Influence of postzygotic reproductive isolation on the interspecific transmission of the paternal sex ratio chromosome in *Trichogramma*

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Abstract

The paternal sex ratio (PSR) chromosome is a supernumerary chromosome that causes the destruction of the paternal chromosome set in the first mitosis in a fertilized egg. It is known from parasitoid wasps in the genera *Nasonia* and *Trichogramma* (Hymenoptera). In these haplodiploids, the egg fertilized by sperm carrying PSR matures as a haploid male that again carries, and is capable of transmitting, the PSR chromosome. Because of its unique transmission behavior, the PSR chromosome may be easily transmitted between species. This study tests whether the interspecific transmission of PSR between *Trichogramma kaykai* Pinto and Stouthamer and *Trichogramma deion* Pinto and Oatman (Hymenoptera: Trichogrammatidae) is affected by two types of postzygotic reproductive isolation, i.e., hybrid inviability and hybrid sterility. The results show that PSR can rescue fertilized eggs that would normally be inviable in the interspecific cross and the rescued eggs develop into male offspring that carry PSR. The results suggest that the two types of postzygotic reproductive isolation have no effect on the transmission of PSR between the two *Trichogramma* species.

Introduction

The paternal sex ratio (PSR) chromosome is a parasitic B chromosome, which is not necessary for the survival of its carrier (Beukeboom & Werren, 2000; Werren & Stouthamer, 2003). The PSR chromosome is known as an extremely selfish genetic element (Werren et al., 1988). When PSR carrying sperm fertilizes an egg, it induces loss of the normal paternal chromosomes during the first mitotic division. The fertilized egg is therefore converted from a diploid egg, which would become a female, into a haploid egg, which then develops into a male harboring PSR (Reed & Werren, 1995; van Vugt et al., 2003; Werren & Stouthamer, 2003). So far, PSR chromosomes have been found naturally in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) and *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae) (Werren & Stouthamer, 2003). The bacterium *Wolbachia* is another sex ratio distorter found in the genus *Trichogramma*, which induces parthenogenesis where infected virgin females give birth to infected daughters from unfertilized eggs (Stouthamer et al., 1999; Huigens & Stouthamer, 2003). In contrast to other *Wolbachia*-infected parthenogens, infected *Trichogramma* females are able to utilize sperm when mated (Stouthamer, 1997). Therefore, when a *Wolbachia*-infected egg is fertilized by sperm harboring PSR, the egg develops into an infected male carrying PSR instead of an infected female (Werren & Stouthamer, 2003).

The PSR chromosome is capable of sometimes moving across species boundaries because of its unique mode of action (Werren & Stouthamer, 2003). The PSR chromosomes of *Nasonia* and *Trichogramma* spp. have been transmitted from their natural hosts to closely related congeners (Dobson & Tanouye, 1998; Huigens, 1998; Hulskes, 2002). The two cases of interspecific transmission of PSR in *Nasonia* and *Trichogramma* are the basis for a recent suggestion to use PSR as a potential agent for biological control of haplodiploid pests and as a vehicle for genetic engineering of haplodiploids (Werren & Stouthamer, 2003).

Two mechanisms of postzygotic reproductive isolation, hybrid inviability and sterility, can separate genetically different populations from each other (Wu & Palopoli, 1994).

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Haldane's rule suggests that either mechanism is more likely to be found in the heterogametic sex (i.e., XY and ZW) than the homogametic sex (i.e., XX and ZZ) when mating between populations/species occurs (Haldane, 1922). Several hypotheses have been proposed to explain Haldane's rule (Wu & Palopoli, 1994).

Even though Haldane's rule is only applicable to species with diplodiploid heterogametic sex determination, sterility or inviability of hybrids is observed in organisms lacking a hemizygous X (Presgraves & Orr, 1998) and in haplodiploids where females develop from fertilized (diploid) eggs and males from unfertilized (haploid) eggs. For example, in *Nasonia*, a haplodiploid wasp genus parasitizing fly pupae, the F_2 hybrid male offspring between *Nasonia vitripennis* and *Nasonia giraulti* suffer from inviability (Breeuwer & Werren, 1995). Another example is found in the *Trichogramma deion* complex, where reproductive incompatibility between two populations results in inviable female offspring, and this cross incompatibility is unidirectional, i.e., the A♀ × B♂ cross produces hybrid female offspring while the B♀ × A♂ cross does not produce hybrid female offspring (Pinto et al., 1991; Stouthamer et al., 1996).

