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

Joke J. F. A. Van Vugt^{1,†}, Hans de Jong² and Richard Stouthamer^{3,*}

¹Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands

²Laboratory of Genetics, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

³Department of Entomology, University of California, Riverside, CA 92521, USA

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. *Eyprepocnemis 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 (Schartl *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

* Author for correspondence (richard.stouthamer@ucr.edu).

† Present address: Department of Molecular Biology, NCMLS, Radboud University, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

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 (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 with infected SW436-1 females. All wasps were reared at $23 \pm 1^\circ\text{C}$ with a light–dark period of 18 h and 6 h. Freshly emerged females were provided with egg-sheets containing *Mamestra brassicae* eggs or irradiated *Ephestia kuehniella* eggs.

(b) Chromosome spreading, probe labelling and fluorescent *in situ* hybridization

Three- to five-day-old *Trichogramma* larvae were dissected from *M. brassicae* eggs and fixed, and the cells were spread as described by Van Vugt *et al.* (2005).

For labelling the 45S rDNA probe we used the standard nick-translation protocol (Roche) with biotin-16-dUTP (Boehringer) on the pTa71 plasmid containing wheat 45S rDNA (Gerlach & Bedbrook 1979). The ITS2 probe was made by PCR labelling with biotin-16-dUTP using 1 μl Chelex extraction (see below) from a *T. kaykai* female as template and the general ITS2 primers (table 1). We used the FISH protocol and image analysis of Van Vugt *et al.* (2005).

(c) Molecular techniques

DNA extraction of individual wasps using Chelex-100 and PCR amplification on 1 μl DNA extract was performed according to Huigens *et al.* (2004). The primers and their corresponding PCR programmes are shown in table 1. PCR products were extracted from agarose (gel extraction kit from QIAGEN), cloned into the pGEM-T vector (Invitrogen) and transformed into XL-2 Blue cells (Stratagene).

The plasmid insert was sequenced using standard plasmid primers. We aligned the obtained sequences in Seqman (DNASTAR Inc.) and analysed all unknown 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.

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 with 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.

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

primer combination	primer sequences	PCR programme
general ITS2	F = TGTCAACTGCAGGACACATG; R = GTCTTGCCTGCTCTGAG	3 min 94°C; 33x (40 s 94°C; 40 s 58°C; 45 s 72°C); 5 min 72°C
PT1	F = ACCCGACTGCTCTCTCGCAAGAG; R = AGCCAGCTATAAAAATAGCGCGCG	3 min 94°C; 35x (1 min 94°C; 1 min 55°C; 1 min 72°C); 5 min 72°C
PT2	F = TAAAAACGAACCTCTCTCGCAAGAG; R = AGCCAGCTATAAAAATAGCGCGCG	3 min 94°C; 40x (1 min 94°C; 1 min 64°C; 1 min 72°C); 5 min 72°C
PT2-kk	F = TAAAAACGAACCTCTCTCGCAAGAG; R = GTCTTGCCTGCTCTGAG	3 min 94°C; 35x (1 min 94°C; 1 min 57°C; 1 min 72°C); 5 min 72°C
PT3	F = CTCTCTACCACCTATCTCTTGC; R = ATTCGAGCTGGCCAATAACACGC	3 min 94°C; 40x (1 min 94°C; 1 min 64°C; 1 min 72°C); 5 min 72°C
<i>T. kaykai</i> -specific	F = ATCTCTTGCTGCTCTCGAGAGG; R = CACACGATAATGATAAACACGCG	3 min 94°C; 35x (1 min 94°C; 1 min 53°C; 1 min 72°C); 5 min 72°C
<i>T. deion</i> -specific	F = TCTGGGCGCTCGTGTCGCTATC; R = GGCCATTATTATAAAAAATAGCGCGC	3 min 94°C; 35x (1 min 94°C; 1 min 55°C; 1 min 72°C); 5 min 72°C

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* 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; Szczerbal & Switonski 2003).

