Selfish element maintains sex in natural populations of a parasitoid wasp

R. Stouthamer¹*, M. van Tilborg¹, J. H. de Jong², L. Nunney³ and R. F. Luck⁴

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

²Department of Plant Sciences, Laboratory of Genetics, Wageningen University, Dreijenlaan 2, 6703HA Wageningen, The Netherlands

³Department of Biology, and ⁴ Department of Entomology, University of California, Riverside, CA 92521, USA

Genomic conflicts between heritable elements with different modes of inheritance are important in the maintenance of sex and in the evolution of sex ratio. Generally, we expect sexual populations to exhibit a 1:1 sex ratio. However, because of their biology, parasitoid wasps often exhibit a female-biased sex ratio. Sex-ratio distorters can further alter this optimum, sometimes leading to the complete loss of sexual reproduction. In the parasitoid wasp *Trichogramma kaykai* ca. 4–26% of females in field populations are infected with a bacterial sex-ratio distorter, *Wolbachia*, allowing virgin mothers to produce daughters. In some micro-Hymenoptera these infections have led to the complete loss of sex, but in field populations of *T. kaykai* the proportion of individuals infected remains relatively stable. We tested several hypotheses to explain this low infection level, including inefficient and horizontal transmission of *Wolbachia*, suppressor genes negating the effect of *Wolbachia* and the presence of male-biasing sex-ratio distorters. Here, a male-biasing sex-ratio distorter, a parasitic B chromosome, causing females to produce only sons, keeps the frequency of *Wolbachia* low. The male-biasing factor of *T. kaykai* is the second known case of a B chromosome manipulating the reproduction of a parasitoid wasp.

Keywords: parthenogenesis; paternal sex ratio; *Wolbachia*; *Trichogramma*; B chromosome; sex-ratio distorters

1. INTRODUCTION

In parasitoid wasps, a female-biased sex ratio is favoured when two conditions are satisfied: first, that one or a few females lay their clutches of eggs in small patches, and, second, that the newly eclosed offspring mate among themselves within a patch before dispersing (Hamilton 1967). These conditions are generally satisfied by tiny wasps in the genus *Trichogramma*, which specialize in parasitizing the eggs of Lepidoptera (Pinto 1999). While a female-biased sex ratio is typical of this genus, a number of these taxa are parthenogenetic (Pinto 1999). In the majority of cases, these taxa are parthenogenetic because bacteria of the genus *Wolbachia* (Rousset *et al.* 1992; Stouthamer *et al.* 1993) infect them. This infection can be cured by heat treatment or antibiotics (Stouthamer *et al.* 1990), resulting in the appearance of males.

The success of parthenogenesis-inducing (PI) *Wolbachia* stems from their ability to exploit the haplodiploid sex-determining mechanism of micro-Hymenoptera. Males normally arise from unfertilized, haploid eggs, whereas females arise from diploid eggs. *Wolbachia* enhance their cytoplasmic transmission by converting unfertilized eggs (sons) into diploid daughters, through a modification of the first mitotic division (Stouthamer & Kazmer 1994). As a result, PI *Wolbachia* are expected to spread through a population, leading to the loss of sexual reproduction, provided that the infection is passed on faithfully and that infected mothers produce more daughters than uninfected mothers (Stouthamer 1997). This phenomenon is not restricted to *Trichogramma* and has been found in a wide range of micro-Hymenoptera taxa (Stouthamer 1997). In

most known cases of PI *Wolbachia* infection, more than 99% of the population consists of infected females and many of these species are completely parthenogenetic (Stouthamer 1997).

In contrast to this general rule, PI *Wolbachia* infection in some *Trichogramma* populations is at a low level. In these populations, infected and uninfected individuals coexist and interbreed. Infected females can mate and incorporate their mate's genome into daughters produced from fertilized eggs while their unfertilized eggs produce homozygous daughters resulting from the effects of *Wolbachia*; these daughters inherit only maternal genes. One such example is *Trichogramma kaykai* from the Mojave Desert in California (Pinto *et al.* 1997) where populations have infection levels of 6–26% (Stouthamer & Kazmer 1994). Here, we present the results of a study to determine what factor prevents the spread of PI *Wolbachia* in these populations, a spread that is expected to occur and lead to the loss of sexual reproduction.

