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Wolbachia-induced parthenogenesis in a genus of phytophagous mites

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The vertically transmitted endosymbiotic bacterium *Wolbachia* modifies host reproduction in several ways in order to enhance its own spread. One such modification results in the induction of parthenogenesis, where males, which are unable to transmit *Wolbachia*, are not produced. Interestingly, parthenogenesis-inducing *Wolbachia* have only been found within haplodiploid insects and it is not known whether this exclusivity is the result of functional constraints of *Wolbachia*. Here we find a unique pattern of *Wolbachia* infection that is associated with parthenogenesis in six species within the phytophagous mite genus *Bryobia*. Through antibiotic treatment we show that, in two species, *Bryobia praetiosa* and an unidentified species, the *Wolbachia* infection is strictly associated with parthenogenesis. Microsatellite loci show the mechanism of parthenogenesis to be functionally apomictic and not gamete duplication, with progeny identical to their infected mother. Crossing experiments within *B. praetiosa* showed no evidence of sexual reproduction. These results are discussed with reference to the distribution of parthenogenesis-inducing *Wolbachia* and the diversification of the *Bryobia* genus.

Keywords: *Wolbachia*; parthenogenesis; *Bryobia*; apomictic; speciation

1. INTRODUCTION

The role that endosymbionts play in evolutionary processes has been largely underestimated. Over the last decade endosymbionts have been implicated in causing various reproductive abnormalities (Stouthamer *et al.* 1999), altering sex determination systems (Rigaud *et al.* 1997), causing changes in ploidy levels and life cycles (Weeks *et al.* 2001) and playing a role in incipient speciation (Breeuwer & Werren 1990; Bordenstein *et al.* 2001). The bacterium *Wolbachia* is the best known of these endosymbionts due to its high prevalence in invertebrates (Jeyaprakash & Hoy 2000; Werren & Windsor 2000) and the many reproductive effects it can induce in its hosts. These reproductive phenotypes include cytoplasmic incompatibility, parthenogenesis induction, the feminization of genetic males and male killing (see Stouthamer *et al.* (1999) for a review of these phenotypes).

Wolbachia are transmitted from host to offspring maternally and, thus, their evolutionary interests lie in improving transmission through females. Each of the reproductive phenotypes results in its spread. However, parthenogenesis induction is the ultimate reproductive manipulation in terms of transmission and spread. Provided vertical transmission is 100%, an infected adult female will produce only infected female progeny, which will in turn produce only infected daughters. Therefore, there is no waste in male production that, in this case, cannot transmit *Wolbachia*. As long as an infected female produces more daughters than an uninfected female, then the infection is expected to spread through a population (Stouthamer 1997).

Interestingly, until now parthenogenesis-inducing *Wolbachia* strains have been restricted to insects and found within the order Hymenoptera (Zchori-Fein *et al.* 1992; Stouthamer 1997; Plantard *et al.* 1998) and recently in a species of thrips (Arakaki *et al.* 2001). This may be due to the mechanism by which they induce parthenogenesis. In the haplodiploid parasitic wasp *Trichogramma* the cytogenetic mechanism of *Wolbachia*-induced parthenogenesis is known to be gamete duplication. Normal meiosis is followed by a fusion of the two nuclei of the first mitotic division, thus restoring diploidy (Stouthamer & Kazmer 1994). This effectively makes all progeny homozygous at all loci throughout their genome. Two things are common in all known cases in which *Wolbachia* induce parthenogenesis in the Hymenoptera and where the mechanism of parthenogenesis has been determined: (i) they have haplodiploid sex determination, where unfertilized eggs develop into haploid males while fertilized eggs develop into diploid females and (ii) diploidy is restored through gamete duplication (Stouthamer *et al.* 1999). Therefore, due to the way in which they induce parthenogenesis, it is possible that parthenogenesis-inducing *Wolbachia* strains are exclusive to haplodiploid reproductive systems.

