The origin of a selfish B chromosome triggering paternal sex ratio in the parasitoid wasp Trichogramma kaykai

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This study uses molecular and cytogenetic methods to determine the origin of a B chromosome in some males of the wasp Trichogramma kaykai. This so-called paternal sex ratio (PSR) chromosome transmits only through sperm and shortly after fertilization triggers degeneration of the paternal genome, while keeping itself intact. The resulting embryos develop into haploid B-chromosome-carrying males. Another PSR chromosome with a very similar mode of action is found in the distantly related wasp Nasonia vitripennis and its origin was traced by transposon similarity to the genus Trichomalopsis, which is closely related to Nasonia. To determine whether both PSR chromosomes have a similar origin we aimed to reveal the origin of the Trichogramma PSR chromosome. Using fluorescent in situ hybridization, we discovered a major satellite repeat on the PSR chromosome, the 45S ribosomal DNA. Analysis of the internal transcribed spacer 2 (ITS2) of this repeat showed the presence of multiple ITS2 sequences on the PSR chromosome resembling either the ITS2 of T. oleae or of T. kaykai. We therefore conclude that the Trichogramma PSR chromosome originates from T. oleae or a T. oleae-like species. Our results are consistent with different origins for the PSR chromosomes in Trichogramma and Nasonia.

Keywords: Trichogramma kaykai; PSR chromosome; B chromosome; chromosome origin; ITS2; rDNA

1. INTRODUCTION

Sex determination in the insect order Hymenoptera is largely based on ploidy level. In general diploid female zygotes develop from fertilized eggs and haploid male zygotes from unfertilized eggs. A large number of heritable factors are known that manipulate this sex determination system to favour their own transmission. These sex ratio distorters can be categorized into two classes: one where the sex ratio distorter is transmitted through females and causes a female-biased sex ratio, and another where the transmitting sex is male, resulting in a male-biased sex ratio (Werren 1987). A well-known female-biasing sex ratio distorter is the parthenogenesis-inducing bacterium Wolbachia (Stouthamer et al. 1999a). This bacterium is only transmitted cytoplasmically and causes abortion of the first mitotic anaphase in unfertilized eggs, resulting in diploid eggs that develop into females (Stouthamer & Kazmer 1994). An example of a male-biasing sex ratio distorter that reduces the ploidy level of fertilized eggs is the paternal sex ratio (PSR) chromosome (Werren et al. 1987). This B chromosome occurs in males and transmits via sperm. Shortly after fertilization, during the first mitotic division in the egg, it condenses the paternal genome into a dense chromatin mass, somehow escaping from condensation itself (Nur et al. 1988; Van Vugt et al. 2003). The egg thus develops into a haploid male with the genome from the mother and the B chromosome from the father. PSR chromosomes have been called extremely selfish because in every generation they destroy the complete chromosome set that allowed them to enter the next generation (Werren et al. 1988).

So far, B chromosomes conferring PSRs have only been found in the parasitoid wasps Nasonia vitripennis and Trichogramma kaykai (Werren & Stouthamer 2003), both belonging to the superfamily Chalcidoidea. Species of the genus Trichogramma are parasitoids of mainly butterfly and moth eggs, while Nasonia species are parasitoids of fly pupae (Whiting 1967; Pinto 1999). Although the two wasps are not closely related, their B chromosomes appear to have identical modes of action (Nur et al. 1988; Van Vugt et al. 2003), suggesting either a common B chromosome ancestor or an independent B chromosome origin with similar mechanisms for paternal genome loss.

B chromosomes originate either from autosomal chromosomes (e.g. Zea mays; Stark et al. 1996) or from sex chromosomes (e.g. Euprepocnemis plorans; López-León et al. 1994), starting as aneuploid chromosomes or as centric fragments caused by cytoplasmic or genome incompatibility (Breeuwer & Werren 1993). Most B chromosomes arise from chromosomes of their host and consequently have an intraspecific origin (Camacho et al. 2000). Some B chromosomes, however, carry non-host DNA and either originated during an interspecific hybridization (Scharl et al. 1995; Perfectti & Werren 2001) or may have been transferred interspecifically from the host of origin to a closely related species.