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 *Dra*I (TTT|AAA), arrow is *Eco*RI (G|AATTC), asterisk is *Xmn*I (GAANN|NNTTC).

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 that on the A chromosomes (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 (Voegelé & 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.

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) $\times$ 100 and *every gap equals 1* (B) % identity = no. of similar bp/(no. of similar bp + no. of gaps + no. of mismatches bp) $\times$ 100.

name ITS2	found in PSR species	identity (%)				
		<i>T. kaykai</i>	<i>T. deion</i>	<i>T. oleae</i>	<i>T. pretiosum</i>	<i>T. nr. pretiosum</i>
A						
PT1	<i>T. kaykai</i>	74.2	87.3	94.5	91.7	91.4
PT2	<i>T. kaykai</i>	72.9	84.2	91.8	89.7	90.0
PT2-kk	<i>T. kaykai</i> , <i>T. deion</i>	80.6	80.7	84.3	84.9	83.8
PT3	<i>T. kaykai</i>	71.6	64.1	70.6	67.8	68.7
B						
PT1	<i>T. kaykai</i>	88.5	94.1	97.7	96.2	97.1
PT2	<i>T. kaykai</i>	89.0	92.6	96.8	95.3	96.3
PT2-kk	<i>T. kaykai</i> , <i>T. deion</i>	94.3	89.2	91.9	91.3	91.0
PT3	<i>T. kaykai</i>	94.1	88.5	90.4	88.0	87.8

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. J. F. A. Van Vugt 2001, 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.

The Dutch Science Foundation (NWO-ALW 810.34.006) is acknowledged for their financial support.

REFERENCES

- Beukeboom, L. W. 1994 Bewildering Bs: an impression of the 1st B-chromosome conference. *Heredity* **73**, 328–336. (doi:10.1038/hdy.1994.140)
- Breeuwer, J. A. J. & Werren, J. H. 1993 Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics* **135**, 565–574.
- Cabrero, J., López-Léon, M. D., Bakkali, M. & Camacho, J. P. M. 1999 Common origin of B chromosome variants in the grasshopper *Eyprepocnemys plorans*. *Heredity* **83**, 435–439. (doi:10.1038/sj.hdy.6885960)
- Camacho, J. P. M., Sharbel, T. F. & Beukeboom, L. W. 2000 B-chromosome evolution. *Phil. Trans. R. Soc. Lond. B* **355**, 163–178. (doi:10.1098/rstb.2000.0556)
- Dhar, M. K., Friebe, B., Koul, A. K. & Gill, B. S. 2002 Origin of an apparent B chromosome by mutation, chromosome fragmentation and specific DNA sequence amplification. *Chromosoma* **111**, 332–340. (doi:10.1007/s00412-002-0214-4)
- Donald, T. M., Houben, A., Leach, C. R. & Timmis, J. N. 1997 Ribosomal RNA genes specific to the B chromosomes in *Brachycome dichromosomatica* are not transcribed in leaf tissue. *Genome* **40**, 674–681. (doi:10.1139/g97-089)
- Dover, G. 1982 Molecular drive: a cohesive mode of species evolution. *Nature* **299**, 111–117. (doi:10.1038/299111a0)
- Elder, J. F. & Turner, B. J. 1995 Concerted evolution of repetitive DNA sequences in eukaryotes. *Quart. Rev. Biol.* **70**, 297–320.
- Gerlach, W. L. & Bedbrook, J. R. 1979 Cloning and characterisation of ribosomal RNA genes from wheat and barley. *Nucl. Acids Res.* **7**, 1869–1885. (doi:10.1093/nar/7.7.1869)
- Huigens, M. E., Almeida, R. P., de Boons, P., Luck, R. F. & Stouthamer, R. 2004 Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc. R. Soc. Lond. B* **271**, 509–516. (doi:10.1098/rspb.2003.2640)
- Jones, R. N. 1995 Tansley review no. 85 B chromosomes in plants. *New Phytol.* **131**, 411–434. (doi:10.1111/j.1469-8137.1995.tb03079.x)