Wolbachia that induce cytoplasmic incompatibility have been inferred as a possible mechanism of speciation by promoting reproductive isolation between host populations (Werren 1998). For example, laboratory experiments with *Nasonia* wasps have demonstrated that *Wolbachia*-induced bidirectional incompatibility between species prevents the formation of hybrids in interspecies matings (Breeuwer & Werren 1990; Bordenstein *et al.* 2001). However, while parthenogenesis-inducing *Wolbachia* effectively induce reproductive isolation within their hosts, their involvement in speciation has only been discussed briefly (Werren 1998). This is probably due to current views on both species concepts of parthenogenetic organisms (Mayr 1963) and the ability of obligate parthenogens to evolve (Darlington 1939; White 1973; Maynard Smith 1978). Nevertheless, parthenogenesis-inducing *Wolbachia* have the potential for generating isolated genotypes/clones rapidly, which, under certain circumstances, could diverge rapidly in both the phenotype and genotype.

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A speciose genus of haplodiploid phytophagous mites, the *Bryobia*, exists within the family of spider mites (Tetranychidae). Approximately 100 nominal species have been described based on their morphology, with many host generalists and specialists found. While little is known about these mites, most are thought to reproduce by thelytokous parthenogenesis (Norton *et al.* 1993; Bolland *et al.* 1998). In fact males have only been reported in two species (Norton *et al.* 1993). A genus consisting of this many parthenogenetic species is highly unusual within the animal kingdom because parthenogenetic species are thought to be evolutionary dead ends that are incapable of evolution (Maynard Smith 1978).

Here we screen six species of *Bryobia* that reproduce by thelytokous parthenogenesis for infection by *Wolbachia*. We then investigate the effects of infection by *Wolbachia* in two of these species, *Bryobia praetiosa* and an undescribed species. *Wolbachia* is shown to be strictly associated with parthenogenesis in both cases. Finally, we investigate (i) the mechanism of *Wolbachia*-induced parthenogenesis and (ii) sperm use by parthenogenetic females in *B. praetiosa*.

2. MATERIAL AND METHODS

(a) Bryobia *collection and DNA extraction*

Samples of *Bryobia* were collected from various host plants within Amsterdam, The Netherlands and identified by Hans Bolland (University of Amsterdam, The Netherlands) based on their morphology. One species remained unidentified and is called *B.* sp. *x* hereafter. Samples of two species, *Bryobia kissophila* and *B. praetiosa*, were collected from three and two additional sites, respectively, within The Netherlands. All samples were frozen at −80 °C until DNA extraction. Genomic DNA was extracted from individual *Bryobia* using the cetyltrimethylammonium bromide (Sigma) method adapted for mites from Weeks *et al.* (2000). A negative control (no mites) was included in each DNA extraction.

Five immature females of *B. praetiosa* from each of three populations (Amsterdam, Enkhuizen and Zaanse Schans) and five immature females of *B.* sp. *x* collected in Amsterdam were also reared as isofemale lines in the laboratory. All 20 lines were reared on detached leaves of the common bean (*Phaseolus vulgaris*, variety 'Arena') in climate rooms (23 °C, 60–80% relative humidity and 16 L:8 D photoperiod). No males were found in any of the lines after five generations, thereby confirming the mode of reproduction to be thelytokous parthenogenesis in both species. These lines were used for the experiments in §s 2c, 2e and 2f.

(b) Wolbachia *PCR assay*

The presence of *Wolbachia* within a *Bryobia* mite was assessed by polymerase chain reaction (PCR) amplification using *Wolbachia*-specific primers. The *ftsZ* primers (491F and 1262R), which amplify 730 bp of the cell division gene (Holden *et al.* 1993) and the primer pairs *wsp*81F and *wsp*619R (Zhou *et al.* 1998), which amplify 590–632 bp of the *wsp* gene, were used in separate PCR amplifications. We used two sets of *Wolbachia*-specific primers in separate reactions for screening samples in order to avoid false negatives. The PCRs were performed in a total volume of 25 µl containing 1 X Super Taq buffer (Sphaero Q, Leiden, The Netherlands), 0.5 mM of each dNTP, 1 unit of Super Taq (Sphaero Q) and 5 µM of each primer. An aliquot of 2.5 mM $MgCl_2$ was added to each reaction for *ftsZ*, while 1.5mM $MgCl_2$ was added for *wsp*. Both amplifications were run on an MJ Research thermal cycler (Waltham, MA, USA) under the following conditions: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, at 55 °C for 1 min and at 72 °C for 1 min and one cycle at 72 °C for 5 min. A negative control was also included in both PCR reactions in which no DNA was added to the PCR mix. Ten microlitres of amplified reaction product was run on a 1.5% agarose gel stained with ethidium bromide after the PCRs in order to determine the presence of an amplified DNA fragment. If a sample was negative for both *Wolbachia*-specific amplifications, then the ITS2 region of the host was amplified using the primers, PCR reactions and cycling parameters from Navajas *et al.* (1998). If this did not yield an amplified product of *ca.* 600 bp, then failure of DNA extraction was assumed and the sample was not included in any further analysis.

