***Wolbachia*-mediated parthenogenesis in the predatory thrips *Frankliniopsis vespiformis* (Thysanoptera: Insecta)**

Norio Arakaki¹*, Takeharu Miyoshi² and Hiroaki Noda²

¹Okinawa Prefectural Agricultural Experiment Station, Sakiyama, Naha, Okinawa 903-0814, Japan

²National Institute of Sericultural and Entomological Science, Owashi, Tsukuba, Ibaraki 305-8634, Japan

Wolbachia are bacterial endosymbionts in arthropods and filarial nematodes. They cause thelytoky, which is a form of parthenogenesis in which females produce females without males, in hymenopteran insects. Infection of this parthenogenesis-inducing *Wolbachia* has been restricted to the order Hymenoptera, but was found in another insect order, Thysanoptera. A parthenogenetic colony of a predatory thrips *Frankliniopsis vespiformis* (Aeolothripidae) possessed B-group *Wolbachia*. Male progeny were produced from this thrips by heat and tetracycline treatments. Males produced motile sperm, which were transferred to the female spermatheca by mating. However, the mating did not affect the sex ratios of the next generation, suggesting that the sperm do not fertilize the eggs.

Keywords: *Frankliniopsis vespiformis*; *Wolbachia*; symbiont; thrips; parthenogenesis; thelytoky

1. INTRODUCTION

Most hymenopteran species show arrhenotoky, which is a form of parthenogenesis in which unfertilized eggs give rise to haploid males and fertilized eggs to diploid females. However, complete parthenogenesis, i.e. thelytoky, in which unfertilized eggs give rise to female offspring, is also found in many hymenopterous lineages (Luck *et al.* 1992). Consequently, thelytokous lines can persist for multiple generations in the absence of males and sexual reproduction (Stouthamer & Kazmer 1994). Thelytoky is often associated with the presence of endosymbiotic bacteria of the genus *Wolbachia* (Stouthamer *et al.* 1990, 1993; Stouthamer & Werren 1993). Most of the known thelytokous forms in *Trichogramma* can be rendered permanently bisexual by the use of antibiotics or exposure to high temperatures (Stouthamer *et al.* 1990), which work to eliminate or suppress the function of *Wolbachia*. Although thelytoky induced by *Wolbachia* has been observed in more than 30 species, it has been restricted to members of the Hymenoptera (Stouthamer 1997; Cook & Butcher 1999). Discovery of *Wolbachia*-induced parthenogenesis in other groups of haplodiploid species has been anticipated since its discovery in hymenopterans. Parthenogenetic thrips (Thysanoptera) or haplodiploid mites (Acari) are considered to be important groups in which to look for parthenogenetic *Wolbachia* outside of the Hymenoptera (Werren 1997; Cook & Butcher 1999).

Arrhenotoky is commonly seen in thrips (Thysanoptera) and thelytoky is also found in some species (Lewis 1973). The predatory thrips *Frankliniopsis vespiformis* (Crawford) is a known natural enemy of mites, leafhoppers and white flies in citrus and avocado groves in Central and South America (Moulton 1932). Both sexes of *F. vespiformis* have been observed in South America (Moulton 1932) and adult females and larvae of this species were recorded in Thailand in 1987 (Okajima *et al.* 1992). This species was also found in Okinawa, Japan, in

June 1996 and seems to have recently inhabited Okinawa Islands (Arakaki & Okajima 1998). The adults of *F. vespiformis* collected in a field in Okinawa in 1997 were all female ($n = 47$) and their offspring were also all female (Arakaki & Okajima 1998). No males appeared in more than ten generations in a laboratory culture. Therefore, it is obvious that this species exhibits thelytoky and propagates parthenogenetically on Okinawa Islands.

In the present study, we examined whether the thelytoky in *F. vespiformis* is caused by microorganisms. The sexual function of antibiotic-induced males and the phylogenetic relationship of *Wolbachia* were also studied.

