic, as well as *Wolbachia*-uninfected populations of *P. mori*. We then carried out round-robin crosses among *Wolbachia*-uninfected populations, *Wolbachia*-infected populations and *Wolbachia*-free populations.

Materials and methods

Mite populations

Mites were collected from mulberry, *Morus bombycis*, at 25 localities from 1993 to 1998 (Figure 1). Laboratory stocks of these populations were separately reared on leaf discs (ca. 25 cm²) of mulberry placed on water-saturated polyurethane mats in plastic dishes (9 cm in diameter). In winter, mulberry plants were cultivated in a greenhouse. All experiments were carried out at 25°C, 60–70% RH and with a 16L–8D photoperiod.

DNA extraction and PCR amplification

The PCR techniques used here were the same as those used by Gomi *et al.* (1997) and Gotoh *et al.* (2003). Two pairs of *Wolbachia*-specific primers were used to detect the presence of *Wolbachia*. One amplifies a part of the 16S rDNA (O'Neill *et al.*, 1992) and the other amplifies the *ftsZ* gene (Holden *et al.*, 1993). A total of 10–20 females were tested for each population. Since CLO (*Cytophaga*-like organisms) in the CFB (*Cytophaga*-*Flavobacterium*-*Bacteroides*) phylum have recently been reported to cause reproductive alteration in host insects and mites (Weeks *et al.*, 2001, 2003; Zchori-Fein *et al.*, 2001; Hunter *et al.*, 2003), mite samples were examined using specific 16S rDNA primers (forward 5'-GGAACCTTACCTGGGCTA-GAATGTATT and reverse 5'-GAGGGTTCTTTCGG-GACGGAAT) based on the 16S rDNA of CLO from *Ixodes scapularis* (Kurtti *et al.*, 1996), *Brevipalpus phoenicis* (Weeks *et al.*, 2001) and *Encarsia* spp. (Zchori-Fein *et al.*, 2001).

Antibiotic treatment

To administer rifampicin to spider mites, small mulberry leaf discs (ca. 1 cm²) were placed on a cotton bed soaked in rifampicin (0.05%, w/v) in plastic dishes (9 cm in diameter) 1 day before the start of rearing. Newly hatched larvae were placed on the leaf discs and distilled

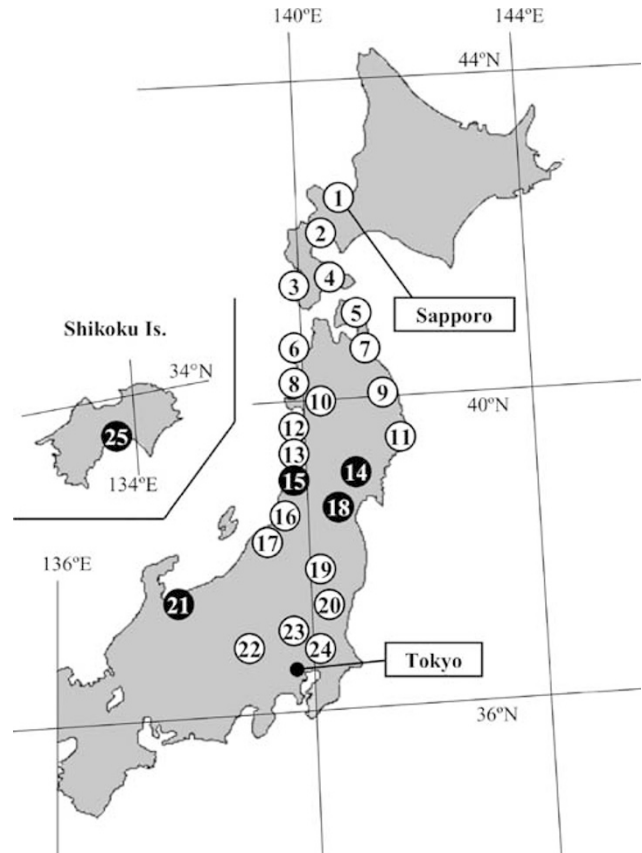


Figure 1 Collection sites and *Wolbachia* infection in *P. mori*. Closed and open circles denote *Wolbachia*-infected and *Wolbachia*-free populations, respectively.

water was added daily to keep the cotton bed wet. The cotton and the leaf discs were replaced every 4 days (see also Gotoh *et al.*, 1995) and the mites were reared under the antibiotic for one generation. Mites were checked for infection of *Wolbachia* by PCR after three generations. Offspring from adults that were found to be uninfected were allowed to mate.

Crossing experiments

In order to determine the reproductive compatibility among the 25 populations, round-robin crosses were carried out on mulberry leaves. Single females in the teleiochrysalis stage from each population were placed on small leaf discs (ca. 4 cm²) with a male adult from each of the populations. Crossing experiments were also carried out between the 25 populations and *Wolbachia*-free populations that were made by treating the *Wolbachia*-infected populations with rifampicin. The males were removed 2 days after adult emergence of the females. After oviposition started, each female was allowed to lay eggs for 5 days and then removed. Eggs on the leaf discs were checked daily to determine hatchability, survival rate and sex ratio. More than seven pairs were examined for each combination of the crosses and a total of 14480 pairs were used for 900 crosses. Experiments were carried out for 3 years from June to October when good mulberry leaves were available.

Crosses were regarded as compatible when (1) egg hatchability was $\geq 94.1\%$, (2) survival rate in immatures

was $\geq 85.0\%$ and (3) the proportion of female adults was $\geq 60.9\%$. These values correspond to the minimal rates previously obtained from intrapopulation crosses (Gotoh and Higo, 1997). Data were analyzed with either the Kruskal–Wallis test or ANOVA and the values were tested by the Scheffé’s test. Arcsin-transformed values for ANOVA were used for analyzing egg hatchability, survival rate and female ratio.