(c) *Antibiotic treatment of adult* B. praetiosa *and* B. *sp.* x

One hundred adult female *B. praetiosa* and 100 adult female *B.* sp. *x* were collected from an egg cohort (50 female mites from an isofemale line of each species in §2 a were allowed to lay eggs for 1 day and then removed) 2 days after their final moult. Sixty adults from each species were then treated with the antibiotic tetracycline. Leaf discs (1 cm diameter) of the common bean *Phaseolus vulgaris* were floated in a solution of 0.15% tetracycline hydrochloride and distilled water and three adult mites per leaf disc were left to feed for 4 days at constant temperature (25 °C) and humidity (60%) under a photoperiod of 16 L:8 D. At the same time 40 adults from each species were set up in exactly the same way, except that no tetracycline was added to the distilled water, to act as controls. The mites were transferred to fresh leaf discs after 4 days and the respective solutions for a further 4 days. The tetracycline-treated mites as well as the control mites were transferred to leaves of *P. vulgaris* after 8 days and left to recover for 24 h at 25 °C (60% humidity and 16 L:8 D photoperiod). Surviving mites from each treatment were then set up as follows: one mite per leaf disc (2 cm diameter) floated on cotton wool soaked in water at 25 °C. The mites were left to lay eggs for 10 days, at which time adults were taken from each leaf disc, leaving the leaf discs and developing eggs at 25 °C. The sex of the progeny was scored upon reaching the adult stage (*ca.* 2 weeks). Samples of the progeny of both species from the tetracycline and control treatments were tested for infection by *Wolbachia* using the protocol outlined in § 2b.

(d) *Microsatellite isolation and amplification*

We screened a library of randomly amplified polymorphic DNA (RAPD) from *B. praetiosa* in order to isolate microsatellite loci using the PCR-based isolation of microsatellite arrays method of Lunt *et al.* (1999). The RAPD library was created using four oligonucleotide primers from the H kit (Operon Technologies, Crawley, West Sussex, UK). The products were then ligated into a pGEM T-vector. Screening was performed as described in Lunt *et al.* (1999) using an unanchored $(CT)_{10}$ oligonucleotide probe in a PCR reaction. Microsatellite loci were extremely abundant in *B. praetiosa*, with a total of 13 positive clones found from 210. The primers were designed for eight loci, but only three gave clear polymorphic amplification products (table 1). The PCR amplifications for each microsatellite locus were performed in a total volume of 20 µl containing 1 X Super Taq buffer (Sphaero Q), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 units of Super Taq polymerase (Sphaero Q) and 5 pmol of each primer. One primer for each microsatellite locus was 5′-end labelled with γ-^{32}P dATP using polynucleotide kinase (NEB,

Table 1. Microsatellite loci isolated from *B. praetiosa*.

(The table shows the primers used, class of repeat array, initial fragment size with flanking regions, annealing temperature (T_a) and Genbank accession numbers.)

locus	primer sequence 5′→3′	repeat array	fragment size (bp)	T_a (°C)	Genbank accession number
Bry1	F – GTAGCGTGATTCATAGATGATCC R – CAGAATTCTTCAGCTACAGGTTC	$(AG)_{21}$	224	55	AY044829
Bry2	F – CAGGTAATATCGATCCCAAAC R – GGATCATTGAGCAAACCAATAC	$(AG)_{30}$	165	55	AY044830
Bry3	F – GTTTCGCTTCTTTTCATTCACG R – CCCATCATTTTAACGCCATCA	$(AG)_{24}$	136	54	AY044831

Beverly, MA, USA). The PCR was run on an MJ Research thermal cycler under the following conditions: one cycle at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, at 54 °C for 30 s and at 72 °C for 30 s and one cycle at 72 °C for 5 min. The products were separated electrophoretically in a denaturing polyacrylamide gel (6%), and then exposed to an autoradiographic film.