Currently, no detailed study has been carried out on the effects that the two types of postzygotic reproductive isolation have on the interspecific transmission of PSR. In this study, we test if reproductive isolation has any effect on the interspecific transmission of PSR between two closely related, sympatric *Trichogramma* species (Pinto et al., 1997; Huigens, 2003). Here, we report that hybrid inviability and sterility between the donor and recipient do not prevent the interspecific transmission of PSR in the two *Trichogramma* species. However, the transmission efficiency is not reciprocal and may be influenced by the donor's host genetic background rather than by reproductive isolation.

Materials and methods

Trichogramma species and lines

Trichogramma deion and *T. kaykai* were originally collected from the Mojave Desert, CA, USA. In the laboratory, the two species were reared on *Ephestia kuehniella* L. (Lepidoptera: Pyralidae) eggs provided by Koppert BV (Berkel en Rodenrijs, The Netherlands) at 23 ± 1 °C, L16:D8, and 60 ± 10% r.h.

In these experiments, nine *Trichogramma* lines were used (Table 1). A *Wolbachia*-infected parthenogenetic (thelytokous) isofemale line of *T. deion* was collected from the Sidewinder Mountains, San Bernardino County, CA, USA (lab code: SW436-1), and is abbreviated to D1T. A sexual (arrhenotokous) isofemale line was derived from D1T by curing the wasps using antibiotics and is abbreviated to D1A. An infected PSR line (lab code: P38d) was obtained by interspecifically mating a D1T female with an infected *T. kaykai* PSR male (K1Tp) and is maintained in the D1T genetic background by mating a D1T female with a conspecific infected PSR male and is abbreviated to D1Tp. Uninfected PSR males were obtained by mating a D1A female with a D1Tp male and are abbreviated to D1Ap. A second sexual isofemale *T. deion* line used in these experiments was collected in Last Chance Canyon, Kern County, CA, USA (lab code: LC151), and is abbreviated to D2A.

Table 1 *Trichogramma* species and lines used for the experiments, indicating the presence of the PSR chromosome and/or *Wolbachia* in males and females of these lines

Species	Lines	Sex	*Wolbachia*	PSR
Trichogramma deion	D1A	♀ and ♂		
	D2A	♀ and ♂		
	D1T	♀	✓	
	D1Ap	♂		✓
	D1Tp	♂	✓	✓
Trichogramma kaykai	KA	♀ and ♂		
	KT	♀	✓	
	K1Ap	♂		✓
	K1Tp	♂	✓	✓

A *Wolbachia*-infected parthenogenetic isofemale line of *T. kaykai* was collected in Last Chance Canyon, Kern County, CA, USA (lab code: LC19-1), and is abbreviated to KT. A sexual isofemale line was derived from KT by antibiotic treatment and is abbreviated to KA. An infected PSR line (lab code: P38k) was maintained in the KT genetic background by mating a KT female with a conspecific infected PSR male and is abbreviated to K1Tp. Uninfected PSR males were obtained by mating a KA female with a K1Tp male and are abbreviated to K1Ap.

Determination of reproductive isolation between the two species

To test if the two species were reproductively isolated, reciprocal matings were performed between the D1A and D2A and KA lines. One day before the individuals emerged, parasitized *E. kuehniella* eggs were placed individually in glass tubes (7.5 × 1 cm). When the wasps emerged, individual virgin females were confined with heterospecific males for about 24 h. In case of intraspecific crosses, each couple was confined for about 4 h. After the mating period, the males were removed from the tubes, and the females were allowed to oviposit in 120–160 *E. kuehniella* eggs for about 72 h. When the offspring emerged, their number and sex were determined under a stereomicroscope (10 × magnification). Because of the haplodiploid sex determination system of these wasps, the outcomes of the interspecific crosses give insights in the type of prezygotic and postzygotic reproductive

Table 2 Methods used for identifying broods that show evidence of pre- and postzygotic reproductive isolation in crosses between *Trichogramma deion* and *Trichogramma kaykai*. A, B: the two lines/species that are crossed; X: the number of fertilized eggs; Y: the number of unfertilized eggs