Results

Wolbachia infection

Five out of the 25 populations of *P. mori* were infected with *Wolbachia*. The primers for a 770-bp fragment of the *ftsZ* gene and an 890-bp fragment of the 16S rDNA amplified DNAs of the expected sizes in the five populations (Figure 1). These five populations were treated with rifampicin and used for crossing experiments. Hence, using 20 *Wolbachia*-uninfected populations, five *Wolbachia*-infected populations and five rifampicin-treated populations, we conducted $30 \times 30 = 900$ crosses (Figure 2). Detailed results of the crossing experiments are available at <http://www.agr.ibaraki.ac.jp/~shokubutu/gotoh/900data.pdf>, from supplementary information on Heredity website, or from the author.

Compatibility in intrapopulation crosses of the five *Wolbachia*-infected populations

Hatchability of eggs, survival rate in immature stages and female ratio were normal, even when infected males of the Sd population were crossed with *Wolbachia*-free (rifampicin-treated) Sd females (Table 1). This suggests that the *Wolbachia* strain infecting the Sd population is modification-negative.

On the other hand, the crosses between *Wolbachia*-infected and rifampicin-treated *Wolbachia*-free mites in the Hy, Ty, Hr and Tr populations showed unidirectional incompatibility; that is, crosses between infected males and uninfected females were incompatible, while the reciprocal crosses were compatible (Table 1). The reproductive incompatibility observed in these crosses was detected by a reduced hatchability and/or male-biased sex ratio. This shows that *Wolbachia* strains in the four *P. mori* populations are modification-positive.

Compatibility among *Wolbachia*-infected populations

All the crosses between the Sd and Hy populations were compatible except for the cross between the Sd R females and the Hy males, which was incompatible (top panel in Table 2). In the crosses between the Sd and Ty populations, compatibility was found only in the crosses in which females were mated with uninfected males

Female	Male	S					H					T					T																	
		d	y	r	r	r	d	y	r	r	r	p	s	n	k	d	k	h	k	m	a	u	h	k	a	s	s	z	w	r	m			
		W	W	W	W	W	R	R	R	R	R																							
Sd (18, Sendai)/W		O	O	X	X	X	O	O	O	X	X	O	O	X	X	O	O	O	X	X	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Hy (14, Hanayama)/W		O	O	O	X	X	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X
Ty (21, Toyama)/W		X	O	O	X	X	O	O	O	X	X	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hr (25, Haruno)/W		X	X	X	O	X	X	X	O	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O
Tr (15, Tsuruoka)/W		X	X	X	X	O	X	X	X	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O
Sd R		O	X	X	X	X	O	O	O	X	X	O	O	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Hy R		O	X	X	X	X	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X
Ty R		X	X	X	X	X	O	O	O	X	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hr R		X	X	X	X	X	X	X	O	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O
Tr R		X	X	X	X	X	X	X	X	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O
Sp (1, Sapporo)		X	X	X	X	X	X	X	X	X	X	O	O	O	O	X	O	O	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Os (2, Oshamanbe)		X	X	X	X	X	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
On (3, Ohnuma)		X	X	X	X	X	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Wk (5, Wakinosawa)		X	X	X	X	X	X	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Hd (4, Hakodate)		O	X	X	X	X	O	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Uk (24, Ushiku)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X
Hh (9, Hachinohe)		O	X	X	X	X	O	O	O	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Mk (11, Miyako)		O	X	X	X	X	O	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Nm (12, Nishime)		O	X	X	X	X	O	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Na (17, N-Asahi)		O	X	X	X	X	O	O	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Iu (6, Ichijura)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Nh (7, Noheji)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Kk (13, Kisakata)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Ya (16, Y-Asahi)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Fs (19, Fukushima)		O	X	X	X	X	O	O	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Hs (8, Hirosaki)		X	X	X	X	X	X	O	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Tz (10, Tazawako)		X	X	X	X	X	X	O	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Iw (20, Iwaki)		X	X	X	X	X	X	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Kr (22, Kiryu)		X	X	X	X	X	X	O	O	O	X	X	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Am (23, Ami)		X	X	X	X	X	X	X	O	O	O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O

Figure 2 Reproductive compatibility among 25 populations and five rifampicin-treated populations of *P. mori*. Circles show compatible crosses ($\geq 94.1\%$ in hatchability of eggs, $\geq 85.0\%$ in survival rate in immatures and $\geq 60.9\%$ in female ratio), and Xs show incompatible crosses. W means *Wolbachia*-infected populations, and R means rifampicin treatment. Localities are shown by abbreviations of two letters. Numbers in parentheses correspond to the site numbers appearing in Figure 1 and full locality names are also shown in parentheses. Crosses enclosed within thick lines indicate *Wolbachia*-uninfected populations whose reproductive incompatibility is mediated by *Wolbachia*, and crosses enclosed within thin lines indicate the respective reciprocal crosses of the crosses enclosed within thick lines.