The origin of the PSR chromosome in N. vitripennis was elucidated by transposon analysis. Long terminal

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repeat (LTR) containing retrotransposons in this chromosome resemble those of the closely related genus *Trichomalopsis* (Chalcidoidea; McAllister 1995; McAllister & Werren 1997). In this study we clarified the origin of the *Trichogramma* PSR chromosome (Stouthamer et al. 2001) using cytogenetic techniques and DNA sequence comparisons. Fluorescent in situ hybridization (FISH) revealed the presence of 45S ribosomal DNA on the *Trichogramma* PSR chromosome. This satellite repeat occurs in tandem arrays of a few hundreds of copies in insect genomes (Long & Dawid 1980) and contains the ribosomal genes 18S, 5.8S and 28S, separated by three spacer regions. These spacer sequences vary considerably between species and are relatively conserved within species, and are therefore favourable targets for species identification. In *Trichogramma*, the internal transcribed spacer 2 (ITS2) sequence is used to discriminate closely related species, and are therefore favourable targets for species identification. In *Trichogramma*, the internal transcribed spacer 2 (ITS2) sequence is used to discriminate closely related species (Stouthamer et al. 1999b). Here, we analysed the ITS2 sequences on the *Trichogramma* PSR chromosome and discovered that this B chromosome originated in a closely related *Trichogramma* species.

2. MATERIAL AND METHODS

(a) *Trichogramma* cultures

All *Trichogramma* lines were collected in the Mojave Desert of California. LC19-1 is a *Wolbachia*-infected *T. kaykai* line, collected in 1995 in Last Chance Canyon, Kern County, and SW436-1 is a *Wolbachia*-infected *T. deion* line, collected in 1996 from the Sidewinder Mountains, San Bernardino County. The *Trichogramma* PSR chromosome originated from a *T. kaykai* male that was collected in 1997 near Danby, San Bernardino County. Strain P1A PSR LC19-1 carrying this PSR chromosome was maintained in a culture with infected LC19-1 females. The PSR chromosome was transmitted to *T. deion* in the laboratory by crossing a male from strain P1A PSR LC19-1 with a SW436-1 female. We maintained the P1A PSR SW436-1 strain in a culture from strain P1A PSR LC19-1 with a SW436-1 female. The PSR chromosome (i.e. 580 bp product on chromosomes 1 and 4, as well as a large signal on the PSR chromosome, covering about two-thirds of this chromosome (figure 1a). The *T. kaykai* ITS2 probe hybridizes to the same chromosome positions as wheat 45S rDNA (figure 1b). This does not necessarily mean *T. kaykai* ITS2 is present on the PSR chromosome, since the *T. kaykai* ITS2 probe may hybridize to an ITS2 sequence residing on the PSR chromosome.

To determine what ITS2 sequences are present on the PSR chromosome, we performed a PCR with the general ITS2 primers positioned in the conserved ribosomal genes flanking ITS2 (table 1). In principle, a single PCR product is produced, because each species generally has a common ITS2 sequence homogenized through concerted evolution. This is true for *Trichogramma* DNA without PSR (i.e. 580 bp product on *T. kaykai* DNA and 510 bp product on *T. deion* DNA), but *Trichogramma* DNA with PSR has two PCR products. The extra PCR products of approximately 500 and 580 bp on *T. kaykai* and *T. deion* DNA with PSR, respectively, suggest the PSR chromosome comprises at least two ITS2 sequences of different length. All PCR products were cloned and at least 10 plasmid clones were sequenced from each PCR product. The sequences of the PCR products of *T. kaykai* and *T. deion* without PSR are identical to the ITS2 sequences of *T. kaykai* and *T. deion*, respectively (figure 2). The extra 500 bp PCR product of *T. kaykai* with PSR consists of four previously unknown ITS2 sequences, which we named PT1 (23 clones), PT2 (2 clones), PT2-kk (2 clones) and PT3 (1 clone), with accession numbers AY845190, AY845191, AY845192 and AY845189, respectively (figure 2). PT2-kk is an ITS2 sequence of which one-third resembles PT2 and the other two-thirds resembles *T. kaykai* ITS2. The extra 580 bp PCR product of *T. deion* with PSR is identical to *T. kaykai* ITS2, while the 510 bp PCR product of *T. deion* with PSR contains two sequences, namely *T. deion* ITS2 (nine clones) and PT2-kk (one clone).