2. MATERIAL AND METHODS

(a) Insects

Adults and larvae of *F. vespiformis* (Crawford) (Thysanoptera: Aeolothripidae) were sampled as parental stock from a wild population on the cucumber *Cucumis sativus* in June 1997 at Okinawa Prefectural Agricultural Experiment Station, Naha. They were maintained at 25 °C in plastic containers (10 cm × 4 cm × 12 cm) with ultraviolet-sterilized eggs of the Mediterranean flour moth *Ephestia kuehniella* (Zeller) as food, leaves of *Kalanchoe tubiflora* (Harvey) Hamet as an oviposition substrate and a piece of slightly twisted tissue paper for shelter and a pupation site.

(b) High-temperature treatment

The thrips were individually reared in glass test-tubes (1.7 cm diameter × 13 cm length) with *E. kuehniella* eggs, a leaf of *K. tubiflora* and a piece of tissue paper. Pupae (at the second pupal stage) were incubated at 35 °C for 24 h and then returned to 25 °C. The number of offspring and their sexes were recorded from 20 heat-treated females.

(c) Antibiotic treatment and sex ratio

Tetracycline hydrochloride (Wako, Osaka, Japan) dissolved in diluted honey solution (50 mg ml⁻¹) was absorbed in cotton and administered to the thrips in two ways. First, tetracycline was given to newly emerged adults for 24 h. Second, the antibiotic was administered at the second-instar larval stage for two days

*Author for correspondence (arakaki@nousi.pref.okinawa.jp).

and at the newly emerged adult stage for one day. The appearance of males in the next generation was observed daily. The effects of four other antibiotics, cephalosporin C, erythromycin, penicillin-G (Sigma, St Louis, MI, USA) and streptomycin sulphate (Meiji, Tokyo, Japan), were also examined. Solutions of each antibiotic (50 mg ml^{-1}) were given at the second-instar larval stage for two days and at the adult stage for one day.

(d) *PCR amplification of Wolbachia genes*

Wolbachia was detected by a diagnostic polymerase chain reaction (PCR) using 99F/994R primers (O'Neill *et al.* 1992) for the 16S rRNA gene (rDNA) and *ftsZ* gene primers (Holden *et al.* 1993) and 81F/691R primers for the *wsp* gene (Zhou *et al.* 1998). Two sets of primer pairs for the *ftsZ* gene, Adf/Adr and Bf/Br (Werren *et al.* 1995), were also used for distinguishing A and B *Wolbachia* groups. Each adult thrips was homogenized with $20 \mu\text{l}$ of STE buffer (100 mM NaCl and 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)) and treated with $2 \mu\text{l}$ of proteinase K (10 mg ml^{-1}) (Sigma) (O'Neill *et al.* 1992). The PCR conditions were heating at 95°C for 30 s, 30–35 cycles at 95°C for 30 s, 52°C for 30 s and 72°C for 90 s, with the final extension at 72°C for 5 min.

A PCR primer set for 16S rDNA, SN910601 and SN920204 (Namba *et al.* 1993) was used for testing infection of phytoplasma under the above-mentioned PCR conditions. A specific primer set for the citrate synthase gene of the genus *Rickettsia* (Regnery *et al.* 1991) was also used for detecting rickettsial infection.

Template samples that did not show any specific DNA bands in the PCR using the primers above were further tested by mitochondrial 12S rDNA primers (Simon *et al.* 1991; O'Neill *et al.* 1992) in order to verify the validity of the template preparation.

(e) *Gene sequencing and phylogenetic analysis*

Bacterial 16S rDNA was amplified from eggs with a pair of general primers for 16S rDNA (fD1 and rP2), which amplify nearly the full length of 16S rDNA (Weisburg *et al.* 1991), in order to examine the micro-organisms in the thrips. The egg templates were prepared using proteinase K as described in § 2(d). The *ftsZ* gene was amplified from adult templates with *ftsZ* f1 primer (Werren *et al.* 1995) and the reverse primer of Holden *et al.* (1993), and the *wsp* gene was amplified with the 81F/691R primers (Zhou *et al.* 1998). The PCR products were cloned into pBluescript II (Stratagene, Cedar Creek, TX, USA) or pGEM (Promega, Madison, WI, USA) and sequence templates were generated by colony PCR using the M13 universal/reverse primer pair. The sequences were determined by the BigDye Terminator Cycle sequencing method with a DNA sequencing system (model 377, PE Applied Biosystems, Foster City, CA, USA). Homology searches of the 16S rDNA sequences in the DNA databases were performed using the BLAST program.