Table 1 Compatibility of intrapopulation crosses for the five *Wolbachia*-infected populations of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Sd × Sd	20	32.4 ± 0.74 a	99.2 ± 0.32 b	98.7 ± 0.46	79.0 ± 0.60 c	O
Sd × Sd R	18	41.6 ± 2.03 b	97.5 ± 0.24 ab	98.6 ± 0.32	71.8 ± 1.37 b	O
Sd R × Sd	30	32.9 ± 1.07 a	96.5 ± 0.44 a	98.3 ± 0.40	66.8 ± 1.01 a	O
Sd R × Sd R	15	32.4 ± 1.60 a	96.7 ± 0.64 a	98.9 ± 0.40	77.4 ± 1.46 c	O
χ ² -value ^c		16.429***	20.273***	1.069 NS	42.847***	
Hy × Hy	15	34.9 ± 2.46 b	97.5 ± 0.78 b	97.6 ± 0.49	83.1 ± 1.29 d	O
Hy × Hy R	17	30.1 ± 1.82 ab	97.7 ± 0.58 b	97.1 ± 0.54	64.9 ± 1.56 b	O
Hy R × Hy	15	26.1 ± 1.44 a	83.8 ± 2.33 a	95.9 ± 1.04	36.3 ± 2.28 a	X
Hy R × Hy R	19	35.1 ± 1.57 b	97.6 ± 0.44 b	98.5 ± 0.38	75.8 ± 1.37 c	O
χ ² -value		15.031**	34.497***	6.689 NS	53.685***	
Ty × Ty	15	32.1 ± 0.96	97.9 ± 0.50	97.8 ± 0.63	81.6 ± 1.26 c	O
Ty × Ty R	24	32.6 ± 1.40	96.8 ± 0.52	96.5 ± 0.68	71.9 ± 1.03 b	O
Ty R × Ty	15	30.3 ± 1.60	96.5 ± 0.35	98.8 ± 0.59	25.5 ± 1.39 a	X
Ty R × Ty R	24	34.0 ± 1.28	97.7 ± 0.59	97.8 ± 0.51	72.7 ± 1.08 b	O
χ ² -value		5.508 NS	5.412 NS	6.511 NS	50.842***	
Hr × Hr	20	32.6 ± 1.06	96.1 ± 0.64	97.0 ± 0.71	70.9 ± 1.33 b	O
Hr × Hr R	18	33.9 ± 1.58	96.4 ± 0.57	98.4 ± 0.41	66.1 ± 1.01 b	O
Hr R × Hr	15	35.5 ± 1.42	97.3 ± 0.75	98.2 ± 0.40	27.2 ± 2.80 a	X
Hr R × Hr R	14	34.8 ± 1.91	98.2 ± 0.44	99.3 ± 0.40	70.4 ± 1.87 b	O
χ ² -value		2.433 NS	5.160 NS	5.699 NS	38.410***	
Tr × Tr	14	41.0 ± 2.31 b	96.7 ± 0.87	93.7 ± 0.90 a	73.6 ± 1.76 b	O
Tr × Tr R	16	32.5 ± 1.72 a	96.2 ± 0.64	98.7 ± 0.45 b	73.6 ± 1.23 b	O
Tr R × Tr	17	30.5 ± 1.50 a	96.3 ± 0.72	99.2 ± 0.38 b	16.4 ± 1.41 a	X
Tr R × Tr R	17	30.7 ± 1.18 a	96.7 ± 0.47	99.8 ± 0.19 b	77.3 ± 1.47 b	O
χ ² -value		12.362**	0.453 NS	33.717***	39.290***	

^aNumber of females tested.

^bCircles show compatible crosses and Xs incompatible ones.

^cMeans differ significantly at $P < 0.01$ (**) and $P < 0.001$ (***) (Kruskal–Wallis test); NS, not significant at $P > 0.05$. Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

(middle panel in Table 2). The Hy and Ty populations were infected with the same strain of *Wolbachia* (bottom panel in Table 2), as shown by the crosses between infected males and infected females being compatible, while it is difficult to explain the properties the *Wolbachia* strain infecting the Sd population. The Sd population seems to harbor a modification-negative (mod−) strain of *Wolbachia* based on its crosses with the Hy populations, while it seems to harbor a modification-positive (mod+) strain based on its crosses with the Ty population. Thus, these results seem to be contradictory.

Crosses between infected mites from the Hr and Tr populations were incompatible, but those between uninfected males and either infected or uninfected females were compatible (Table 3). This shows that the Hr and Tr populations were infected with different *Wolbachia* strains.

Table 4 shows the results of the crosses among the Sd, Hy, Hr and Tr populations. All the crosses between the Sd population and either the Hr or Tr population were incompatible and female ratios were significantly lower (Sd × Hr: ANOVA, $F_{9,157} = 81.327$, $P < 0.001$; Sd × Tr: ANOVA, $F_{9,154} = 154.960$, $P < 0.001$) than those in the intrapopulation crosses. Incompatibility was also ob-

served even when rifampicin-treated individuals were crossed. Similarly, the crosses between the Hy population and either the Hr or Tr population were incompatible (Table 4). The female ratios were significantly lower (Hy × Hr: ANOVA, $F_{9,148} = 94.923$, $P < 0.001$; Hy × Tr: ANOVA, $F_{9,162} = 85.145$, $P < 0.001$) than those in each intrapopulation cross. These results imply that the incompatibility observed in crosses between either the Sd or Hy population and either the Hr or Tr population is due to nuclear–nuclear interactions rather than to the microorganisms.

The Ty and Hr populations harbored different *Wolbachia* strains (Table 5), because the crosses between infected males and infected females from these two populations were incompatible but the crosses between *Wolbachia*-free males and either of *Wolbachia*-infected females or *Wolbachia*-free females were compatible. In contrast, none of the crosses between the Ty and Tr populations were compatible (Table 5), implying that the incompatibility between them is caused by nuclear factors. The female ratios in the crosses between Ty and Tr populations were significantly lower (ANOVA, $F_{9,190} = 83.539$, $P < 0.001$) than those in the intrapopulation crosses.