T. kaykai is a minute parasitic wasp that oviposits in eggs of a lyceanid butterfly, *Apodemia mormo deserti*. It usually lays a clutch of four or five eggs per butterfly egg and *Wolbachia* infection appears to have little influence on clutch size. While infected females produce only daughters, uninfected females generally produce a female-biased clutch consistent with Hamilton's model (Hamilton 1967), yielding sex ratios ranging from 22–29% males (Stouthamer & Kazmer 1994). In this system, we would expect the PI *Wolbachia* infection to spread and sexual reproduction to be lost. Several processes potentially stabilize the proportions of infected and uninfected individuals. These include inefficient transmission of the bacterium, the evolution of a suppressor gene that kills the bacterium or negates its effect, and the presence of some other non-Mendelian balancing factor.

*Author for correspondence (richard.stouthamer@users.ento.wau.nl).

Figure 1. Equilibrium infection frequency (I) in females as a function of the fitness cost (ω) associated with the *Wolbachia* infection for different transmission fidelities of PI *Wolbachia* (α). ω is expressed as the offspring production of infected females relative to that of uninfected females. α is the proportion of infected offspring from an infected mother. Values of α range from 0.85 to 0.99. The fertilization proportion of eggs ($1-r$) was assumed to be 0.75 as found in field populations of *Trichogramma kaykai*. All females are assumed to have mated.

2. BACTERIAL TRANSMISSION AS A FACTOR INFLUENCING WOLBACHIA INFECTION LEVELS

Inefficient transmission causes some offspring of infected females to lose their infection. Such losses are expected, particularly in older or heat-stressed females (Cabello & Vargas 1985; Legner 1985), and can stabilize a polymorphism if infected females produce more daughters than uninfected females (Stouthamer 1997). Using the proportion of eggs fertilized by uninfected females in field populations of *T. kaykai* (75%), figure 1 shows equilibrium values for a range of infection costs and *Wolbachia* transmission efficiencies. For the range of infection frequencies found in the field (5–20%, lower left of each curve in figure 1), the steep slopes indicate that slight changes in either transmission efficiency or infection cost would cause the infection to become either very common or extinct. We do not find this in our field populations: all nine samples of more than 100 broods per population over the period 1988–1997 had infection frequencies in the range 4–14% (data not shown).

We recently discovered that the PI *Wolbachia* is not only transmitted from mother to daughter but infective transmission can occur when a host egg is parasitized by both an infected and an uninfected female (Huigens *et al.* 2000). In approximately 37% of such super-parasitized eggs horizontal transmission of the PI *Wolbachia* takes place resulting in the infection of the offspring of the uninfected mother. The addition of horizontal transmission to the inefficient-transmission model above will always increase the infection frequency in the population, making it even more difficult to attain the relatively low infection levels we find in the field. Thus, other factors must stabilize the infection frequencies at the levels found in the field.

Figure 2. The equilibrium frequency of *Wolbachia* infection (I) in females in the presence of a dominant suppressor allele. The reduction in survival of the suppressor genotypes is given by c and the proportion of eggs remaining unfertilized is given by r .

3. SUPPRESSOR GENES AS A FACTOR PREVENTING THE FIXATION OF WOLBACHIA

Alleles suppressing *Wolbachia*'s effect (suppressor genes) are favoured in populations that contain infected individuals because of the high fitness accruing to males in populations with a *Wolbachia*-induced female bias (Stouthamer 1997). Such a variant could invade either a polymorphic population or a population fixed for *Wolbachia*. For example, consider an all-female population. A new mutant (dominant or recessive, since *Wolbachia* assures that all new mutations are immediately homozygous) that suppresses *Wolbachia* infection causes unmated females to produce only sons. Such males are highly fit, since every female in the population is a potential mate, hence the suppressor allele would spread. Any male immigrant, regardless of genotype, would have this advantage when entering a completely infected population. However, the advantage of a non-suppressor allele is short lived since the beneficial trait (maleness) is not heritable. All of his grand-offspring would be infected females. In contrast, a male immigrant carrying a suppressor allele produces cured daughters (dominant allele) or granddaughters (recessive allele), allowing spread of the suppressor allele.

Since *Wolbachia* is a maternally transmitted, cytoplasmic factor, it cannot benefit from male fitness; thus, the evolution of suppression arises from a nuclear–cytoplasmic conflict (Cosmides & Tooby 1981). Suppressor genes act either by killing the bacteria or by negating their effect. Several cases have been reported in which suppressors have evolved against cytoplasmic sex-ratio distorters (Cavalcanti *et al.* 1957; Rigaud & Juchault 1993). The consequences of a hypothetical suppressor have also been modelled for male-killing bacteria (Randerson *et al.* 2000).