The nucleotide sequences of the *wsp* genes were aligned with CLUSTAL X (Thompson *et al.* 1997) and 561 bases of the *wsp* genes in B-group *Wolbachia* were analysed (Zhou *et al.* 1998). A maximum-parsimony analysis, as implemented in PAUP v. 4.0b2 (Swofford 1999), was performed based on a heuristic search and a bootstrap analysis was performed with 1000 replications. A maximum-likelihood analysis was performed with PUZZLE v. 4.02 (Strimmer & Von Haessler 1996) based on the gamma-distributed rates.

(f) *Sexual function of males*

The mating potential of the antibiotic-induced males was tested. One zero-day-old virgin female and two males were

Figure 1. Emergence pattern of adult progeny produced by 20 tetracycline-treated females of *F. vespiformis*.

confined in a test tube in order to observe their mating behaviour. Females which mated with males were dissected and the presence of sperm in their spermathecae was examined under a microscope.

The effects of mating on the sex ratio of the next generation of the thrips were then examined for the tetracycline treatment. Twenty females were used for each of four groups: (i) untreated virgin females as the control, (ii) antibiotic-treated virgin females, (iii) untreated and mated females, and (iv) antibiotic-treated and mated females. Tetracycline (50 mg ml^{-1} honey solution) was given to newly emerged, zero-day-old females for 24 h. The females were allowed to mate by confining a newly emerged female in the test tube with two males from antibiotic-treated colonies. The antibiotic-treated and mated females were obtained by tetracycline treatment and mating in this order.

3. RESULTS

(a) *High-temperature treatment*

A one-day exposure of the pupal stage of *F. vespiformis* to 35°C induced male progeny in one female adult out of 20 females. This female adult produced three males in eight progeny.

(b) *Effect of antibiotic treatment*

The females treated with tetracycline for 24 h ($n=20$) mainly produced male progeny in the first half of the ovipositional period (figure 1). The proportion of male progeny was 78.7% and the mean number of progeny per female was 31.3 ± 14.1 (mean $\pm$ s.d.), which was not significantly different from untreated females (27.2 ± 9.6) (one-way ANOVA, $p > 0.05$). Female progeny emerged in small numbers in the first three days and emerged again from the seventh day. Untreated females ($n=20$) produced no males.

When tetracycline was given at both the second-instar larval and adult stages ($n=6$), all of the females produced all-male progeny except one, which produced two females out of 22 progeny. The mean number of progeny was 12.3 ± 7.4 , which was smaller than in untreated females (*t*-test, $p < 0.01$). This was presumably due to the long exposure to tetracycline.

Four other antibiotics, cephalosporin C, erythromycin, penicillin-G and streptomycin sulphate, were also given. These antibiotic treatments did not induce the production of male progeny except for 1 penicillin-treated female out of 15, which produced 6 males out of 11 offspring.

Figure 2. Maximum-likelihood tree of *wsp* DNA sequence data of B-group *Wolbachia* with A-group *Wolbachia* of *Glossina austeni* and *Drosophila simulans* as outgroups. The *Wolbachia* strains are represented by the name of their host species. DNA database accession numbers are given in parentheses. The program used was PUZZLE v. 4.02 with gamma-distributed rates. The numbers at the front indicate the quartet puzzling support values and those at the back the bootstrap values calculated by a maximum-parsimony analysis of PAUP v. 4.02b.

(c) PCR detection of micro-organisms

Tetracycline-sensitive microorganisms seem to have an influence on the production of male progeny. Phytoplasma and rickettsia often infect insects and are well known as being sensitive to tetracycline. Therefore, these infections were tested in the laboratory-cultured colony by diagnostic PCR. A phytoplasma-specific primer set, SN910601–SN920204, did not amplify specific products. A specific primer for the citrate synthase gene of rickettsia also did not give positive products. Diagnostic PCRs for 16S rDNA, the *ftsZ* gene and the *wsp* gene of *Wolbachia* were all positive. Two primer sets for the *ftsZ* gene, Adf/Adr and Bf/Br, which are specific for A and B groups, respectively (Werren *et al.* 1995), both amplified the expected-sized DNA in the laboratory-cultured colony. However, newly collected field thrips possessed only B-group *Wolbachia*.