Table 2 Compatibility among three *Wolbachia*-infected populations of *P. mori* (Sd, Hy and Ty)

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Sd × Hy	23	25.8±1.48 a	98.7±0.50 b	93.7±1.04	76.2±0.77 c	O
Sd × Hy R	15	25.2±0.92 a	96.9±0.67 ab	96.3±0.95	66.6±1.53 b	O
Sd R × Hy	15	30.9±2.18 a	91.6±2.93 a	96.9±0.88	36.6±2.57 a	X
Sd R × Hy R	16	26.1±1.46 a	98.1±0.59 ab	97.0±0.69	67.4±1.50 bc	O
Hy × Sd (1) ^c	16	31.9±1.44 a	97.6±0.73 ab	96.3±0.64	65.0±1.30 b	O
Hy × Sd (2)	14	30.2±1.85 a	96.8±0.68 ab	93.2±1.36	71.9±1.21 bc	O
Hy × Sd R	17	26.4±1.42 a	98.0±0.64 ab	97.0±0.61	69.7±1.30 bc	O
Hy R × Sd (1)	12	23.6±0.65 a	95.8±1.19 ab	96.8±1.04	63.9±1.71 b	O
Hy R × Sd (2)	11	23.4±1.20 a	97.2±0.69 ab	94.1±1.45	67.3±1.79 bc	O
Hy R × Sd R	14	22.9±0.93 a	98.7±0.59 b	96.7±0.73	68.5±1.49 bc	O
χ ² -value ^d		33.186***	18.525*	15.687 NS	82.916***	
Sd × Ty (1)	25	32.6±0.59 ab	98.7±0.34 b	98.1±0.45 bc	7.3±0.97 a	X
Sd × Ty (2)	22	35.0±1.71 b	96.8±0.30 b	99.6±0.27 c	22.9±1.70 b	X
Sd × Ty R	16	30.8±1.68 ab	95.8±0.57 b	99.5±0.29 c	65.6±1.84 d	X
Sd R × Ty	19	33.2±1.78 ab	98.5±0.50 b	99.4±0.35 c	36.6±2.37 c	O
Sd R × Ty R	15	30.2±1.36 ab	96.7±0.60 b	99.1±0.39 c	71.3±1.66 d	O
Ty × Sd (1)	15	32.9±0.87 ab	99.2±0.35 b	97.5±0.61 bc	20.1±1.48 ab	X
Ty × Sd (2)	19	30.8±1.09 ab	96.9±0.56 b	99.3±0.33 c	29.1±2.39 bc	X
Ty × Sd (3)	16	27.9±1.38 ab	83.4±2.35 a	94.3±1.18 ab	30.3±3.48 bc	X
Ty × Sd R	17	34.4±1.68 ab	96.9±0.50 b	98.2±0.46 c	71.3±1.39 d	O
Ty R × Sd (1)	16	31.2±1.26 ab	97.2±0.55 b	98.3±0.81 c	37.3±2.98 c	X
Ty R × Sd (2)	12	25.3±1.50 a	77.9±4.90 a	93.0±1.23 a	40.0±2.09 c	X
Ty R × Sd R	14	30.8±1.83 ab	94.8±1.09 b	98.4±0.47 c	67.3±1.48 d	O
χ ² -value		29.186**	85.094***	58.336***	171.337***	
Hy × Ty	18	30.5±1.25	96.5±0.60 b	97.9±0.63 a	75.8±1.43 c	O
Hy × Ty R	18	33.2±1.68	97.3±0.42 b	97.2±0.35 a	78.1±1.32 c	O
Hy R × Ty	15	37.2±1.90	73.6±4.12 a	98.5±0.67 a	55.6±2.98 b	X
Hy R × Ty R	17	33.8±1.65	97.2±0.53 b	99.1±0.35 a	71.0±1.78 c	O
Ty × Hy	17	32.9±1.41	97.1±0.47 b	99.3±0.40 a	77.7±1.83 c	O
Ty × Hy R	21	30.6±0.89	96.5±0.61 b	98.3±0.51 a	74.1±1.21 c	O
Ty R × Hy	15	31.5±2.20	91.0±3.33 b	98.6±0.47 a	35.0±3.62 a	X
Ty R × Hy R	20	30.5±1.46	96.5±0.48 b	98.8±0.38 a	74.0±1.23 c	O
χ ² -value		11.303 NS	34.332***	17.296*	73.071***	

^aNumber of females tested.

^bCircles show compatible crosses and Xs incompatible ones.

^cNumerals in parentheses indicate the number of trials.

^dMeans differ significantly at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) (Kruskal–Wallis test); NS, not significant at $P > 0.05$.

Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

Table 3 Compatibility between the *Wolbachia*-infected Hr and Tr populations of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Hr × Tr	14	31.2±1.08 a	94.5±0.94	97.8±0.71 a	35.1±2.02 b	X
Tr × Hr	18	30.1±1.17 a	96.7±0.61	97.3±0.55 a	19.1±1.83 a	X
Hr × Tr R	19	33.2±1.51 a	97.7±0.54	98.3±0.58 a	77.4±1.52 c	O
Tr × Hr R	18	27.1±1.28 a	97.5±0.54	98.3±0.46 a	72.6±1.30 c	O
Hr R × Tr	18	32.1±1.38 a	97.6±0.52	99.5±0.30 a	18.6±2.05 a	X
Tr R × Hr	21	28.6±1.17 a	97.6±0.46	98.9±0.52 a	14.8±1.61 a	X
Hr R × Tr R	17	31.3±2.03 a	96.7±0.50	99.2±0.35 a	75.4±1.42 c	O
Tr R × Hr R	19	30.8±0.97 a	96.6±0.30	98.2±0.41 a	70.4±1.77 c	O
χ ² -value ^c		12.700*	10.739 NS	14.830*	101.448***	

^aNumber of females tested.

^bCircles show compatible crosses and Xs incompatible ones.