(e) *Mode of parthenogenesis in* B. praetiosa

In order to assess the mode of parthenogenesis in *B. praetiosa*, we used the microsatellites developed in § 2d for comparing the genotypes of adults and their offspring, as well as the offspring produced from tetracycline-treated adults. For instance, if the mode of parthenogenesis were gamete duplication then we would expect only homozygous progeny for any microsatellite locus from an infected female. However, if the mode of parthenogenesis were functionally apomictic, then we would expect heterozygosity to be maintained in the progeny from an infected heterozygous female. Five immature females were reared in isolation on leaf discs (2 cm diameter) floated on cotton wool soaked in water at 25 °C (60% humidity and 16 L:8 D photoperiod). After *ca.* 20 eggs were laid per female, the adults were removed and frozen at −80 °C until DNA extraction. The progeny were then reared through to the adult stage and surviving mites were frozen at −80 °C. In addition, four adult females were treated with tetracycline as in § 2c and then reared in isolation as previously until *ca.* 20 eggs were laid per female and subsequently frozen at −80 °C. The progeny were reared through to the adult stage and surviving mites were also frozen at −80 °C. DNA was extracted from all frozen mites as in § 2a and genotyped for three microsatellite loci.

(f) *Sperm use by parthenogenetic females of* B. praetiosa*: testing for sexual reproduction*

In order to determine whether parthenogenetic females of *B. praetiosa* are able to accept and use sperm from males produced by tetracycline-treated females, pair matings were performed and F_1 offspring were obtained. The maternal and paternal lines were chosen such that they carried different alleles for one or more of the three microsatellite loci. Males for the paternal lines were generated as in § 2c. Ten females from an isofemale line collected from Enkhuizen, The Netherlands, were pair mated to 10 males generated from an isofemale line collected from Amsterdam, The Netherlands (see § 2a). Copulation was confirmed at least once for all 10 single pair matings. The F_1 offspring were then genotyped in order to determine their maternal and/or paternal alleles.

3. RESULTS

(a) Wolbachia *infection frequency within* Bryobia

Six species of *Bryobia* were collected from different host plants within Amsterdam, The Netherlands, with one species unable to be identified (table 2). All six species tested positive for infection with *Wolbachia*. In fact, all mites tested from each species were infected with *Wolbachia* (table 2). All individuals from two additional populations of *B. praetiosa* (collected from Enkhuizen and Zaanse Schans) and three additional populations of *B. kissophila* (collected from Enkhuizen, Utrecht and Groningen) also tested positive for *Wolbachia*. No uninfected *Bryobia* mites were found in a total of 516 tested from 11 populations. The five identified species of *Bryobia* are known to reproduce by thelytokous parthenogenesis (Norton *et al.* 1993) and we also confirmed this for the unidentified species *B.* sp. *x*, as well as reconfirming it for *B. praetiosa*. No males were found in any collections. The data suggest an association between infection with *Wolbachia* and thelytokous parthenogenesis in *Bryobia*.

(b) *Tetracycline treatment*

Tetracycline-treated females from *B. praetiosa* and *B.* sp. *x* produced significantly more male progeny than the control treatments (one-way analysis of variance (ANOVA) after arcsine transformation, $F_{1,74} = 172.85$ and $p < 0.001$ and $F_{1,73} = 161.31$ and $p < 0.001$, respectively) (table 3). Approximately 50% of the progeny were male (ranging from 0 to 100% male progeny from an individual tetracycline-treated female) for both species, while no males were found in either of the control treatments (100% vertical transmission of *Wolbachia*). Sexual *Bryobia* species are known to reproduce by haplodiploidy, where unfertilized eggs develop as haploid males while fertilized eggs develop into diploid females. This reproductive mode is characteristic of their family, the Tetranychidae. Therefore, if *Wolbachia* were causing the thelytokous parthenogenesis within *B. praetiosa* and *B.* sp. *x*, we would expect that curing the infection would lead to male production as their eggs would be unfertilized and, therefore, haploid. However, we find here that female progeny are still produced by tetracycline-treated females. If the adults were fully cured by tetracycline treatment then we would not expect to find any female progeny. A possible reason for this result is that there is not a 100% cure rate

Table 2. *Wolbachia* infection status in six species of mites from the genus *Bryobia*.