Parents	Offspring		
♀ × ♂	No. ♀	No. ♂	Classification
Virgin A		X + Y	
A × A	X	Y	
A × B		X + Y	Evidence for prezygotic isolation
A × B	<X	Y	Evidence for incomplete postzygotic isolation
A × B		Y	Evidence for complete postzygotic isolation

isolation (see Table 2). If females that had been confined with heterospecific males only produced sons in numbers similar to the total offspring production of conspecific unmated females, we assumed that no mating had taken place. The offspring number and sex ratio of these broods were classified as cases of prezygotic reproductive isolation (Table 2). If females that had been confined with heterospecific males only produced sons in numbers similar to that of mated conspecific females, we assumed that mating had taken place and the females used the sperm to fertilize their eggs, and that these fertilized eggs died. The offspring number and sex ratio of these broods were classified as cases of complete postzygotic isolation, i.e., hybrid inviability. The production of a daughter by a female mated with a heterospecific male is evidence of hybrid formation, as female offspring develop from fertilized eggs. This case was classified as incomplete postzygotic isolation (Table 2).

Determination of hybrid status of female offspring in interspecific crosses

When an uninfected female confined with a heterospecific normal male produced female offspring, we assumed that the female offspring were hybrids. To determine if the females were indeed hybrids, we used four *Trichogramma*-specific microsatellite loci (Table 3). The temperature profile of the polymerase chain reaction (PCR) was 94 °C for 5 min (1 cycle); 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min (33 cycles) on a thermocycler (Mastercycler® gradient, Eppendorf, Hamburg, Germany).

Table 3 Primers used for detecting PSR and determining the hybrid status of female offspring produced in intraspecific crosses in *Trichogramma* spp.

Primers		Sequences
PSR		F: 5′-ATGACAATTCCGAATATGTAAC-3′
		R: 5′-ACGGTGATACGTAGCGAGAA-3′
Microsatellites	AC150	F: 5′-CATAACTCGTGCGTGTATTTTT-3′
		R: 5′-GAACTCGATTCCTTACGCGT-3′
	TGA22	F: 5′-GACTCTCGAGGTAGTGGCGG-3′
		R: 5′-GAGTTCTAGCTGTGCATTATAA-3′
	TG123	F: 5′-GACTATCCGACTCGCTTTAGC-3′
		R: 5′-CCCTCGTTGCTTTAGATTATCT-3′
	TTG49	F: 5′-GTAGTCTGGTTTTCGATTCCCA-3′
		R: 5′-TCCCCGACCTATCGATTTTCC-3′

Determination of the interspecific transmission of the PSR chromosome

To determine the efficiency of interspecific transmission of PSR, females of the three isofemale lines were mated to heterospecific PSR males. One day before the individuals of the three isofemale lines emerged, parasitized *E. kuehniella* eggs were placed individually in glass tubes (7.5 × 1 cm). On emergence, individual females were confined with heterospecific infected and uninfected PSR males for about 24 h. After the mating period, the males were removed from the tubes, and the females were allowed to oviposit in 120–160 *E. kuehniella* eggs for about 72 h.

If infected females confined with a heterospecific PSR male produced male offspring, the male offspring were assumed to harbor PSR. Females that produced all-female offspring were assumed to have remained unmated and were excluded from our analysis of the transmission efficiency.

For the uninfected line D2A, our mating procedure was modified. In this line, we could not use the offspring sex ratio to distinguish between females that had mated with a PSR male and those that remained unmated, as both of them would produce only male offspring (Table 2). Therefore, following confinement with a male, females were monitored and only those that were observed mating were used. Subsequently, their offspring were tested for the presence of PSR using PCR with PSR-specific primers (Table 3) (van Vugt, 2005). The temperature profile used was 94 °C for 2 min (1 cycle); 94 °C for 1 min, 61 °C for 1 min, and 72 °C for 1 min (35 cycles) on a thermocycler. In addition, we tested if infection with *Wolbachia* of the PSR males had an effect on the interspecific transmission by comparing the transmission efficiency of PSR via *Wolbachia*-infected PSR males with that via *Wolbachia*-uninfected ones.

Statistics

To analyze the number of offspring, t-test or analysis of variance (ANOVA) were used. Tukey's b post hoc comparison

tests were done to determine if there was variation in the number of offspring in the same maternal group. Mann–Whitney U-tests and Kruskall–Wallis tests were applied for the analysis of sex ratio and fertilization rate. The cluster analysis (Sokal & Rohlf, 1995), a method to find relatively homogeneous clusters of cases, based on the variation of the number of offspring, was applied to identify the coexistence of pre- and postzygotic isolation in interspecific crosses. All the analyses were performed using SPSS version 10.0.