^cMeans differ significantly at $P < 0.05$ (*) and $P < 0.001$ (***) (Kruskal–Wallis test); NS, not significant at $P > 0.05$. Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

Table 4 Compatibility of crosses between either of the Sd or Hy populations and either of the Hr or Tr populations of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Sd × Hr	15	28.8 ± 1.69 a	95.9 ± 0.87 a	99.4 ± 0.38 c	15.8 ± 1.92 ab	X
Hr × Sd	16	31.3 ± 1.68 a	95.7 ± 0.68 a	99.0 ± 0.40 c	26.6 ± 2.42 bcd	X
Sd × Hr R	15	24.7 ± 1.53 a	96.6 ± 0.79 a	94.4 ± 1.06 ab	12.8 ± 2.13 ab	X
Hr × Sd R	17	26.4 ± 1.76 a	89.6 ± 2.87 a	96.7 ± 0.61 bc	37.1 ± 3.85 d	X
Sd R × Hr	14	26.5 ± 1.44 a	96.1 ± 1.10 a	96.1 ± 0.71 abc	10.5 ± 2.79 a	X
Hr R × Sd	17	24.5 ± 0.73 a	92.8 ± 2.13 a	94.0 ± 1.05 ab	21.6 ± 1.87 abc	X
Sd R × Hr R	15	23.9 ± 0.80 a	96.4 ± 0.81 a	96.7 ± 0.92 bc	21.3 ± 2.84 abc	X
Hr R × Sd R	18	25.3 ± 1.15 a	92.6 ± 1.70 a	92.1 ± 0.87 a	30.6 ± 2.73 cd	X
χ ² -value ^c		18.806**	14.540*	47.037***	50.372***	
Sd × Tr	15	35.1 ± 2.36	96.7 ± 0.82	98.0 ± 0.61	38.4 ± 2.30 c	X
Tr × Sd	18	32.4 ± 1.42	97.3 ± 0.76	97.9 ± 0.64	13.2 ± 1.44 a	X
Sd × Tr R	18	34.4 ± 1.74	96.6 ± 0.64	99.1 ± 0.37	37.1 ± 2.78 c	X
Tr × Sd R	16	29.3 ± 1.11	96.6 ± 0.89	98.6 ± 0.47	10.4 ± 1.13 a	X
Sd R × Tr	15	31.1 ± 1.69	95.2 ± 0.73	97.9 ± 0.57	18.1 ± 1.81 ab	X
Tr R × Sd	16	32.3 ± 1.07	96.8 ± 0.51	97.9 ± 0.50	14.4 ± 1.59 a	X
Sd R × Tr R	15	30.7 ± 0.88	96.2 ± 0.36	99.6 ± 0.28	28.1 ± 2.78 bc	X
Tr R × Sd R	17	30.2 ± 1.17	95.2 ± 0.81	99.0 ± 0.40	11.3 ± 0.97 a	X
χ ² -value		9.928 NS	11.158 NS	11.138 NS	80.320***	
Hy × Hr	16	27.7 ± 1.61 a	92.2 ± 1.83 b	96.2 ± 0.95	2.7 ± 1.37 a	X
Hr × Hy	15	27.4 ± 1.68 a	67.9 ± 4.04 a	95.7 ± 1.28	21.6 ± 3.95 b	X
Hy × Hr R	13	24.8 ± 1.44 a	96.2 ± 1.19 b	94.6 ± 1.15	13.4 ± 2.46 ab	X
Hr × Hy R	16	31.9 ± 1.44 a	94.7 ± 2.43 b	96.5 ± 0.82	47.3 ± 2.30 d	X
Hy R × Hr	14	25.9 ± 1.40 a	93.1 ± 2.92 b	95.6 ± 0.98	6.9 ± 2.36 a	X
Hr R × Hy	16	24.5 ± 0.88 a	88.0 ± 2.37 b	94.6 ± 1.19	21.2 ± 1.88 b	X
Hy R × Hr R	15	28.3 ± 1.88 a	97.5 ± 0.50 b	94.7 ± 0.91	26.4 ± 3.00 bc	X
Hr R × Hy R	18	25.8 ± 0.81 a	95.2 ± 1.03 b	95.7 ± 0.87	37.0 ± 2.35 cd	X
χ ² -value		16.820*	40.005***	3.898 NS	83.279***	
Hy × Tr	17	36.2 ± 2.03	96.5 ± 0.76 b	98.2 ± 0.35	16.7 ± 2.11 abc	X
Tr × Hy	20	28.6 ± 1.01	96.6 ± 0.55 b	98.2 ± 0.49	21.7 ± 1.87 abc	X
Hy × Tr R	18	29.4 ± 1.52	94.4 ± 1.09 b	95.5 ± 1.04	24.8 ± 3.28 abc	X
Tr × Hy R	20	30.4 ± 1.10	94.9 ± 1.47 b	98.1 ± 0.46	25.3 ± 2.89 bc	X
Hy R × Tr	16	29.5 ± 1.97	95.4 ± 0.84 b	98.3 ± 0.51	12.2 ± 0.86 a	X
Tr R × Hy	16	29.5 ± 1.47	96.5 ± 0.55 b	98.3 ± 0.50	14.4 ± 1.36 ab	X
Hy R × Tr R	16	33.6 ± 2.01	92.7 ± 2.08 b	98.3 ± 0.47	20.7 ± 2.22 abc	X
Tr R × Hy R	20	30.7 ± 1.51	76.1 ± 3.46 a	99.0 ± 0.41	27.9 ± 2.72 c	X
χ ² -value		13.179 NS	33.338***	11.972 NS	29.326***	

^aNumber of females tested.^bCircles show compatible crosses and Xs incompatible ones.^cMeans differ significantly at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) (Kruskal–Wallis test); NS, not significant at $P > 0.05$. Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

Compatibility among the 30 populations including the five rifampicin-treated populations