BLASTN search with PT1, PT2 and PT3 in the GenBank database of NCBI reveals PT1 and PT2 most resemble *T. oleae* ITS2 and PT3 most resembles *T. kaykai* ITS2. DNA sequence identity for PT1, PT2, PT2-kk and PT3 with closely related ITS2 sequences were calculated using two different formulae (table 2). The difference between both formulae is the gap value. The first formula weighs a gap by its size in basepairs, while in the second formula each gap, independent of its size, is weighed as one. Using either formula, PT1 and PT2 have the highest similarity with *T. oleae* ITS2 and PT3 most resembles *T. kaykai* ITS2.

The plasmid insert was sequenced using standard plasmid primers. We aligned the obtained sequences in Seqman (DNASTAR Inc.) and analysed all known ITS2 sequences in a BLASTN search (NCBI). Restriction enzyme digestion was performed with 5 U restriction enzyme and 1.5 μl reaction buffer on 10 μl PCR product in a total volume of 15 μl for 1 h at 37°C.

3. RESULTS

(a) 45S rDNA and multiple ITS2 sequences on the paternal sex ratio chromosome

FISH with the wheat 45S rDNA probe clearly shows large fluorescent signals at the ends of the short arms of the A chromosomes 1 and 4, as well as a large signal on the PSR chromosome, covering about two-thirds of this chromosome (figure 1a). The *T. kaykai* ITS2 probe hybridizes to the same chromosome positions as wheat 45S rDNA (figure 1b). This does not necessarily mean *T. kaykai* ITS2 is present on the PSR chromosome, since the *T. kaykai* ITS2 probe may hybridize to an ITS2 sequence resembling this sequence.

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Table 1. ITS2 primer combinations and their PCR programmes. F, forward primer; R, reverse primer.

<table>
<thead>
<tr>
<th>primer combination</th>
<th>primer sequences</th>
<th>PCR programme</th>
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<tr>
<td>general ITS2</td>
<td>F = TGTCAACTGCAGGACAGATG; R = GTCTTGCTGCTTGAG</td>
<td>3 min 94°C; 33x (40 s 94°C; 40 s 58°C; 45 s 72°C); 5 min 72°C</td>
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<tr>
<td>PT1</td>
<td>F = ACCGGACTGCTCCTGCAAGAG; R = AGCCAGCTATTAAATAGGGCGCG</td>
<td>3 min 94°C; 35x (1 min 94°C; 1 min 55°C; 1 min 72°C); 5 min 72°C</td>
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<tr>
<td>PT2</td>
<td>F = TAAAAACGAATCTCTGCAAGAG; R = AGCCAGCTATTAAATAGGGCGCG</td>
<td>3 min 94°C; 40x (1 min 94°C; 1 min 64°C; 1 min 72°C); 5 min 72°C</td>
</tr>
<tr>
<td>PT2-kk</td>
<td>F = TAAAAACGAATCTCTGCAAGAG; R = GTCTTGCTGCTTGAG</td>
<td>3 min 94°C; 40x (1 min 94°C; 1 min 64°C; 1 min 72°C); 5 min 72°C</td>
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<tr>
<td>T. kaykai-specific</td>
<td>F = ATCTCTGCTGCTGCTCAGGAG; R = ATTCGAGCTGGCCAATAACGC</td>
<td>3 min 94°C; 35x (1 min 94°C; 1 min 53°C; 1 min 72°C); 5 min 72°C</td>
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<tr>
<td>T. deion-specific</td>
<td>F = TCTGGGCGCTGTGCTGCTATCC; R = GGCAATTATTATTTAAAAATAGGGCG</td>
<td>3 min 94°C; 35x (1 min 94°C; 1 min 55°C; 1 min 72°C); 5 min 72°C</td>
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Figure 1. Karyogram of *T. kaykai*: (a) labelled with 45S rDNA from wheat; (b) labelled with ITS2 from *T. kaykai*.

(b) **Paternal sex ratio specificity of the new ITS2 sequences**

We designed specific primers for each ITS2 sequence to determine whether the unknown ITS2 sequences are located on the PSR chromosomes (table 1). The *T. kaykai*-specific ITS2 primers not only amplify PCR products on *T. kaykai* DNA with and without PSR, but also on *T. deion* DNA with PSR. Sequencing of all these products shows the *T. kaykai* ITS2 sequence and we can therefore conclude that the PSR chromosome has *T. kaykai* ITS2. The *T. deion*-specific ITS2 primers only generate a product on *T. deion* DNA.