- Leach, C. R., Houben, A., Field, B., Pistrick, K., Demidov, D. & Timmis, J. N. 2006 Molecular evidence for transcription of genes on a B chromosome in *Crepis capillaris*. *Genetics* **171**, 269–278. (doi:10.1534/genetics.105.043273)
- Lim, K. Y., Kovarik, A., Matyasek, R., Bezdek, M., Lichtenstein, C. P. & Leitch, A. R. 2000 Gene conversion of ribosomal DNA in *Nicotiana tabacum* is associated with undermethylated, decondensed and probably active gene units. *Chromosoma* **109**, 161–172.
- Long, E. O. & Dawid, I. B. 1980 Repeated genes in eukaryotes. *Ann. Rev. Biochem.* **49**, 727–764. (doi:10.1146/annurev.bi.49.070180.003455)
- López-León, M. D., Neves, N., Schwarzach, T., Heslop-Harrison, J. S., Hewitt, G. M. & Camacho, J. P. M. 1994 Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. *Chrom. Res.* **2**, 87–92. (doi:10.1007/BF01553487)
- López-León, M. D., Cabrero, J. & Camacho, J. P. M. 1999 Unusually high amount of inactive ribosomal DNA in the grasshopper *Stauroderus scalaris*. *Chrom. Res.* **7**, 83–88. (doi:10.1023/A:1009270310092)
- Maluszynska, J. & Schweizer, D. 1989 Ribosomal RNA genes in *Crepis capillaris* detected by non-radioactive *in situ* hybridization. *Heredity* **62**, 59–65. (doi:10.1038/hdy.1989.8)
- Marschner, S., Meister, A., Blattner, F. R. & Houben, A. 2007 Evolution and function of B chromosome 45S rDNA sequences in *Brachycome dichromosomatica*. *Genome* **50**, 638–644. (doi:10.1139/G07-048)
- McAllister, B. F. 1995 Isolation and characterization of a retroelement from B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. *Insect Mol. Biol.* **4**, 253–262. (doi:10.1111/j.1365-2583.1995.tb00031.x)
- McAllister, B. F. & Werren, J. H. 1997 Hybrid origin of a B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. *Chromosoma* **106**, 243–253. (doi:10.1007/s004120050245)
- Nur, U., Werren, J. H., Eickbush, D. G., Burke, W. D. & Eickbush, T. H. 1988 A 'selfish' B chromosome that enhances its transmission by eliminating the paternal genome. *Science* **240**, 512–514. (doi:10.1126/science.3358129)
- Perfecti, F. & Werren, J. H. 2001 The interspecific origin of B chromosomes: experimental evidence. *Evolution* **55**, 1069–1073. (doi:10.1554/0014-3820(2001)055 [1069: TIOOBC]2.0.CO;2)
- Pinto, J. D. 1999 Systematics of the North American species of *Trichogramma* (Hymenoptera: Trichogrammatidae). *Mem. Entomol. Soc. Washington* **22**, 1–287.
- Pinto, J. D., Oatman, E. R. & Platner, G. R. 1986 *Trichogramma pretiosum* and a new cryptic species occurring sympatrically in Southwestern North America (Hymenoptera: Trichogrammatidae). *Ann. Entomol. Soc. Am.* **79**, 1019–1028.
- Pinto, J. D., Platner, G. R. & Sassaman, C. A. 1993 Electrophoretic study of two closely related species of North American *Trichogramma*: *T. pretiosum* and *T. deion* (Hymenoptera: Trichogrammatidae). *Ann. Entomol. Soc. Am.* **86**, 702–709.
- Pinto, J. D., Stouthamer, R. & Platner, G. R. 1997 A new cryptic species of *Trichogramma* (Hymenoptera: Trichogrammatidae) from the Mojave Desert of California as determined by morphological, reproductive and molecular data. *Proc. Entomol. Soc. Washington* **99**, 238–247.
- Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J., Schmid, M. & Parzefall, J. 1995 Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**, 68–71. (doi:10.1038/373068a0)
- Schilthuizen, M. & Stouthamer, R. 1997 Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. *Proc. R. Soc. Lond. B* **264**, 361–366. (doi:10.1098/rspb.1997.0052)
- Schubert, I. & Wobus, U. 1985 *In situ* hybridization confirms jumping nucleolus organizing regions in *Allium*. *Chromosoma* **92**, 143–148. (doi:10.1007/BF00328466)
- Stark, E. A., Connerton, I., Bennett, S. T., Barnes, S. R., Parker, J. S. & Forster, J. W. 1996 Molecular analysis of the structure of the maize B-chromosome. *Chrom. Res.* **4**, 15–23. (doi:10.1007/BF02254939)
- Stitou, S., De La Guardia, R. D., Jiménez, R. & Burgos, M. 2000 Inactive ribosomal cistrons are spread throughout the B chromosomes of *Rattus rattus* (Rodentia, Muridae): implications for their origin and evolution. *Chrom. Res.* **8**, 305–311. (doi:10.1023/A:1009227427575)
- Stouthamer, R. & Kazmer, D. J. 1994 Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* **370**, 317–327.
- Stouthamer, R., Breeuwer, J. A. J. & Hurst, G. D. D. 1999a *Wolbachia pipiensis*: microbial manipulator of arthropod reproduction. *Ann. Rev. Microbiol.* **53**, 71–102. (doi:10.1146/annurev.micro.53.1.71)
- Stouthamer, R., Hu, J., Van Kan, F. J. P. M., Platner, G. R. & Pinto, J. D. 1999b The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *Biocontrol* **43**, 421–440. (doi:10.1023/A:1009937108715)
- Stouthamer, R., Van Tilborg, M., De Jong, J. H., Nunney, L. & Luck, R. F. 2001 Selfish element maintains sex in natural populations of a parasitoid wasp. *Proc. R. Soc. Lond. B* **268**, 617–622. (doi:10.108/rspb.2000.1404)
- Szczerbal, I. & Switonski, M. 2003 B chromosomes of the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides* Gray) contain inactive NOR-like sequences. *Caryologia* **56**, 213–216.
- Van Vugt, J. J. F. A., Salverda, M., De Jong, J. 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. (doi:10.1139/g03-044)
- Van Vugt, J. J. F. A., De Nooijer, S., Stouthamer, R. & De Jong, H. 2005 NOR activity and repeat sequence characterization of the paternal sex ratio chromosome of the parasitoid wasp *Trichogramma kaykai*. *Chromosoma* **114**, 410–419.
- Voegelé, J. & Pointel, G. 1979 *Trichogramma oleae*, n. sp., espèce jumelle de *Trichogramma evanescens* Westwood (Hym., Trichogrammatidae). *Ann. Societe Entomol. de France* **15**, 643–648.
- Werren, J. H. 1987 The coevolution of autosomal and cytoplasmic sex ratio factors. *J. Theoret. Biol.* **124**, 317–334. (doi:10.1016/S0022-5193(87)80119-4)
- Werren, J. H. & Stouthamer, R. 2003 PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements. *Genetica* **117**, 85–101. (doi:10.1023/A:1022368700752)
- Werren, J. H., Nur, U. & Eickbush, D. 1987 An extrachromosomal factor which causes loss of paternal chromosomes. *Nature* **327**, 75–76. (doi:10.1038/327075a0)
- Werren, J. H., Nur, U. & Wu, I. 1988 Selfish genetic elements. *Trends Ecol. Evol.* **3**, 297–302. (doi:10.1016/0169-5347(88)90105-X)
- Whiting, A. R. 1967 The biology of the parasitic wasp *Mormoniella vitripennis* (= *Nasonia vitripennis*). *Quart. Rev. Biol.* **43**, 333–406.