Reproductive incompatibility mediated by *Wolbachia* was found in crosses between 17 *Wolbachia*-uninfected populations and four infected populations (represented by the region containing Xs enclosed in a thick line in Figure 2). Females from these 17 uninfected populations were incompatible when crossed with males of *Wolbachia*-infected populations, but they were compatible when crossed with rifampicin-treated males. The reciprocal crosses between females of either *Wolbachia*-infected or rifampicin-treated populations and males of *Wolbachia*-uninfected populations were compatible (these crosses are shown by circles enclosed by a thin line in Figure 2). As the most typical examples, the crosses between two

Wolbachia-uninfected populations (Hs, Tz) and four *Wolbachia*-infected populations (Hy, Ty, Hr and Tr) are shown in Table 6. In haplodiploid species, such as spider mites, incompatibility is usually revealed by male-biased offspring with little or no egg mortality. However, in some crosses, the eggs of *P. mori* failed to hatch, as was reported in the CI of *Tetranychus* species (Breeuwer, 1997).

The *Wolbachia*-infected Sd population was bidirectionally compatible with 11 *Wolbachia*-uninfected populations (Figure 2), which raises the possibility that this population harbors a modification-negative strain of *Wolbachia* (but see below). It was considered that the remaining bidirectional incompatibility observed among *Wolbachia*-uninfected populations (shown by Xs in

Table 5 Compatibility between the *Wolbachia*-infected Ty population and either of the Hr or Tr populations of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Ty × Hr	16	30.1 ± 1.64	96.6 ± 0.52	97.5 ± 0.59 a	22.8 ± 3.05 a	X
Hr × Ty	14	33.6 ± 1.06	95.2 ± 0.85	98.1 ± 0.80 a	25.2 ± 4.34 a	X
Ty × Hr R	19	29.2 ± 1.09	96.9 ± 0.64	99.3 ± 0.32 a	70.1 ± 1.21 b	O
Hr × Ty R	18	32.0 ± 1.22	96.5 ± 0.31	99.5 ± 0.29 a	73.4 ± 2.30 b	O
Ty R × Hr	24	29.9 ± 1.01	96.6 ± 0.36	99.8 ± 0.17 a	20.0 ± 1.94 a	X
Hr R × Ty	20	32.9 ± 2.03	97.1 ± 0.92	98.9 ± 0.38 a	17.4 ± 2.60 a	X
Ty R × Hr R	18	31.6 ± 1.19	97.0 ± 0.41	99.2 ± 0.45 a	71.4 ± 1.32 b	O
Hr R × Ty R	16	33.6 ± 1.50	96.9 ± 0.47	97.6 ± 0.55 a	71.2 ± 1.76 b	O
χ ² -value ^c		12.368 NS	8.482 NS	25.636***	109.245***	
Ty × Tr	26	32.1 ± 1.39 a	95.4 ± 0.60 b	97.0 ± 0.61	42.9 ± 2.91 c	X
Tr × Ty	31	43.5 ± 1.95 b	94.4 ± 0.69 ab	97.4 ± 0.74	11.5 ± 1.13 a	X
Ty × Tr R	15	31.8 ± 1.22 a	96.8 ± 0.46 b	99.0 ± 0.48	29.7 ± 2.50 b	X
Tr × Ty R	28	30.1 ± 1.46 a	96.0 ± 0.49 b	98.4 ± 0.55	14.2 ± 1.20 a	X
Ty R × Tr	20	35.9 ± 1.56 ab	90.6 ± 1.68 a	97.8 ± 0.68	16.8 ± 2.67 a	X
Tr R × Ty	17	32.3 ± 1.56 a	96.7 ± 0.42 b	98.7 ± 0.48	13.8 ± 1.18 a	X
Ty R × Tr R	14	35.9 ± 2.79 ab	96.7 ± 0.89 b	98.9 ± 0.42	30.1 ± 2.60 b	X
Tr R × Ty R	20	29.3 ± 1.04 a	96.8 ± 0.48 b	98.3 ± 0.60	18.9 ± 1.73 ab	X
χ ² -value		37.730***	21.329**	9.236 NS	85.787***	

^aNumber of females tested.

^bCircles show compatible crosses and Xs incompatible ones.

^cMeans differ significantly at $P < 0.01$ (**) and $P < 0.001$ (***) (Kruskal-Wallis test); NS, not significant at $P > 0.05$. Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

Figure 2) was due to genetic differences, that is, nuclear genes.

Crosses between the *Wolbachia*-infected Sd population and the *Wolbachia*-uninfected Sp and Os populations

As shown in Figure 2, the Sd population resulted in unidirectional incompatibility in crosses with the Sp or Os population (as shown by four diagonal cells). The crosses between females of either the Sd or Sd R population and males of either the Sp or Os population were compatible, but the reciprocal crosses were incompatible (Table 7). The incompatibility was shown by a reduced hatchability and/or a male-biased sex ratio. The crosses with the Sp population appeared to be more incompatible than the crosses with the Os population. This incompatibility closely resembled the incompatibility observed in *Amphitetranychus quercivorus*, which was due to a nuclear–cytoplasmic interaction (Gotoh et al (1995); in that paper, *A. quercivorus* was referred to as *Tetranychus quercivorus*).