Results

Hybrid formation in interspecific crosses

The results of the test for reproductive isolation between *T. kaykai* and *T. deion* are shown in Table 4. All the interspecific crosses are divided into two classes: those showing evidence of prezygotic reproductive isolation and those showing evidence of postzygotic reproductive isolation. Females that did not mate or did not fertilize any eggs are identified by all-male broods with offspring numbers similar to those produced by virgin females (cluster analysis) (Table 4).

D1A female group. In the interspecific cross between D1A♀ and KA♂, some of the fertilized eggs developed into female offspring (Table 4). The females carried the microsatellite markers of both parents. Their color phenotype was similar to the yellow color of *T. kaykai* females, while *T. deion* females were brown. These females were sterile and carried a very low complement of 1–7 eggs (n = 5) in their ovaries. Males of both species introduced into a vial containing a hybrid female were immediately attracted to, and mated with, these hybrid females within 30 min (n of hybrid♀ × D1A♂ = 8 and n of hybrid♀ × KA♂ = 11). Even though they were sterile and carried few eggs in their ovaries, they showed normal egg-laying behavior (n = 7) when given *E. kuehniella* eggs. In the D1A♀ × KA♂ cross, eight females that produced a normal brood size compared with the reference brood size of virgin D1A♀ and that of the D1A♀ × D1A♂ cross were indicative of prezygotic reproductive isolation (ANOVA, P>0.1, Tukey's b-test) (Tables 2 and 4).

In the postzygotic reproductive isolation cases, we assumed that the fertilization rate in the D1A♀ × KA♂ cross was the same as in the control D1A♀ × D1A♂ cross. The mean number of 8.8 male offspring in the D1A♀ × KA♂ cross was similar to the number of males found in the D1A♀ × D1A♂ cross (t-test, P>0.1). However, the number of female offspring differed substantially, being 24.2 in the D1A♀ × D1A♂ cross and only 3.8 in the D1A♀ × KA♂ cross. This indicates that about 84% of the fertilized eggs died during development in the interspecific cross (Table 4 and Figure 1). The relative compatibility of an interculture cross (A♀ × B♂) is defined as (mean proportion of female offspring of A♀ × B♂) × 100/(mean proportion of female offspring of A♀ × A♂) (Pinto et al., 1991; Pinto et al., 1997). In the D1A♀ × KA♂ cross the relative compatibility was 42.1%.

Table 4 Reproductive isolation between the arrhenotokous lines of *Trichogramma deion* (D1A, D2A) and *Trichogramma kaykai* (KA). Total number of offspring was compared in all the crosses that shared the same maternal line; total number of offspring followed by the same letter and sharing the same maternal line indicates no significant difference. For D1A: ANOVA, P<0.001, Tukey's b-test a and b; for D2A: ANOVA, P<0.001, Tukey's b-test c and d; for KA: ANOVA, P<0.001, Tukey's b-test e and f

	Parents		Offspring			
Classification	♀ × ♂	No. of crosses	Mean ± SD of ♀	Mean ± SD of ♂	Mean ± SD of ♀ + ♂	% males
Control	D1A♀ virgin	19	0	31.0 ± 6.6	31.0 ± 6.6a	100
	D1A × D1A	18	24.2 ± 8.2	9.2 ± 2.7	33.4 ± 8.9a	27.6
	D2A♀ virgin	20	0	23.5 ± 13.5	23.5 ± 13.5c	100
	D2A × D2A	11	18.3 ± 7.1	9.4 ± 3.8	27.6 ± 10.3c	33.9
	KA♀ virgin	19	0	33.8 ± 11.5	33.8 ± 11.5e	100
	KA × KA	18	22.1 ± 8.9	6.9 ± 5.9	29.1 ± 9.6e	23.9
Prezygotic reproductive isolation	D1A × KA	8	0	29.4 ± 8.3	29.4 ± 8.3a	100
	D2A × KA	13	0	24.9 ± 5.3	24.9 ± 5.3c	100
	KA × D1A	6	0	32.0 ± 6.0	32.0 ± 6.0e	100
	KA × D2A	2	0	30.5 ± 10.6	30.5 ± 10.6e	100
Postzygotic reproductive isolation	D1A × KA	12	3.8 ± 2.5	8.8 ± 1.8	12.6 ± 3.3b	69.5
	D2A × KA	9	0	8.7 ± 2.9	8.7 ± 2.9d	100
	KA × D1A	10	0	4.4 ± 1.8	4.4 ± 1.8f	100
	KA × D2A	8	0	4.4 ± 1.7	4.4 ± 1.7f	100