Figure 3. Crossing protocol for the detection of *Wolbachia*-suppressing alleles in field populations of *Trichogramma kaykai*. Infected females (superscript i) produce all-female broods. Field-collected all-female broods (generation F₁) were used to search for polymorphic suppressor alleles segregating in their parents (generation P). Subsequently, each female of the all-female brood was allowed to produce offspring (F₂) as a virgin. If some of the females from an all-female brood (F₁) produced all-male offspring (F₃), the brood was classified as a potential suppressor brood and the F₃ and F₄ were derived to determine the presence of suppressor alleles. u, uninfected individual; i, infected individual; s, recessive suppressor allele; S, dominant suppressor allele; +, wild-type non-suppressor allele.

The equilibrium frequency of *Wolbachia* infection (I) in the presence of a dominant suppressor allele can be calculated. Assume that females carrying the suppressor allele are cured of any *Wolbachia* infection but at a cost (c) that lowers the survival of suppressor genotypes to $1 - c$. The equilibrium is strongly dependant upon the proportion of eggs that remain unfertilized (r) if $r > 0.3$, otherwise r has little effect. The infection frequency in females, valid for small c , is given by

$$I = \{3(1+c)r - (1-c) + \sqrt{[(9-46c+41c^2)r^2 - 6(1-4c+c^2)r + (1-c)^2]}/\{2(2-c)r\}. \quad (1)$$

Modelling such a PI *Wolbachia* case (figure 2) shows that suppression is relatively ineffective if r is high, i.e. if uninfected populations are male biased. If r is low, as is the case with *T.kaykai* ($r < 0.3$) then *Wolbachia* can be completely eliminated.

Elimination of *Wolbachia* is prevented if suppression has a fitness cost (see figure 2). Even a very small cost stabilizes the coexistence of the suppressor allele and the

infection. With a cost of less than 5%, the expected proportion of infected females is below 15% and very insensitive to changes in r over a broad range of fertilization frequencies (*ca.* $0 < r < 0.3$). At higher values of r , the proportion of infected females rapidly increases, as it does in the no-cost scenario. Sib mating, typically 54–65% in uninfected *T. kaykai* populations (Stouthamer & Kazmer 1994), has little effect on the expected proportion of infected females in the presence of a suppressor allele, provided that sib mating does not markedly decrease the likelihood of males subsequently mating with infected females.

Suppressor genes, if present, can be detected in field populations using the crossing protocols described in figure 3. Infected females (superscript i) produce all-female broods. Field-collected all-female broods (generation F₁) were used to search for polymorphic suppressor alleles segregating in their parents (generation P). These female offspring were isolated and produced offspring as virgins (generation F₂). An all-male F₂ brood could be the result of a suppressor allele segregating in an F₁ family

Figure 4. Equilibrium frequency for *Wolbachia* infection in females and paternal sex ratio (PSR) in males. Model (see §4) based on the assumptions that infected and uninfected females produce equal numbers of offspring; both infected and uninfected females mate and fertilize their eggs at a frequency of 0.75; infected females emerging in a brood with only PSR males sib-mate with a PSR brother with a frequency of 100%, due to the male bias of such broods; and the transmission efficiencies of the parthenogenesis-inducing *Wolbachia* and the PSR factor are 100%. In populations consisting of PSR males, normal males, infected females and normal females, the solid line indicates the PSR frequency in males and the dotted line indicates the *Wolbachia* infection in females. In populations consisting of PSR males, normal males and normal females, the dashed line indicates the PSR frequency in males.

(potential suppressor family). In order to detect recessive suppressors (*s*) we allowed F_2 females from each potential suppressor family to reproduce as virgins. If a recessive suppressor was present, some of the F_2 virgin females should produce all-male broods (generation F_3). In order to detect dominant suppressors we mated some of the F_2 males with infected (*i*) females. A dominant suppressor (*S*) present in the male should result in some uninfected (*u*) females in the all-female F_3 . These uninfected F_3 virgins should only produce male offspring. During the field seasons 1996 and 1997 we collected a total of 2422 broods, out of which 442 produced only female F_1 offspring, out of which 28 produced potential suppressor families. Out of these 28 families we tested 13 for suppressors (13 families for recessive suppressors with a total of 83 F_2 virgin females tested, and six families tested additionally for dominant suppressors, with a total of 41 F_2 males tested). We found no evidence for suppressor alleles. We concluded that suppressor genes are unlikely to be a major factor stabilizing the coexistence of infected and uninfected *T. kaykai* populations in the Mojave Desert.