Discussion

Using 25 *P. mori* populations, we performed 900 combinations (30 × 30) of crosses, which included 600 (25 × 25–25) interpopulation crosses (= 300 (600/2) combinations of pairs of different populations (Figure 2)). The observed incompatibility in the crosses can be interpreted as the consequence of either nuclear–nuclear interactions, nuclear–cytoplasmic interactions or *Wolbachia* infection (see below). Bidirectional incompatibility caused by nuclear–nuclear interactions was observed in 99 combinations of interpopulations (99/300 = 0.33; shown by Xs enclosed in a double line excluding the

two combinations of diagonal cells); five out of 10 combinations among the five *Wolbachia*-eliminated populations ((5 × 5–5)/2) (shown by hatching), 45 out of 190 combinations among the 20 *Wolbachia*-uninfected populations ((20 × 20–20)/2) (enclosed in a dotted line) and 49 out of 100 combinations between *Wolbachia*-eliminated and -uninfected populations (5 × 20; enclosed in a dashed line in Figure 2). Although a few female progeny were produced in these crosses, nearly one-third of the combinations were incompatible, based on the nuclear background. This shows that there is genetic divergence among *P. mori* populations even when mites were collected from the same host plant, mulberry. We did not detect any geographical trends in the distribution of bidirectionally compatible populations. This is probably due to frequent transportation of mulberry trees, on which *P. mori* populations were collected, over the past 100 years as the sericulture industry was developed in Japan.

The incompatibility of the five *Wolbachia*-infected populations with the 25 different *P. mori* populations was complex. The Sd population did not show any *Wolbachia*-mediated incompatibility with the uninfected populations. The other four *Wolbachia*-infected populations (Hy, Ty, Hr and Tr) induced unidirectional incompatibility when their males were crossed with females of *Wolbachia*-uninfected populations. However, this *Wolbachia*-mediated incompatibility was not observed in all combinations of the populations between infected males and uninfected females. The Tr population, for example, was incompatible with three uninfected populations, whereas the Hy population was incompatible with 16 uninfected populations (Figure 2). This difference in the number of incompatible combinations may be due to different affinities of *Wolbachia* for

Table 6 Compatibility of crosses between males of four *Wolbachia*-infected populations (Hy, Ty, Hr and Tr) and females of two *Wolbachia*-uninfected populations (Hs and Tz) of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Hs × Hy	16	28.6 ± 1.33	94.6 ± 1.19	96.5 ± 0.73	47.5 ± 2.76	X
Hs × Hy R	12	26.6 ± 1.28	97.3 ± 0.61	94.6 ± 1.31	66.5 ± 1.57	O
Z ^c		-0.933 NS	-2.062*	-1.011 NS	-4.295***	
Tz × Hy	14	23.0 ± 0.78	95.1 ± 1.44	97.2 ± 0.86	37.7 ± 3.22	X
Tz × Hy R	15	22.9 ± 0.71	98.3 ± 0.57	95.9 ± 1.18	63.8 ± 1.53	O
Z		-0.000 NS	-1.831 NS	-0.549 NS	-4.587***	
Hs × Ty	11	31.7 ± 1.15	66.3 ± 4.47	95.8 ± 0.94	65.5 ± 2.80	X
Hs × Ty R	19	27.3 ± 1.33	96.0 ± 0.75	98.9 ± 0.46	76.5 ± 1.33	O
Z		-2.369*	-3.659***	-2.540*	-3.436***	
Tz × Ty	17	33.3 ± 0.61	56.5 ± 2.67	97.2 ± 0.77	48.4 ± 2.82	X
Tz × Ty R	16	29.3 ± 1.73	95.9 ± 0.63	98.8 ± 0.54	78.6 ± 1.35	O
Z		-2.349*	-4.901***	-1.526 NS	-4.794***	
Hs × Hr	17	30.9 ± 1.31	97.0 ± 0.57	99.3 ± 0.42	15.2 ± 1.24	X
Hs × Hr R	16	28.6 ± 1.35	97.9 ± 0.59	99.6 ± 0.29	76.3 ± 1.20	O
Z		-1.211 NS	-0.985 NS	-0.346 NS	-4.901***	
Tz × Hr	21	30.2 ± 1.22	96.7 ± 0.57	98.6 ± 0.39	19.2 ± 2.04	X
Tz × Hr R	19	29.5 ± 1.27	95.6 ± 0.64	98.8 ± 0.42	77.1 ± 1.65	O
Z		-0.354 NS	-1.034 NS	-0.302 NS	-5.405***	
Hs × Tr	15	31.3 ± 1.30	52.9 ± 2.09	96.8 ± 0.93	53.2 ± 2.27	X
Hs × Tr R	19	29.1 ± 1.20	97.0 ± 0.53	98.5 ± 0.42	70.8 ± 1.21	O
Z		-1.026 NS	-4.952***	-1.317 NS	-4.684***	
Tz × Tr	16	33.3 ± 0.64	99.2 ± 0.35	98.5 ± 0.47	39.6 ± 2.23	X
Tz × Tr R	17	29.6 ± 1.13	96.2 ± 0.43	98.4 ± 0.52	77.7 ± 1.04	O
Z		-2.386*	-3.999***	-0.200 NS	-4.901***	

^aNumber of females tested.^bCircles show compatible crosses and Xs incompatible ones.^cMeans differed significantly at $P < 0.05$ (*) and $P < 0.001$ (***) (Mann-Whitney *U* test). NS: not significant at $P > 0.05$.**Table 7** Compatibility of crosses between *Wolbachia*-infected Sd population and *Wolbachia*-uninfected Sp and Os populations of *P. mori*