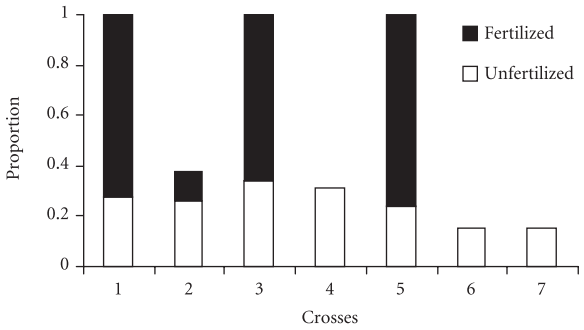

Figure 1 Estimated fertilization rate and mortality rate of fertilized eggs in the homospecific and heterospecific *Trichogramma* crosses (♀ × ♂). Cross 1: D1A × D1A; 2: D1A × KA; 3: D2A × D2A; 4: D2A × KA; 5: KA × KA; 6: KA × D1A; and 7: KA × D2A.

D2A female group. Like D1A females, in the D2A♀ × KA♂ cross, 13 out of 22 females produced only sons in numbers similar to that produced by virgin D2A♀; consequently, these 13 had not mated and were cases of prezygotic reproductive isolation (Tables 2 and 4). The remaining nine D2A females also produced no female offspring, and the number of male offspring they produced was similar to the number of male offspring produced in the D2A♀ × D2A♂ cross (t-test, P>0.1). This implies that all eggs fertilized by heterospecific sperm died (Table 4). These nine crosses were classified as cases of postzygotic reproductive isolation. In the D2A♀ × KA♂ cross, the relative compatibility was 0%.

KA female group. In the KA♀ × D1A♂ and KA♀ × D2A♂ crosses, all fertilized eggs were believed to have died (Figure 1). In these crosses, six and two females, respectively, produced normal brood sizes (cluster analysis) compared with the reference brood size of the KA♀ × KA♂ cross, indicative of prezygotic reproductive isolation (Tables 2 and 4).

The fertilization rates of the KA♀ × D1A♂ and KA♀ × D2A♂ crosses, where reproductive isolation appeared to be postzygotic, were not different from that of the KA♀ × KA♂ cross (Table 4). This was supported by the fact that there was no difference in the number of male offspring in the KA♀ × KA♂ cross and those of the KA♀ × D1A♂ and KA♀ × D2A♂ crosses (ANOVA, P>0.1) (Table 4). The KA♀ × D1A♂ and KA♀ × D2A♂ crosses both had a relative compatibility of 0%.

Interspecific transmission of PSR

D1T female group. In D1T♀ × K1Tp♂ and D1T♀ × K1Ap♂ crosses, almost all eggs fertilized by sperm carrying PSR were thought to survive because the total numbers of offspring produced in these crosses were not different from that produced by D1T♀ × D1A♂ (ANOVA, P>0.1) (Table 5 and Figure 2). There was no difference in the sex ratio and the number of offspring between the D1T♀ × K1Tp♂ and D1T♀ × K1Ap♂ crosses (Mann–Whitney U-test, P>0.1, and t-test, P>0.1, respectively). This implies that *Wolbachia* infection did not interfere with the K1Tp males' ability to transmit the PSR factor to their offspring.