Eggs from the laboratory-cultured colony were examined using the two primer sets for the *ftsZ* gene in order to clarify the origin of A-group *Wolbachia*. The eggs were not infected with A-group *Wolbachia*, indicating that the laboratory colony was only infected with B-group *Wolbachia*, as was the case with the field population. The A-group *Wolbachia* was found from the eggs of *E. kuehniella*, which were provided to the thrips as food.

Male and female progenies produced by females treated with tetracycline for 24 h were tested for infection of B-group *Wolbachia* by PCR. Thirty-four out of 70 males

(48.6%) and 51 out of 69 females (73.9%) were infected with B-group *Wolbachia*.

(d) Wolbachia gene sequence and phylogenetic analysis

Bacterial 16S rDNA was amplified from eggs using the general primers for 16S rDNA and single-pass sequencing of the 5'- or 3'-region (*ca.* 500 nucleotides) of the amplified 16S rDNA was performed in order to examine the bacterial species in the eggs of the thrips. Twenty-one clones were sequenced. Homology searches indicated that the sequences of 16 clones gave the highest similarity score with *Wolbachia* and the other five sequences with *Acinetobacter* (two clones), *Methylobacterium*, *Burkholderia* and *Sphingomonas*.

Two kinds of sequence were found for each gene in the analysis of the nucleotide sequences of the *ftsZ* and *wsp* genes: one was that of the A group and the other that of the B group. The sequences of the A group were identical to those from the *E. kuehniella* that was used as food for the thrips. The nucleotide sequence of the *wsp* genes amplified from newly collected females in the field was identical to that of the B-group *Wolbachia* in the laboratory-cultured colony (DNA database accession numbers AB045314 for the *ftsZ* gene and AB045315 for the *wsp* gene).

The nucleotide sequences of the *wsp* gene of 23 *Wolbachia* from the B group and two outgroups from the A group

Table 1. *Effect of mating in female F. vespiformis on the sex ratio of the next generation*

(The mean ($\pm$ s.d.) longevity of females and the mean number of offspring were not significantly different at the 5% level by one-way ANOVA. The mean ($\pm$ s.d.) percentages of males followed by the same superscripted letters were not different at the 5% level by Scheffé's multiple comparisons. Statistical analysis of the mean percentages of males was conducted after arcsine transformation.)

treatment	number of female replicates	mean longevity of females	mean number of offspring	mean % of males
virgin (control)	20	30.7 $\pm$ 9.8	27.2 $\pm$ 9.6	0 ^a
mated	20	28.5 $\pm$ 9.7	26.9 $\pm$ 7.8	0 ^a
antibiotic virgin	20	26.2 $\pm$ 9.0	31.3 $\pm$ 14.1	78.7 $\pm$ 17.9 ^b
antibiotic and mated	20	32.1 $\pm$ 8.5	26.8 $\pm$ 9.5	80.0 $\pm$ 15.2 ^b

were aligned and analysed. The maximum-parsimony and maximum-likelihood analyses indicated similar topologies. The closest relative obtained in the likelihood analysis was male-killing *Wolbachia* of *Acraea encedon* (figure 2). *Wolbachia* from *Torymus bedeguaris*, *Tribolium confusum* and *Laodelphax striatellus* were phylogenetically close to the *Wolbachia* of *F. vespiformis*.

(e) Sexual function of males

All females copulated with males within 3 min when they were confined in a test-tube with the males. The mated pair remained in copula for 28.6 $\pm$ 5.3 min (mean $\pm$ s.d.) ($n=20$). This mating behaviour appears to be normal. Actively mobile sperm were found in the spermathecae of dissected females ($n=10$).

The effects of mating on the sex ratios were compared between the four different treatments (table 1). Virgin females that were untreated with tetracycline produced no male progeny. Untreated females did not produce any male progeny after mating. In contrast, tetracycline-treated females gave birth to male progeny with and without mating (80.0 and 78.7%, respectively). There was no significant difference in the mean percentage of male production between mating and non-mating females (Scheffé's multiple comparisons, $p > 0.05$). There were no significant differences in the mean longevity and number of offspring produced between the four treatments (one-way ANOVA, $p > 0.05$).