4. MALE-BIASING SEX-RATIO DISTORTERS AS A FACTOR PREVENTING THE FIXATION OF *WOLBACHIA*

Another potential stabilizing mechanism is the presence of paternal sex ratio (PSR) factors (Werren 1991). PSR factors are transmitted through males, since eggs fertilized with sperm from PSR-infected males

become male instead of female. PSR factors are known in only two parasitoid species, *Nasonia vitripennis* (Werren 1991) and *Encarsia pergandiella* (Hunter *et al.* 1993). In *N. vitripennis*, the PSR factor is a B chromosome that converts the diploid fertilized egg into a male haploid egg by destroying the paternal set of chromosomes, with the exception of itself (Werren 1991). This extremely selfish genetic element is a parasitic chromosome that enhances its own transmission by destroying all the chromosomes it contacts (Werren *et al.* 1988).

The equilibrium frequencies of PSR and PI *Wolbachia* in populations can be calculated. It is assumed that both infected and uninfected females mate and fertilize their eggs at a frequency of 0.75 (Stouthamer & Kazmer 1994). Infected females emerging in a brood with only PSR males are assumed to sib-mate with a PSR brother at a frequency of 100%, due to the male bias of such broods. Uninfected females are assumed to mate with a brother at a frequency *s*. Transmission efficiencies of the PI *Wolbachia* and the PSR factor are assumed to be 100%. Under these circumstances it can be shown that the equilibrium *Wolbachia*-infection frequency, *I*, among females is

$$I = 1 - [(1 + (1 - r)(1 - s)p)(1 - p) / ((1 - p)r + (p - 1 + r)(1 - r)(1 - s) + (1 - p) \times ((1 - r)(1 - s)p + (1 - p)))] \quad (2)$$

and the equilibrium PSR frequency, *p*, among males is

$$p = r / (r + (1 - r)s). \quad (3)$$

In the absence of *Wolbachia* the equilibrium PSR frequency (p^*) shifts to

$$p^* = (1 - 2r - s(1 - r)) / ((1 - r)(1 - s)). \quad (4)$$

This equilibrium exists ($p^* > 0$) if $r < (1 - s) / (2 - s)$.

If we assume that *Wolbachia*-infected and *Wolbachia*-uninfected eggs fertilized by PSR sperm develop into PSR-carrying males, modelling shows that this factor can stabilize *Wolbachia*-infection frequencies at approximately the level found in the field (figure 4).

We searched for such a PSR factor by mating males from field-collected all-male broods with PI *Wolbachia*-infected females. If these males are normal, such matings should yield only female offspring, since both fertilized and unfertilized infected eggs develop into females. In contrast, if these males are PSR carriers then fertilized infected eggs should develop into males and unfertilized infected eggs should develop into females, giving a sex ratio of 25% females. We collected 46 all-male broods from several sites and in 35 of these broods at least one of the males induced their *Wolbachia*-infected mates to produce mostly male offspring (table 1). This result suggests that the males carried a PSR-like factor. We maintained at least one line of each suspected PSR brood for more than three generations to confirm the stability of the trait. In all cases, these lines continued to produce mostly male offspring. Further, we sought evidence of a B chromosome in the PSR males. We used DNA-specific fluorochrome (DAPI) to determine the chromosome numbers in PSR and normal males. Samples were prepared by fixing the larvae in a drop of Carnoy's fixative (acetic acid:ethanol = 1:3) for 3 min, then

Table 1. *Brood composition of Apodemus mormo eggs parasitized by Trichogramma kaykai*

(Eggs were collected at various localities in the Mojave Desert from April to July 1997. Infected broods are all-female broods in which at least one of the offspring produced daughters when allowed to reproduce as a virgin. PSR broods are all-male broods in which at least one of the offspring, when crossed with an infected female, produced mainly sons. All other broods were classified as sexual broods. To determine the level of PSR infection in males, males were classified as PSR males if they induced the production of male offspring by an infected female. Those that failed to produce offspring were classified as unknown but for the calculation of male PSR frequency were assigned as PSR in the same proportion as the successfully tested males from the same location.)