Table 5 Crosses between *Trichogramma deion* and *Trichogramma kaykai* to determine the level of intra- and interspecific transmission of PSR. Total number of offspring was compared in all the crosses that shared the same maternal line; total number of offspring followed by the same letter and sharing the same maternal line indicates no significant difference. For D1T: ANOVA, P<0.001, Tukey's b-test a, b, and c; for D2A: ANOVA, P>0.1, Tukey's b-test d; for KT: ANOVA, P<0.001, Tukey's b-test e, f, and g

Parents		Offspring			
♀ × ♂	No. of crosses	Mean ± SD of ♀	Mean ± SD of ♂	Mean ± SD of ♀ + ♂	% males
D1T♀ virgin	19	19.5 ± 4.6	0	19.5 ± 4.6a	0
D1T × D1A	19	24.4 ± 5.4	0	24.4 ± 5.4b	0
D1T × D1Tp	22	19.6 ± 11.0	9.1 ± 8.1	29.7 ± 8.9c	31.5
D1T × D1Ap	11	13.1 ± 5.2	19.7 ± 5.4	32.8 ± 5.2c	60.1
D1T × K1Tp	17	4.9 ± 2.2	18.8 ± 4.3	23.7 ± 4.3b	79.4
D1T × K1Ap	15	5.1 ± 3.3	17.1 ± 4.7	22.2 ± 5.4ab	76.9
D2A♀ virgin	18	0	27.9 ± 7.3	27.9 ± 7.3d	100
D2A × D2A	18	16.7 ± 4.9	8.5 ± 2.7	25.2 ± 5.4d	33.8
D2A × K1Tp	21	0	27.1 ± 5.4	27.1 ± 5.4d	100
D2A × K1Ap	17	0	27.7 ± 9.7	27.7 ± 9.7d	100
KT♀ virgin	19	17.6 ± 3.2	0	17.6 ± 3.2e	0
KT × KA	19	26.2 ± 5.1	0	26.2 ± 5.1f	0
KT × K1Tp	22	4.6 ± 2.5	21.5 ± 6.2	26.1 ± 6.8f	82.2
KT × K1Ap	11	4.8 ± 2.2	21.0 ± 10.5	25.8 ± 10.1f	81.3
KT × D1Tp	7	4.0 ± 6.3	1.6 ± 0.8	5.6 ± 6.0 g	28.2
KT × D1Ap	7	4.3 ± 5.1	1.9 ± 1.1	6.1 ± 4.5 g	30.2

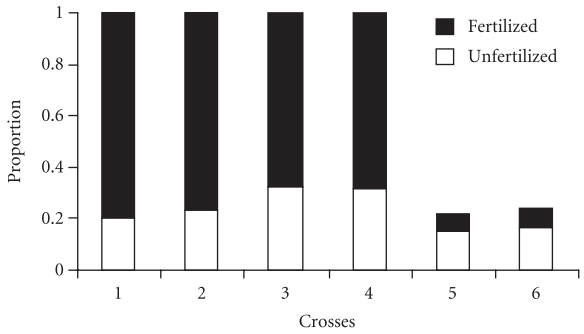

Figure 2 Transmission efficiency of PSR and mortality rate of fertilized eggs in the homospecific and heterospecific *Trichogramma* crosses (♀ × ♂). Cross 1: D1T × K1Tp; 2: D1T × K1Ap; 3: D2A × K1Tp; 4: D2A × K1Ap; 5: KT × D1Tp; and 6: KT × D1Ap. In the figure, the bars of D2A × K1Tp and D2A × K1Ap are based on the PCR results. The other bars are based on the sex ratio in Table 2.

D2A female group. The brood sizes of the D2A♀ × K1Tp♂ and D2A♀ × K1Ap♂ crosses were not different from that of D2A♀ × D2A♂, implying that all the fertilized eggs survived in the heterospecific crosses (ANOVA, $P>0.1$) (Table 5). In the D2A♀ × K1Tp♂ cross, 117 male offspring (five broods), and in the D2A♀ × K1Ap♂ cross, 123 male offspring (five broods), were used to determine the proportion of PSR among them. Of these, 78 (67.5%) and 84 (68.3%) of these male offspring, respectively, were identified as carrying PSR. The fertilization rates, i.e., the proportion of PSR, of the two crosses were not different from that of D2A♀ × D2A♂ (Kruskall–Wallis test, $P>0.1$).

KT female group. Regardless of the infection status of the heterospecific PSR males, most eggs fertilized by heterospecific PSR sperm were thought to die (ANOVA, $P<0.01$) (Table 5 and Figure 2). There was no difference in the sex ratio and the number of offspring between the KT♀ × D1Tp♂ and KT♀ × D1Ap♂ crosses (Mann–Whitney U-test, $P>0.1$, and t-test, $P>0.1$, respectively). The similarity of the transmission rate of PSR determined from the sex ratio in the KT♀ × D1Tp♂ and KT♀ × D1Ap♂ crosses implied that there was no effect of *Wolbachia* infection on the ability of the PSR males to transmit PSR and that there was no interaction effect of the different PI *Wolbachia* of the father (i.e., the *deion-Wolbachia*) and *Wolbachia* of the mother (i.e., the *kaykai-Wolbachia*).