Cross	n ^a	No. of eggs/female	Hatchability (%)	Survival rate in immature stages (%)	% Female offspring	Compatibility ^b
<i>Female × male</i>						
Sd × Sp	12	34.7 ± 0.54 c	99.5 ± 0.35 b	99.3 ± 0.39	63.9 ± 1.27 cde	O
Sd × Os	18	32.7 ± 0.86 bc	99.5 ± 0.27 b	97.8 ± 0.41	73.8 ± 0.59 e	O
Sd R × Sp	15	27.9 ± 1.67 ab	97.8 ± 0.61 b	97.0 ± 0.85	64.3 ± 1.70 cde	O
Sd R × Os	12	23.9 ± 0.96 a	97.6 ± 0.62 b	95.1 ± 1.16	67.4 ± 1.99 de	O
Sp × Sd	38	34.9 ± 0.87 c	45.0 ± 2.53 a	94.3 ± 1.03	7.9 ± 2.97 a	X
Sp × Sd R	11	24.3 ± 0.85 a	71.9 ± 3.52 a	95.3 ± 1.33	25.4 ± 2.71 b	X
Os × Sd	15	35.0 ± 1.37 c	99.0 ± 0.38 b	97.0 ± 0.84	51.3 ± 1.05 c	X
Os × Sd R	13	23.2 ± 0.81 a	97.9 ± 0.71 b	96.5 ± 0.92	51.7 ± 2.12 cd	X
χ ² -value ^c		73.544***	106.117***	12.966 NS	115.745***	

^aNumber of females tested.^bCircles show compatible crosses and Xs incompatible ones.^cMeans differ significantly at $P < 0.001$ (***) (Kruskal-Wallis test); NS, not significant at $P > 0.05$. Data are shown as mean ± SE. Values in a column followed by different letters are significantly different at $P < 0.05$ (Scheffé's test).

the nuclear genes of the uninfected populations; it suggests the presence of 'rescue genes' in the host genome.

The compatibilities among the five *Wolbachia*-infected populations were relatively complicated, apparently because nuclear genes of these populations also affected their compatibilities. Bidirectional compatibility was observed in crosses between Sd and Hy, and between Hy and Ty. The *Wolbachia* strain of the Hy and Ty populations was identical as far as compatibility and *wsp* sequence are concerned (Gotoh *et al.*, 2003). Crosses between Sd and Ty were bidirectionally incompatible, although their *wsp* sequences are identical. This suggests that the host genotype may influence the *Wolbachia* effect. Thus, even though *Wolbachia* have identical *wsp* sequences (Gotoh *et al.*, 2003), they may interact differently with different host genotypes, resulting in different crossability patterns, as has been reported in butterflies (Jiggins *et al.*, 2002). The contradictory results in compatibility of the Sd population are discussed below. In contrast, crosses between Sd and Ty, between Ty and Hr and between Hr and Tr became reciprocally compatible after the antibiotic treatment, indicating that the incompatibility in these populations was due to infection by a different strain of *Wolbachia*.

The Sd population was bidirectionally compatible with the Hy population as well as with the 11 *Wolbachia*-uninfected populations, but was bidirectionally incompatible with the Ty population. Furthermore, the Sd males were compatible with both Sd R and Hy R females. These results can be summarized as follows: *Wolbachia* of the Sd population behave as a modification-negative strain to the Hy population and 11 *Wolbachia*-uninfected populations, whereas the bacteria behave as a modification-positive strain only to the Ty population. The incompatibility observed in crosses between the Sd and Ty populations may be due to different intensities of the modification function included in males through an affinity between genotypes. The *wsp* gene sequences appear to rule out the possibility of double infection of *Wolbachia* against the Sd and Ty populations (Gotoh *et al.*, 2003). We also failed to detect CFB (CLO) in these populations. CFB are also maternally transmitted bacteria that cause a diverse array of reproductive manipulations, including male killing, parthenogenesis, feminization and CI in insects and mites (Hurst *et al.*, 1999; Weeks *et al.*, 2001; Zchori-Fein *et al.*, 2001; Hunter *et al.*, 2003).

Incompatibility based on the nuclear–cytoplasmic interaction was observed between Sp and Sd and between Os and Sd (Table 7). The southern Sd population showed unidirectional incompatibility when crossed with the northern Sp or Os populations; the Sp or Os females were incompatible with the *Wolbachia*-eliminated Sd males (Sd R males), but the reciprocal crosses were compatible (Table 7). The Sd population was infected with *Wolbachia* but this unidirectional incompatibility was not due to *Wolbachia*. A similar result was obtained in crosses between the Tsukuba (36°N) and Sapporo (43°N) populations of *A. quercivorus* (Gotoh *et al.*, 1995). In *A. quercivorus*, the Sapporo females were incompatible with the Tsukuba males, which resulted in a low egg hatchability and a male-biased sex ratio, whereas the reciprocal crosses were compatible and produced normal progenies with a female-biased sex ratio. This unidirec-

tional incompatibility is considered to be due to the negative interaction between the nucleus of the Tsukuba males and the cytoplasm of the Sapporo females (Gotoh *et al.*, 1995). For *P. mori*, the northern Sp and Os females were incompatible with the southern Sd and Sd R males, but the reciprocal crosses were compatible (Table 7). Although the hatchability and the sex ratio observed in the incompatible crosses were higher in *P. mori* than those in *A. quercivorus*, the nuclear–cytoplasmic interactions appear to be responsible for the unidirectional incompatibility between Sd and Sp (Os) populations.

Similar complicated crossing types in a species have mainly been reported in *Culex pipiens* (Laven, 1967; Magnin *et al.*, 1987) and *Drosophila simulans* (Mercot *et al.*, 1995; Rousset and Solignac, 1995), although the latter has been well worked out. Crossing experiments between European, African and American populations of *Culex pipiens* disclosed all types of compatibility: bidirectional compatibility, unidirectional incompatibility and bidirectional incompatibility in the crosses with other populations (Laven, 1959, 1967). A highly complicated incompatibility pattern was observed in *P. mori* along with the combination of nuclear–nuclear and nuclear–cytoplasmic interactions. The complexity of CI observed in some insect species makes it difficult to understand the mechanisms of CI clearly. Future individual approaches to the study of nuclear–nuclear incompatibility, nuclear–cytoplasmic incompatibility and CI should gradually unravel the complexity and contribute for elucidation of the mechanisms.

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