(All species were collected from various sites and hosts throughout The Netherlands. The host plant from which *B.* sp. *x* was collected was not identified.)

species	collection site	host plant	reproductive mode	individuals screened for *Wolbachia*	individuals infected with *Wolbachia*
B. kissophila	Amsterdam	*Hedera helix* (English ivy)	asexual	96	96
	Enkhuizen	*Hedera helix*	asexual	48	48
	Utrecht	*Hedera helix*	asexual	48	48
	Groningen	*Hedera helix*	asexual	48	48
B. praetiosa	Amsterdam	*Holcus lanatus* (grass)	asexual	72	72
	Enkhuizen	*Holcus lanatus*	asexual	48	48
	Zaanse Schans	*Holcus lanatus*	asexual	24	24
B. graminum	Amsterdam	*Lolium perenne* (ryegrass)	asexual	48	48
B. rubrioculus	Amsterdam	*Malus domesticus* (apple tree)	asexual	24	24
B. neopraetiosa	Amsterdam	*Trifolium* spp. (clover)	asexual	36	36
B. sp. *x*	Amsterdam	—	asexual	24	24

Table 3. Sex ratio of the progeny produced by *B. praetiosa* and *B.* sp. *x* females treated with tetracycline.

(Females were treated with 0.15% tetracycline hydrochloride for 6 days and left to lay eggs for 10 days. $^{*}p < 0.001$ (one-way ANOVA after arcsine transformation).)

species	treatment	number of females	mean number of progeny per female ± s.d.	% female progeny	% male progeny
B. praetiosa	tetracycline	41	24.02 ± 20.81	51.6	48.4*
	control	35	32.50 ± 7.89	100.0	0.0
B. sp. *x*	tetracycline	40	16.33 ± 10.42	50.1	49.9*
	control	35	23.58 ± 6.80	100.0	0.0

and that *Wolbachia* are still transmitted to some progeny, which develop as females. In order to test this, we checked a sample of male and female progeny for infection by a PCR for both species. All female progeny tested from tetracycline-treated adults of *B. praetiosa* and *B.* sp. *x* (80 and 72, respectively) were positive for infection with *Wolbachia*, while all male progeny tested from tetracycline-treated females of *B. praetiosa* and *B.* sp. *x* (52 and 36, respectively) were negative for *Wolbachia* infection. All female progeny tested from control treatments for *B. praetiosa* and *B.* sp. *x* (72 and 72, respectively) were also positive for infection with *Wolbachia*. These results show a strong causal relationship between *Wolbachia* infection and thelytokous parthenogenesis in *B. praetiosa* and *B.* sp. *x*.

(c) *Mode of parthenogenesis in* B. praetiosa

The method of *Wolbachia*-induced parthenogenesis in *B. praetiosa* appears to be functionally apomictic, with the maternal genotypes of three microsatellite loci passed unchanged to all progeny (table 4). All female parents were heterozygous for at least two loci and so all were informative with respect to the mechanism of parthenogenesis. Vertical transmission of *Wolbachia* was again 100%, with no male progeny from females that were not treated with tetracycline (females 1–5 in table 4). All four tetracycline-treated females produced both male and female progeny (females 6–9 in table 4). The female progeny were identical to their mother in genotype (maintaining heterozygosity). Only one allele was found at a locus for all male progeny and alleles from a heterozygous locus in a parent segregated in a 1:1 ratio in male progeny (table 4). This confirms haploidy in male *B. praetiosa*. Further work, including cytological experiments, is needed in order to distinguish between apomictic parthenogenesis and premeiotic doubling as the mechanism of *Wolbachia*-induced parthenogenesis. Functionally, both lead to the maternal genotype being passed on to all offspring. However, one is essentially a mitotic division while the other is meiotic.

(d) *Sperm use by parthenogenetic females of* B. praetiosa*: testing for sexual reproduction*

Wolbachia-induced parthenogenetic females of *B. praetiosa* copulated with males (in the same way as other mite species of the Tetranychidae family copulate), but did not use their sperm for fertilizing their eggs. All progeny from 10 single pair matings showed maternal genotypes (table 5), with only female progeny produced. No paternal alleles were found in their progeny. Therefore, there was no evidence that parthenogenetic females and cured males could undergo sexual reproduction.

4. DISCUSSION

The results presented here show a unique pattern of *Wolbachia* infection, which is associated with parthenogenesis in the mite genus *Bryobia*. We have shown that, for two species, *B. praetiosa* and *B.* sp. *x*, this infection has a

Table 4. Genotypes of nine *B. praetiosa* females and their parthenogenetically produced progeny for three microsatellite loci.