Discussion

Reproductive incompatibility between *T. deion* and *T. kaykai* occurred at three levels: prezygotic reproductive isolation, hybrid inviability, and hybrid sterility. There was a substantial level of prezygotic reproductive isolation between these two species. For *T. deion* females, eight out of 20 D1A females and 13 out of 20 D2A females appeared not to have mated or at least did not use the sperm of the KA (*T. kaykai*) male. In the reciprocal cross, the numbers were six out of 16 when exposed to D1A males, and two out of 10 when exposed to D2A males. Postzygotic isolation generally manifested itself in the almost complete inviability of the fertilized eggs. The exception was the production of some hybrid female offspring in the D1A♀ × KA♂ cross. All these hybrid females were sterile. Because the production of hybrid females was not reciprocal, there must be some influence of cytoplasmic factors, possibly mitochondria, on allowing the hybrids to survive as suggested by Breeuwer & Werren (1995). The sterility of the hybrid females may be caused by the incompatibility of the two nuclear genomes or by some nucleocytoplasmic interaction. Another possible cause of inviability may be the inability of the paternally derived microtubule organizing centers (Tram & Sullivan, 2000) of species A to function properly in the cytoplasmic/nuclear background of species B. The mechanisms proposed here for the explanation of the hybrid females are speculative, and cytogenetic studies need to be done to understand the exact nature of the non-reciprocal hybrid formation between the two species.

We made additional crosses with three isofemale lines to test if the hybrid production by *T. deion* females in the interspecific crosses was related with the fact that the line was originally infected with *Wolbachia*. However, two out of three originally uninfected *T. deion* lines also produced females when mated with *T. kaykai* males (data not shown). Therefore, hybrid formation might occur between many lines of these species, although no hybrids were found in an earlier study (Pinto et al., 1997). Interestingly, hybrid formation was only observed in matings between allopatric populations. Crossing patterns within the species *T. deion* showed a large amount of variation in compatibility (Stouthamer et al., 1996). Geographic variation may exist in the expression of the hybrid females. More data are needed to determine if this is indeed the case.

Results showed that postzygotic reproductive isolation did not appear to have an effect on the interspecific transmission of PSR. However, in the transmission of PSR, the asymmetry between these species was pronounced. The transmission of PSR from *T. kaykai* males to *T. deion* females was more efficient than the transmission in the opposite direction, which may not be so surprising as PSR originated from *T. kaykai*. In the cross between *T. deion* PSR males and *T. kaykai* females, many of the fertilized eggs appeared to die, but in the reciprocal cross between *T. kaykai* PSR males and *T. deion* females, many of the fertilized eggs were rescued. This difference might be caused

by the inefficient transmission of PSR in *T. deion* (Jeong, 2004). Even so, in this study, about 90% of the fertilized eggs in KT female mated to D1Tp males or D1Ap males seemed to die. This mortality was very high and was most likely caused by an additional effect of the cross incompatibility between *T. kaykai* females and *T. deion* males, which was stronger than the rescuing ability of PSR.

In *Nasonia*, PSR was successfully transmitted from *N. vitripennis* to *N. giraulti* and subsequently to *N. longicornis* (Dobson & Tanouye, 1998). In that system, the donor species and recipient species produced hybrid offspring, although there was haploid hybrid inviability in the F_2 between *N. vitripennis* and *N. giraulti* (Breeuwer & Werren, 1995). The PSR chromosome was not transmitted from *N. vitripennis* to *N. longicornis* due to prezygotic reproductive isolation between the two species (van den Assem & Werren, 1994; Dobson & Tanouye, 1998).

The ability of PSR to move across species boundaries is remarkable, and therefore a PSR-like factor is considered as a potential biological control agent or as a vehicle for genetic modification in haplodiploids (Werren & Stouthamer, 2003). A PSR-like factor is expected to occur in more haplodiploid organisms than the two cases recognized so far (Werren & Stouthamer, 2003). At present, the studies of PSR in *Nasonia* and this study show that PSR can be transmitted interspecifically when there is limited prezygotic reproductive isolation between the donor and the recipient species.