The ITS2 primers unique for PT1 and PT2 amplify a product on *Trichogramma* DNA with PSR and not on *Trichogramma* DNA without PSR. Sequencing of these DNA fragments confirms they are PT1 and PT2. We therefore conclude that PT1 and PT2 are located on the *Trichogramma* PSR chromosome only.

The PT2-kk specific primers produce bands on *T. kaykai* and *T. deion* DNA with PSR and on *T. deion* DNA without PSR, which would imply the presence of PT2-kk on the PSR chromosome and the *T. deion* genome. However, sequencing of the products on *T. deion* DNA with and without PSR resulted only in the *T. deion* ITS2 sequence. Furthermore, restriction enzyme digestion with DraI, which digests PT2-kk and not *T. deion* ITS2, shows that the products on *T. deion* DNA are not digested and the products on *T. kaykai* DNA with PSR are digested. Apparently, the PT2-kk primers are not specific for PT2-kk, because they also amplify *T. deion* ITS2. Still we can conclude from the results on *T. kaykai* DNA with PSR that PT2-kk is present on the *Trichogramma* PSR chromosome. Alternatively, PT2-kk may be the result of a chimeric PCR product when both the *T. kaykai* ITS2 and the PT2 ITS sequences are present in a template.

The PT3-specific primers generate PCR products on *T. kaykai* DNA with and without PSR, and on *T. deion* DNA with PSR, while a weak product that varies in size is observed on *T. deion* DNA without PSR. These results suggest that PT3 is present on both the PSR chromosome and the *T. kaykai* genome. Sequencing of PCR products from *T. kaykai* DNA with and without PSR, and from *T. deion* DNA with PSR, reveals the PT3 sequence. We were not able to sequence the PCR product from *T. deion* DNA without PSR, because the product was too weak and the size of the product varied too much. Restriction enzyme digestions of the *T. kaykai* PCR products with and without PSR with EcoRI and XmnI results in the PT3 digestion pattern for both the products. These results prove that PT3 is present on both the PSR chromosome and the genome of *T. kaykai*.

4. DISCUSSION

Extensive studies on rDNA in B chromosomes have demonstrated that a large number of B chromosomes contain 45S rDNA cistrons (Maluszynska & Schweizer 1989; López-León et al. 1994, 1999; Jones 1995; Donald et al. 1997; Cabrero et al. 1999; Stitou et al. 2000; Dhar et al. 2002; Szczepula & Switonski 2003).
There are a number of possible explanations for this phenomenon: (i) 45S rDNA is more prone to chromosome breakage, possibly because its location is usually at the end of a chromosome; (ii) 45S rDNA may be susceptible to meiotic isolation, because it has little or no crossing-overs, disjoins later in anaphase I and has a different timing of expression than the rest of the genome; (iii) B chromosomes without 45S rDNA may arise, but then easily obtain hypertransposable rDNA sequences (Schubert & Wobus 1985; Beukeboom 1994; Jones 1995).

The ITS2 sequence has been examined in previous studies for three B chromosomes containing 45S rDNA. Two of them differed in only 2 bp (0.9%) from the ITS2 sequence on their host genome (Donald et al. 1997; Marschner et al. 2007), whereas in Crepis capillaries two ITS2 sequences on the B chromosome were found that differed in 11 bp (4.8%) and 14 bp (6.1%) from the ITS2 sequence on the A chromosome (Leach et al. 2006). Here we show that the PSR chromosome in T. kaykai has at least four ITS2 sequences that are not only very different from the host ITS2, but also from any ITS2 sequence. These data imply that the ITS2 sequences on the PSR chromosome either change very fast or that they had a very long time to change.

The presence of PT1 and PT2 on the PSR chromosome suggests that these ITS2 sequences originated from T. oleae. Trichogramma kaykai and T. oleae both belong to the T. deion/T. pretiosum species complex (Pinto et al. 1986, 1993, 1997; Pinto 1999; Stouthamer et al. 1999b). Geographically, however, both species are very distant from each other. Trichogramma oleae has so far only been reported in Tunisia, former Yugoslavia and France (Vogelé & Pointel 1979; Schilthuizen & Stouthamer 1997), while T. kaykai has only been reported in the Mojave Desert (Pinto 1999). Trichogramma pretiosum and T. near pretiosum overlap geographically with T. kaykai (Pinto et al. 1986, 1993, 1997; Stouthamer et al. 1999b) and their ITS2 resemble PT1 and PT2 second-most. It is therefore tempting to consider that T. pretiosum or T. near pretiosum is linked to the origin of the Trichogramma PSR chromosome. Another possibility is that an unknown or extinct Trichogramma species was involved in the origin of this PSR chromosome.