collection location	number of broods collected	sexual broods			infected broods (all-female)		PSR broods (all-male)				sex-ratio-distorter frequency in population	
		percentage of total broods	sex ratio (% males)	total progeny	percentage of total broods	total progeny	percentage of total broods	total progeny	number tested	PSR males (%)	infection among female offspring (%)	PSR among male offspring (%)
Danby	25	80.0	24.1	87	4.0	5	16.0	14	14	71.4	7.0	28.6
Last Chance Canyon	788	93.0	25.4	3450	3.6	113	3.4	113	95	80.0	4.2	9.1
Panamint Valley	63	90.5	24.4	279	9.5	26	0.0	0	0	0.0	11.0	0.0
Mojave River	29	89.7	26.3	110	6.9	6	3.5	4	2	50.0	6.9	9.1
Yucca Valley	123	92.7	22.5	479	4.9	16	2.4	9	7	100.0	4.1	6.1

dissecting the larvae in a drop of 60% acetic acid for 3 min and finally spreading the cells by adding a drop of ice-cold Carnoy's fixative. After air-drying, the chromosomes were stained with DAPI in the anti-fading agent Vectashield[®] (Vector Laboratories, Burlingame, CA, USA) ($1 \mu\text{g ml}^{-1}$). Photographs were taken under a fluorescence microscope and selected negatives were scanned and processed with image-analysis software to create the karyotypes. Consistent with the *Nasonia* PSR case, we found a small extra chromosome in PSR males (figure 5). To test whether the PSR factor also destroys the paternal set of chromosomes, we crossed PSR males with *Wolbachia*-infected females from two lines. We then compared a microsatellite marker of the male offspring with that of its mother and father. In both crosses, the marker in the PSR male reflected the mother's genotype; thus, the chromosome carrying the paternal microsatellite marker had been destroyed, again consistent with the mode of action of the *Nasonia* PSR. Thus, all of our evidence implicates the presence of a PSR B chromosome that destroys the paternal chromosomes in *T. kaykai* populations.

5. DISCUSSION

Our results show that out of the factors that can contribute to the stable coexistence of infected and uninfected wasps in the population, the PSR factor appears to be the major contributor to the coexistence. Without the PSR factor, the PI *Wolbachia* infection could go to fixation in the population, leading to a loss of sexual reproduction. Therefore, because of the presence of the PSR factor, normal sexual reproduction is maintained in part of the population.

Modelling shows that the PSR factor of *Trichogramma* is able to maintain itself in uninfected *T. kaykai* populations. The combination of a fertilization frequency of 75% with the sib-mating levels in these populations (54–65%) permits the PSR to invade a sexual population (figure 4).

Figure 5. Late prophase chromosome karyotypes of seven-day-old male *Trichogramma kaykai* larvae from (a) an antibiotic-cured line, LC19-1, originally established from an infected female collected at El Paso Mountains, Kern County, CA, USA in May 1995, and (b) a cross between an infected LC19-1 female and a PSR male, P1-A, originally collected as part of an all-male brood at Danby, San Bernardino County, CA, USA in April 1997 and maintained by crossing the males with infected females from line LC19-1. Chromosomes were ordered in sequence of decreasing length. The line intersects the chromosomes at the putative position of the centromere.

However, the *Trichogramma* PSR is even more successful in the presence of a sex-ratio distorter, such as *Wolbachia*, that forces females to produce all-female broods (figure 4). PSR-infection frequencies in our field populations (table 1) are generally lower than those predicted by the model. While our methodology may

have underestimated the frequency of PSR males in the Mojave populations, other factors may also be acting.

Finding a third case of PSR-like factors in wasps indicates that such elements may be more common than previously assumed, especially in species with haplodiploid sex determination. Many of the cases where field-collected females are reported to be virgins because they produce only sons (Hardy & Godfray 1990) may very well be cases of PSR. Our results demonstrate the independent B-chromosome evolution in a second family of Hymenoptera. The mode of action of PSR restricts its presence to species with a haplodiploid sex-determination system. Haplodiploid sex determination is also found in other arthropod taxa, such as spider mites, thrips and whiteflies. Some members of these taxa are serious agricultural pests; PSR-like factors have potential for their control.

The destruction of the paternal chromosome set in haplodiploid species facilitates the spread of PSR elements across species boundaries, either in nature or by laboratory manipulation (McAllister & Werren 1997; Dobson & Tanouye 1998). The genome of the male donor species is destroyed by the element before it can lead to any inter-specific incompatibility. This suggests that in nature the element may have evolved in a lineage quite distant from the one currently infected, as was also found for the *Nasonia* PSR (McAllister & Werren 1997).