4. DISCUSSION

Exposure to a high temperature (35 °C) at the pupal stage and tetracycline treatment of newly emerged adults produced male offspring in *F. vespiformis* (figure 1). This result is quite similar to the case of the thelytoky in wasps induced by *Wolbachia* and strongly suggests that the thelytoky in *F. vespiformis* was induced by microorganisms. Tetracycline was quite effective for male production when mother thrips were treated at the second-instar larval

and adult stages. In contrast, the other four antibiotics did not effectively destroy thelytokous reproduction in these thrips. Tetracycline is primarily used for mycoplasmas, chlamydiae and rickettsiae (Conte & Barriere 1988). Mycoplasmas that infect insects are usually phytoplasmas, which are plant pathogens transmitted by insect vectors (Lee *et al.* 1998) and spiroplasmas that symbiotically infect arthropods (Williamson *et al.* 1998). The diagnostic PCR for phytoplasmas was negative and microscopic observation under high magnification failed to detect spiral micro-organisms, which is a characteristic of spiroplasmas (H. Noda, unpublished observation). Rickettsiae, which are pathogenic or symbiotic and belong to more than ten genera, are often found in ticks (Sonenshine 1993; Noda *et al.* 1997), but insect-infecting rickettsiae are usually members of the genera *Rickettsia* or *Wolbachia*. A primer specific for the citrate synthase of micro-organisms belonging to the genus *Rickettsia* failed to amplify the DNA. Finally, three pairs of *Wolbachia*-specific primers all amplified the DNA products. Therefore, *Wolbachia* is a highly probable candidate for the thelytoky-inducing micro-organism in *F. vespiformis*.

Thelytoky-inducing micro-organisms are probably inherited from generation to generation and transmitted through the ovary into the eggs. We partially sequenced the PCR products amplified from eggs with bacterial 16S rDNA general primers. The sequences of 16 clones out of 21 were those of *Wolbachia*. The other five sequences gave high similarity scores with *Acinetobacter*, *Methylobacterium*, *Burkholderia* and *Sphingomonas*. These micro-organisms may have been introduced by attaching to the surface of the eggs, because arthropod infection and vertical transmission of these micro-organisms are not usual, as far as the authors are aware. The present results indicate that *Wolbachia* causes parthenogenesis in *F. vespiformis* since *Wolbachia* is widely distributed as a causative agent of thelytoky in hymenopterous insects and *F. vespiformis* has a reproductive manner similar to hymenopterans (haplodiploid reproduction). The causative agent of the thelytoky in *F. vespiformis* was the B-group *Wolbachia*, which is phylogenetically close to that of *A. encedon*, *T. bedeguaris*, *T. confusum* and *L. striatellus*. The lineage of these five *Wolbachia* includes those that show different phenotypes, i.e. cytoplasmic incompatibility (*T. confusum* and *L. striatellus*), male killing (*A. encedon*) and parthenogenesis (*F. vespiformis*). The parthenogenesis-inducing *Wolbachia* in *Trichogramma* spp. or *Encarsia formosa* were of different lineages, suggesting multiple occurrence of parthenogenesis-inducing *Wolbachia* in the past.

Wolbachia in eggs are considered to cause segregation failure of the two sets of chromosomes in the first mitotic division of unfertilized eggs (Stouthamer & Kazmer 1994). This results in thelytokous females, which have diploid nuclei. A similar cytological and biochemical mechanism could be involved in the thelytoky in *F. vespiformis*, because both groups have a haplodiploid sex determination system. It is apparent that the eggs that received a certain amount of *Wolbachia* from an antibiotic-treated female developed as females due to the influence of *Wolbachia*. In contrast, the eggs that received no *Wolbachia* or received only a small amount produced male progeny (figure 1). The antibiotic-treated females mostly produced male offspring in the first half of the