If the PSR chromosome originated in intraspecific hybridization, it must have originated in a T. oleae-like species. Later the PSR chromosome would then have been transferred from this species to T. kaykai via an interspecific cross during which the PSR chromosome could have helped to overcome genome incompatibility.

Figure 2. Alignment of Trichogramma ITS2 sequences. Identities are denoted by dots. A dash denotes a gapped position. The T. pretiosum ITS2 sequence is from strain PRV4 from Riverside, CA (accession number U76226; Pinto et al. 1997). The T. oleae ITS2 sequence is from strain ‘Tunisia’ (accession number U74601; Schilthuizen & Stouthamer 1997). Restriction enzyme digestion sites: diamond is DdeI (TTTAAA), arrow is EcoRI (G|AATTCC), asterisk is XmnI (GAANN|NNTTCC).
If so, the presence of PT2-kk, PT3 and T. kaykai ITS2 on the PSR chromosome indicates that this chromosome has obtained or is still obtaining parts of the genomic DNA from T. kaykai. The PSR chromosome could also have originated in an interspecific cross between T. kaykai and a T. oleae-like species, possibly triggered by cytoplasmic or genome incompatibility. Even then it appears more probable that only the T. oleae-like species contributed to the B chromosome origin, because the autosomes of T. kaykai would probably have remained intact.

In general only one ITS2 sequence is present on a chromosome and usually also in a complete genome, caused by intra- and interlocus homogenization, respectively (Dover 1982; Elder & Turner 1995). However, on the PSR chromosome at least five ITS2 sequences are found. Does this mean the PSR chromosome is still in the process of homogenization to a single ITS2 sequence or is there no ITS2 homogenization on this chromosome? The fact that PT2-kk consists of both PT2 and T. kaykai ITS2, and the presence of multiple ITS2 sequences, informs us that DNA rearrangements and changes occur on the PSR chromosome. A possible explanation is that these rearrangements and changes occur faster than homogenization. Possible absence of homogenization could be explained if the rDNA on the B chromosome is inactive, as shown in Nicotiana tabacum, where homogenization of rDNA only occurs when the rDNA is active during interphase (Lim et al. 2000). Our previous observation that only the T. kaykai ITS2 on the PSR chromosome is transcribed, whereas the other ITS2 sequences are inactive and PT3 is only weakly transcribed, might explain the absence of ITS2 homogenization on this chromosome (Van Vugt et al. 2005).

The ITS2 sequences on the PSR chromosomes of Trichogramma do not resemble Nasonia ITS2. Also the Nasonia PSR-specific transposons and repetitive sequences are not located on the Trichogramma PSR chromosome (J. F. A. Van Vugt, unpublished data). From this we conclude that both PSR chromosomes have a different structure and therefore probably a different origin. The independent origin of two B chromosomes with a similar mode of paternal genome loss suggests either the existence of a simple molecular mechanism, like the involvement of only a single gene or chromosome part, or the existence of multiple possibilities to achieve paternal genome loss in the fertilized egg. Future studies should focus on the discovery of the exact molecular mechanisms of both PSR chromosomes.

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REFERENCES


Table 2. DNA sequence identity of unknown ITS2 sequences. Percentage of DNA sequence identity of PT1, PT2, PT2-kk and PT3 with closely related Trichogramma ITS2 sequences calculated with two formulae: Every gap equals its size in bp (A) % identity = no. of similar bp/(no. of similar bp + no. of gaps bp + no. of mismatches bp) × 100 and every gap equals 1 (B) % identity = no. of similar bp/(no. of similar bp + no. of gaps + no. of mismatches bp) × 100.

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<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>PT1</td>
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</tr>
<tr>
<td>PT2</td>
<td>T. kaykai</td>
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<td>PT2-kk</td>
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</tr>
<tr>
<td>PT3</td>
<td>T. kaykai</td>
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<td>B</td>
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