(Alleles are indicated by their length in base pairs (bp), with males being haploid and therefore carrying only one allele per locus. Females 1–5 were not treated with antibiotics while females 6–9 were treated with tetracycline as in § 2b. Numbers in parentheses indicate the number of progeny with that genotype.)

female	tetracycline treatment	microsatellite locus	parental genotype (bp)	progeny genotypes	
				female	male
1–4	no	Bry1	214/240	214/240 (76)	—
		Bry2	157/161	157/161 (76)	—
		Bry3	144/148	144/148 (76)	—
5	no	Bry1	240/248	240/248 (21)	—
		Bry2	151/167	151/167 (21)	—
		Bry3	144/144	144/144 (21)	—
6	yes	Bry1	214/240	214/240 (8)	214 (6)
					240 (4)
		Bry2	157/161	157/161 (8)	157 (5)
					161 (5)
		Bry3	144/148	144/148 (8)	144 (6)
					148 (4)
7	yes	Bry1	214/240	214/240 (11)	214 (4)
					240 (4)
		Bry2	157/161	157/161 (11)	157 (4)
					161 (4)
		Bry3	144/148	144/148 (11)	144 (5)
					148 (3)
8	yes	Bry1	240/248	240/248 (13)	240 (3)
					248 (4)
		Bry2	151/167	151/167 (13)	151 (3)
					167 (4)
		Bry3	144/144	144/144 (13)	144 (7)
9	yes	Bry1	214/240	214/240 (14)	214 (3)
					240 (2)
		Bry2	157/161	157/161 (14)	157 (3)
					161 (2)
		Bry3	144/148	144/148 (14)	144 (4)
					148 (1)

Table 5. Genotypes of progeny at three microsatellite loci from crosses between two strains of *B. praetiosa*.

(The parental genotypes for loci Bry1, Bry2 and Bry3 are for maternal A = 214/240, 157/161 and 144/148 and for paternal B = 248, 151 and 144, paternal C = 248, 167 and 144 and paternal D = 240, 151 and 144, respectively. All progeny alleles were from maternal origin. All progeny carried the maternal genotype A, with no paternal alleles observed.)

cross number	parental genotypes		number of male progeny	number of female progeny	progeny genotypes at locus			number per genotype
	female	male			Bry1	Bry2	Bry3	
1	A	B	0	10	214/240	157/161	144/148	10
2	A	B	0	12	214/240	157/161	144/148	12
3	A	B	0	15	214/240	157/161	144/148	15
4	A	C	0	15	214/240	157/161	144/148	15
5	A	C	0	15	214/240	157/161	144/148	15
6	A	B	0	15	214/240	157/161	144/148	15
7	A	D	0	8	214/240	157/161	144/148	8
8	A	D	0	15	214/240	157/161	144/148	15
9	A	C	0	15	214/240	157/161	144/148	15
10	A	D	0	12	214/240	157/161	144/148	12

causal relationship with thelytokous parthenogenesis. Although we have not tested this here, it is likely that, in the other four species, i.e. *Bryobia graminum*, *Bryobia rubrioculus*, *Bryobia neopraetiosa* and *Bryobia kissophila*, the *Wolbachia* infection also induces thelytokous parthenogenesis.

In all cases so far established within the Hymenoptera, *Wolbachia* infection generally results in gamete duplication as the cytogenetic mechanism for the restoration of diploidy (Stouthamer 1997). This mechanism is relatively rare within parthenogenetic arthropods (Suomalainen

et al. 1987) and is generally associated with *Wolbachia* infection and the Hymenoptera. The mechanism of parthenogenesis in *B. praetiosa* is not gamete duplication, but functionally apomictic, with all progeny identical in genotype to their mother and heterozygosity being maintained. This has important implications for parthenogenesis-inducing *Wolbachia* and the distribution of their hosts. It has been thought that parthenogenesis-inducing *Wolbachia* may be functionally restricted to haplodiploid systems (Stouthamer 1997; Stouthamer *et al.* 1999). Even though we have also found parthenogenesis-inducing *Wolbachia* in another haplodiploid species, the mechanism of parthenogenesis is different and suggests that they do not have to be restricted to haplodiploid systems. For example, apomictic parthenogenesis is the most common form of parthenogenesis within diplo-diploid arthropods (Suomalainen *et al.* 1987). Although it would be difficult to prove the presence of parthenogenesis-inducing *Wolbachia* within a diplodiploid species (as unfertilized eggs would not develop), our results suggest that functionally they can be responsible.