ovipositional period. The antibiotic seemed to be metabolized in the treated females and the *Wolbachia* density appeared to recover in the later stage of oviposition, resulting in female production in the later days (figure 1). *Wolbachia* appeared to degenerate greatly in the thrips when tetracycline treatment was applied at both the second larval instar and adult stages, since most of the progeny were males. *Wolbachia* appeared to propagate during post-embryonic development of the progeny from the small population that was transmitted from the mother to her eggs. This is why *Wolbachia* was detected in the male progeny by diagnostic PCR. In contrast, the PCR did not detect *Wolbachia* in some female progeny (18 out of 69) from antibiotic-treated mothers. A similar result was obtained in the parasitic wasp *Telenomus nawai* (Arakaki *et al.* 2000), suggesting that the parthenogenesis is not closely related to the density of *Wolbachia* in some eggs. This phenomenon raises the relevant question of what sort of molecules influence gamete duplication in the first mitotic division.

The males of *F. vespiformis* successfully mated and transmitted motile sperm to the spermathecae of females. However, the mated females produced all-female progeny, which seemed to be due to thelytokous reproduction (table 1). In contrast, antibiotic-treated females produced many male progeny irrespective of mating or non-mating (80.0 and 78.7%, respectively), thus showing no significant difference in the sex ratios between non-mated and mated females. If the mating had been functionally effective, the male ratio in the offspring should have been lower for the mated females than the unmated females. This lack of mating function was further supported by the result that no females appeared from the mated females that were tetracycline treated at both the second larval instar and adult stages (data not shown). In this respect, a similar phenomenon of lack of participation of sperm in egg fertilization has also been reported in a parasitic hymenopteran, *Aphytis lingnanensis* (Zchori-Fein *et al.* 1995). In these cases, it is unclear whether the sperm are non-functional or the eggs lack a certain factor that is essential for fertilization. The latter appears to be the case in *T. nawai*. The eggs from arrhenotokous females of this species were successfully fertilized by the sperm of males that appeared from a thelytokous colony produced by antibiotic treatment, but those from thelytokous females were not (Arakaki *et al.* 2000). Lack of ability to transmit sperm to females has been reported in *E. formosa* (Zchori-Fein *et al.* 1992). In contrast, these reproductive barriers are not observed in *Trichogramma* species. Males from some thelytokous *Trichogramma* species are able to produce offspring normally when mated with their conspecific females (Orphanides & Gonzalez 1970) and antibiotic treatment transforms the thelytokous colony into an arrhenotokous one (Stouthamer *et al.* 1990; Stouthamer & Kazmer 1994). The reason for the inability of successful fertilization in host insects bearing thelytoky-inducing *Wolbachia* is unknown. It will be important to elucidate what kind of reproductive barriers prevent the successful completion of fertilization in *Wolbachia*-mediated parthenogenetic colonies in future studies.

We thank Mr H. Shiroma and Dr Q. Zhang for their assistance in the experiments and to Dr N. Lo for his helpful discussion

regarding the phylogenetic analyses. Gratitude is also extended to anonymous referees for their valuable suggestions. This work was partly supported by a grant from the Program for Promotion of Basic Research Activities for Innovative Bioscience to H.N.