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References

van den Assem J & Werren JH (1994) A comparison of the courtship and mating behavior of three species of *Nasonia* (Hymenoptera, Pteromalidae). Journal of Insect Behavior 7: 53–66.

Beukeboom LW & Werren JH (2000) The paternal-sex-ratio (PSR) chromosome in natural populations of *Nasonia* (Hymenoptera: Chalcidoidea). Journal of Evolutionary Biology 13: 967–975.

Breeuwer JAJ & Werren JH (1995) Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. Genetics 49: 705–717.

Dobson SL & Tanouye MA (1998) Interspecific movement of the paternal sex ratio chromosome. Heredity 81: 261–269.

Haldane JBS (1922) Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12: 101–109.

Huigens ME (1998) Identification of PSR-carrying *Trichogramma* species. MSc Thesis. Wageningen University, Wageningen, The Netherlands.

Huigens ME (2003) On the evolution of *Wolbachia*-induced parthenogenesis in *Trichogramma* wasps. PhD Thesis. Wageningen University, Wageningen, The Netherlands.

Huigens ME & Stouthamer R (2003) Parthenogenesis associated with *Wolbachia*. Insect Symbiosis (ed. by K Bourtzis & TA Miller), pp. 247–266. CRC Press, FL, USA.

Hulskes A (2002) The origin of the paternal sex ratio chromosome in *Trichogramma kaykai*. MSc Thesis. Wageningen University, Wageningen, The Netherlands.

Jeong G (2004) Evolutionary interactions between sex ratio distorters and their hosts. PhD Thesis. Wageningen University, Wageningen, The Netherlands.

Pinto JD, Stouthamer R & Platner GR (1997) A new cryptic species of *Trichogramma* (Hymenoptera: Trichogrammatidae) from the Mojave Desert of California as determined by morphological, reproductive and molecular data. Proceedings of Entomological Society of Washington 99: 238–247.

Pinto JD, Stouthamer R, Platner GR & Oatman ER (1991) Variation in reproductive compatibility in *Trichogramma* and its taxonomic significance (Hymenoptera: Trichogrammatidae). Annals of the Entomological Society of America 84: 37–46.

Presgraves DC & Orr HA (1998) Haldane's rule in taxa lacking a hemizygous X. Science 282: 952–954.

Reed KM & Werren JH (1995) Induction of paternal genome loss by the paternal sex ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): a comparative study of early embryonic events. Molecular Reproduction and Development 40: 408–418.

Sokal RR & Rohlf FJ (1995) Biometry, 3rd edn. Freeman, New York, NY, USA.

Stouthamer R (1997) *Wolbachia*-induced parthenogenesis. Influential passengers: inherited microorganisms and arthropod reproduction (ed. by SL O'Neill, AA Hoffmann & JH Werren), pp. 102–124. Oxford University Press, NY, USA.

Stouthamer R, Breeuwer JAJ & Hurst GDD (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annual Review of Microbiology 53: 71–102.

Stouthamer R, Luck RF, Pinto JD, Platner GR & Stephen B (1996) Non-reciprocal cross-incompatibility in *Trichogramma deion*. Entomologia Experimentalis et Applicata 80: 481–489.

Tram U & Sullivan W (2000) Reciprocal inheritance of centrosomes in the parthenogenetic hymenopteran *Nasonia vitripennis*. Current Biology 10: 1413–1419.

van Vugt JJFA (2005) Mode of action, origin and structure of the paternal sex ratio chromosome in the parasitoid wasp *Trichogramma kaykai*. PhD Thesis. Wageningen University, Wageningen, The Netherlands.

van Vugt JJFA, Salverda M, de Jong H & Stouthamer R (2003) The paternal sex ratio chromosome in the parasitic wasp

Trichogramma kaykai condenses the paternal chromosomes into a dense chromatin mass. Genome 46: 580–587.

Werren JH, Nur U & Wu CI (1988) Selfish genetic elements. Trends in Ecology and Evolution 3: 297–302.

Werren JH & Stouthamer R (2003) PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements. Genetica 117: 85–101.

Wu CI & Palopoli MF (1994) Genetics of postmating reproductive isolation in animals. Annual Review of Genetics 27: 283–308.