Parthenogenesis-inducing *Wolbachia* has been found in over 40 species of Hymenoptera, with 13 of these being from the genus *Trichogramma*. The genus *Trichogramma* presents an interesting case because, unlike most other infected Hymenopteran species, fixation of parthenogenesis-inducing *Wolbachia* is relatively rare within a species (Stouthamer 1997). Many *Trichogramma* species can maintain both arrhenotokous (uninfected) and thelytokous (infected) forms, with both still capable of sexual reproduction. Uninfected males can mate with infected females and cured females can mate with uninfected males. In most other Hymenopteran species infected with parthenogenesis-inducing *Wolbachia*, infected individuals have lost the ability to undergo sexual reproduction (Zchori-Fein *et al.* 1992, 2000; Stouthamer 1997; Hunter 1999; Stary 1999).

We found no evidence that *B. praetiosa* was capable of sexual reproduction in this study. Infected individuals were observed to copulate with cured males. However, no genetic recombination was evident in their progeny, with all progeny having identical genotypes to their mother. There are a number of reasons why sexual reproduction may not have occurred here, including the following.

(i) *Bryobia praetiosa* may have lost the ability to reproduce sexually due to relaxation of selection for sex and subsequent deterioration of either male or female traits governing sexual reproduction (Pijls *et al.* 1996). In Hymenopteran species where infection frequencies are as high as those we have found here, loss of sexual reproduction is quite common (Stouthamer 1997).

(ii) The strains crossed may have been too genetically dissimilar and, therefore, no fertilization took place. The strains were collected from two geographically distinct populations and were chosen because they carried different alleles at all three microsatellite loci. This may have rendered them incapable of successful fertilization.

(iii) The males used in our experiments may have been sterile due to an incomplete cure rate of *Wolbachia*. Intersexes, where the offspring are partly male and partly female, are commonly found in other parthenogenesis-inducing *Wolbachia*-infected hosts (Stouthamer *et al.* 1999). Our males may have appeared morphologically male, but may have been missing the vital organs necessary for sexual reproduction.

(iv) Functionally, sexual reproduction between infected females and cured males may not be possible due to the cytogenetic mechanisms of parthenogenesis induction. If the mechanism precludes meiosis (i.e. it is purely apomictic) then fertilization may be redundant.

We cannot distinguish between any of the above four hypotheses, as all could be equally possible. Further work from either a phylogenetic perspective, population genetic perspective or more intensive crossing experiments is needed before we can accurately determine whether *B. praetiosa* is capable of sexual reproduction.

Our results raise questions that are important in both *Wolbachia* research and evolution within parthenogenetic organisms. First, how many species in the genus *Bryobia* are infected with parthenogenesis-inducing *Wolbachia* and are the infections fixed? We have found that samples of *B. kissophila* and *B. praetiosa* from various populations collected from Australia, North America and three additional countries in Europe are also fixed for infection by *Wolbachia*, with no males found (A. R. Weeks and J. A. J. Breeuwer, unpublished data). This provides strong evidence that, in these two species, infection by parthenogenesis-inducing *Wolbachia* is fixed. Little is known about the reproductive biology for many of the species within the *Bryobia* genus. However, males have been found in only two out of 100 nominal species (Bolland *et al.* 1998). It is possible and likely that many more *Bryobia* species are infected with parthenogenesis-inducing *Wolbachia*. This raises the possibility that *Wolbachia* is a causal agent in the radiation of the *Bryobia* genus.

Finally, parthenogenetic species have long been labelled as evolutionary dead ends that are incapable of evolving (Darlington 1939; White 1973; Maynard Smith 1978). These extant theories are based on the perception that most parthenogenetic taxa have close sexual relatives and are short-lived on an evolutionary time-scale. Yet much evidence now exists contrary to these theories (Weeks & Hoffmann 1998; Welch & Meselson 2000) and here we have a genus that possibly supports the view that parthenogenetic taxa can be evolutionarily successful, at least in the short term. Understanding how this genus has diversified can potentially provide insight into the evolutionary consequences of parthenogenesis and the adaptive significance of sexuality.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.