REFERENCES

- Arakaki, N. & Okajima, S. 1998 Notes on the biology and morphology of a predatory thrips, *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Aeolothripidae): first record from Japan. *Entomol. Sci.* **1**, 359–363.
- Arakaki, N., Noda, H. & Yamagishi, K. 2000 *Wolbachia*-induced parthenogenesis in the egg parasitoid *Telenomus nawai*. *Entomol. Exp. Appl.* **96**, 177–184.
- Conte Jr, J. E. & Barriere, S. L. 1988 *Manual of antibiotics and infectious diseases*. Philadelphia, PA: Lea & Febiger.
- Cook, J. M. & Butcher, R. D. J. 1999 The transmission and effects of *Wolbachia* bacteria in parasitoids. *Res. Pop. Ecol.* **41**, 15–28.
- Holden, P. R., Brookfield, J. F. Y. & Jones, P. 1993 Cloning and characterization of a *ftsZ* homologue from a bacterial symbiont of *Drosophila melanogaster*. *Mol. Gen. Genet.* **240**, 213–220.
- Lee, I.-M., Gundersen-Rindal, D. E., Davis, R. E. & Bartoszyk, I. M. 1998 Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int. J. Syst. Bacteriol.* **48**, 1153–1169.
- Lewis, T. 1973 *Thrips—their biology, ecology and economic importance*. New York: Academic Press.
- Luck, R. F., Stouthamer, R. & Nummey, L. 1992 Sex determination and sex ratio patterns in parasitic hymenoptera. In *Evolution and diversity of sex ratio in haplodiploid insects and mites* (ed. D. L. Wrench & M. A. Ebbert), pp. 442–476. New York: Chapman & Hall.
- Moulton, D. 1932 The Thysanoptera of South America. *Rev. Entomol.* **2**, 464–465.
- Namba, S., Kato, S., Iwanami, S., Oyaizu, H., Shiozawa, H. & Tsuchizaki, T. 1993 Detection and differentiation of plant-pathogenic mycoplasma-like organisms using polymerase chain reaction. *Phytopathology* **83**, 786–791.
- Noda, H., Munderloh, U. G. & Kurtti, T. J. 1997 Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl. Environ. Microbiol.* **63**, 3926–3932.
- Okajima, S., Hirose, Y., Kajita, H., Takagi, M., Naponpeth, B. & Buranapanichpan, S. 1992 Thrips on vegetable in South Asia. *Appl. Entomol. Zool.* **27**, 300–303.
- O'Neill, S. L., Giordano, R., Colbert, A. M. E., Karr, T. L. & Robertson, H. M. 1992 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl Acad. Sci. USA* **89**, 2699–2702.
- Orphanides, G. M. & Gonzalez, D. 1970 Identity of a uniparental race of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). *Annals Entomol. Soc. Am.* **63**, 1784–1785.
- Regnery, R. L., Spruill, C. L. & Plikaytis, B. D. 1991 Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J. Bacteriol.* **173**, 1576–1589.
- Simon, C., Franke, A. & Martin, A. 1991 The polymerase chain reaction: DNA extraction and amplification. In *Molecular techniques in taxonomy* (ed. G. H. Hewitt, A. W. B. Johnston & J. P. W. Young), pp. 329–355. Berlin: Springer.
- Sonenshine, D. E. 1993 *Biology of ticks*, vol. 2. New York: Oxford University Press.
- Stouthamer, R. 1997 *Wolbachia*-induced parthenogenesis. In *Influential passengers: inherited microorganisms and invertebrate reproduction* (ed. S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 102–124. New York: 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. & Werren, J. H. 1993 Microbes associated with parthenogenesis in wasps of the genus *Trichogramma*. *J. Invertebr. Pathol.* **61**, 6–9.
- Stouthamer, R., Luck, R. F. & Hamilton, W. D. 1990 Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera: Trichogrammatidae) 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* **631**, 66–68.
- Strimmer, K. & Von Haessler, A. 1996 Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* **13**, 964–969.
- Swofford, D. L. 1999 *PAUP*: phylogenetic analysis using parsimony*, v. 4.0b2. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997 The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucl. Acids Res.* **25**, 4876–4882.
- Weisburg, W. G. S., Barns, S. M., Pelletier, D. A. & Lane, D. L. 1991 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.
- Werren, J. H. 1997 Biology of *Wolbachia*. *A. Rev. Entomol.* **42**, 587–609.
- Werren, J. H., Zhang, W. & Guo, L. R. 1995 Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc. R. Soc. Lond. B* **261**, 55–71.
- Williamson, D. L. (and 12 others) 1998 Revised group classification of the genus *Spiroplasma*. *Int. J. Syst. Bacteriol.* **48**, 1–12.
- Zchori-Fein, E., Rousch, R. T. & Hunter, M. S. 1992 Male production induced by antibiotic treatment in *Encarsia formosa*, an asexual species. *Experientia* **48**, 102–105.
- Zchori-Fein, E., Faktor, O., Zeidan, M., Gottlieb, Y., Czosnek, H. & Rosen, D. 1995 Parthenogenesis-inducing microorganisms in *Aphis* (Hymenoptera: Aphelinidae). *Insect Mol. Biol.* **4**, 173–178.
- Zhou, W., Rousset, F. & O'Neill, S. 1998 Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc. R. Soc. Lond. B* **265**, 509–515.