We thank J. F. Bennett, L. Bolijn, B. Deijkers, H. van Heest, J. Honda, M. van Kasteren, M. Loeber-Stouthamer, M. Schilthuisen and C. A. Stouthamer-Bennett for helping with the collections and culturing of the wasps. J. D. Pinto, G. R. Platner and A. B. Koopmanschap identified the wasps for us. G. R. Pratt showed us around the desert and advised us on good localities to collect the butterfly eggs. P. van Baarlen assisted in the microscopy work to visualize the wasp chromosomes. We thank G. D. D. Hurst, F. Vavre and M. E. Huigens for many inspiring conversations on the topic of genomic conflicts. This work was supported in part by NATO (R.S. and R.F.L.), the Dutch Organisation for Scientific Research (R.S.) and the Wageningen Fund (M.v.T.).

REFERENCES

- Cabello, T. & Vargas, P. 1985 Temperature as a factor influencing the form of reproduction of *Trichogramma cordubensis*. *Z. Angew. Entomol.* **100**, 434–441.
- Cavalcanti, A. L. G., Falcao, D. N. & Castro, D. L. 1957 Sex-ratio in *Drosophila prosaltans*—a character due to interaction between nuclear genes and cytoplasmic factors. *Am. Nat.* **91**, 327–329.
- Cosmides, L. M. & Tooby, J. 1981 Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**, 83–129.
- Dobson, S. L. & Tanouye, M. A. 1998 Interspecific movement of the paternal sex ratio chromosome. *Heredity* **81**, 261–269.
- Hamilton, W. D. 1967 Extraordinary sex ratios. *Science* **156**, 477–488.
- Hardy, I. C. W. & Godfray, H. C. J. 1990 Estimating the frequency of constrained sex allocation in field populations of Hymenoptera. *Behaviour* **114**, 137–147.
- Huigens, M. E., Luck, R. F., Klaassen, R. H. G., Maas, M. F. P. M., Timmermans, M. J. T. N. & Stouthamer, R. 2000 Infectious parthenogenesis. *Nature* **405**, 178–179.
- Hunter, M. S., Nur, U. & Werren, J. H. 1993 Origin of males by genome loss in an autoparasitoid wasp. *Heredity* **70**, 162–171.
- Legner, E. F. 1985 Natural and induced sex ratio changes in populations of thelytokous *Muscidifurax uniraptor*. *Annls Entomol. Soc. Am.* **78**, 398–402.
- McAllister, B. F. & Werren, J. H. 1997 Hybrid origin of a B chromosome (PSR) in the parasitic wasp *Nasonia vitripennis*. *Chromosoma* **106**, 243–253.
- Pinto, J. D. 1999 Systematics of the North American species of *Trichogramma*. *Mem. Entomol. Soc. Wash.* **22**, 1–287.
- Pinto, J. D., Stouthamer, R. & Platner, G. R. 1997 A new cryptic species of *Trichogramma* from the Mojave desert, California as determined by morphological, reproductive and molecular data. *Proc. Entomol. Soc. Wash.* **99**, 238–247.
- Randerson, J. P., Smith, N. G. C. & Hurst, L. D. 2000 The evolutionary dynamics of male-killers and their hosts. *Heredity* **84**, 152–160.
- Rigaud, T. & Juchault, P. 1993 Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare*. *Genetics* **133**, 247–252.
- Rousset, F., Bouchon, D., Pintureau, B., Juchault, P. & Solignac, M. 1992 *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc. R. Soc. Lond. B* **250**, 91–98.
- Stouthamer, R. 1997 *Wolbachia*-induced parthenogenesis. In *Influential passengers, inherited microorganisms and arthropod reproduction* (ed. S. C. O'Neill, J. Werren & A. A. Hoffmann), pp. 102–124. Oxford University Press.
- Stouthamer, R. & Kazmer, D. J. 1994 Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* **73**, 317–327.
- Stouthamer, R., Luck, R. F. & Hamilton, W. D. 1990 Antibiotics cause parthenogenetic *Trichogramma* to revert to sex. *Proc. Natl Acad. Sci. USA* **87**, 2424–2427.
- Stouthamer, R., Breeuwer, J. A. J., Luck, R. F. & Werren, J. H. 1993 Molecular identification of microorganisms associated with parthenogenesis. *Nature* **361**, 66–68.
- Werren, J. H. 1991 The paternal sex ratio chromosome of *Nasonia*. *Am. Nat.* **137**, 392–402.
- Werren, J. H., Nur, U. & Wu, C. I. 1988 Selfish genetic elements. *Trends Ecol. Evol.* **3**, 297–302.