Characteristics, Phenotype, and Transmission of *Wolbachia* in the Sweet Potato Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), and Its Parasitoid *Eretmocerus* sp. nr. *emiratus* (Hymenoptera: Aphelinidae)

ELAD CHIEL,[1,2,3] SUZANNE E. KELLY,[2] ALEXANDRE M. HARRIS,[2] MARCO GEBIOLA,[2,4] XIANCHUN LI,[2] EINAT ZCHORI-FEIN,[5] AND MARTHA S. HUNTER[2]

Environ. Entomol. 43(2): 353–362 (2014); DOI: http://dx.doi.org/10.1603/EN13286

ABSTRACT *Wolbachia* is a common intracellular bacterial endosymbiont of insects, causing a variety of effects including reproductive manipulations such as cytoplasmic incompatibility (CI). In this study, we characterized *Wolbachia* in the whitefly *Bemisia tabaci* and in the whitefly parasitoid *Eretmocerus* sp. nr. *emiratus*. We also tested for horizontal transmission of *Wolbachia* between and within trophic levels, and we determined the phenotype of *Wolbachia* in *E.* sp. nr. *emiratus*. Using multilocus sequence typing and phylogenetic analyses, we found that *B. tabaci* and *E.* sp. nr. *emiratus* each harbor a different and unique strain of *Wolbachia*. Both strains belong to the phylogenetic supergroup B. No evidence for horizontal transmission of *Wolbachia* between and within trophic levels was found in our study system. Finally, crossing results were consistent with a CI phenotype; when *Wolbachia*-infected *E.* sp. nr. *emiratus* males mate with uninfected females, wasp progeny survival dropped significantly, and the number of females was halved. This is the first description of CI caused by *Wolbachia* in the economically important genus *Eretmocerus*. Our study underscores the expectation that horizontal transmission events occur rarely in the dynamics of secondary symbionts such as *Wolbachia*, and highlights the importance of understanding the effects of symbionts on the biology of natural enemies.

KEY WORDS cytoplasmic incompatibility, *Encarsia*, multilocus sequence typing, symbiont

Wolbachia (α-Proteobacteria) is arguably the most common bacterial symbiont, estimated to infect ≈40% of terrestrial arthropod species (Zug and Hammerstein 2012) and 66% of insect species (Hilgenboecker et al. 2008). This maternally inherited, intracellular symbiont is traditionally divided into eight supergroups, designated A–H, based on phylogenetic analysis of sequences from the 16S rRNA and the *ftsZ* and *wsp* genes. In 2006, Baldo et al. launched the *Wolbachia* multilocus sequence typing (MLST)—a robust classifying system that provides strain typing based on variation in five conserved housekeeping genes (*ftsZ*, *gatB*, *coxA*, *hcpA*, and *fbpA*), and is the current standard for *Wolbachia* identification (Baldo et al. 2006).

Wolbachia is known for the multiple ways by which it promotes its own transmission, most notably the reproductive manipulations that result in an increased proportion or fitness of *Wolbachia*-carrying females (Werren et al. 2008). Types of reproductive manipulation include cytoplasmic incompatibility (CI), parthenogenesis, feminization, and male-killing. In CI, uninfected females produce few or no offspring when they mate with infected males, thereby providing a selective advantage to infected females. In haplodiploid insects, CI results in a male-biased sex ratio, as fertilized, incipient female eggs will either die or lose the paternal set of chromosomes and develop into male progeny. Besides reproductive manipulation, *Wolbachia* is an obligate nutritional symbiont in filarial nematodes and bed bugs (Hosokawa et al. 2010, Saridaki and Bourtzis 2010) and in other cases where it is facultative, may contribute to the fitness of hosts by enhancing pathogen resistance or nutrient provisioning (Hedges et al. 2008, Brownlie et al. 2009, Saridaki and Bourtzis 2010).

Here we studied *Wolbachia* sequence types (STs), transmission, and phenotype in the sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and in its parasitoid *Eretmocerus* sp. nr. *emiratus* (Zolnerowich and Rose) (Hymenoptera: Aphelinidae).

The sweet potato whitefly, *B. tabaci*, feeds on phloem sap of numerous host plants and is a major pest

[1] Current Address: Department of Biology and Environment, University of Haifa-Oranim, Tiv'on, Israel.
[2] Department of Entomology, 410 Forbes Bldg., University of Arizona, Tucson, AZ.
[3] Corresponding author, e-mail: elad_c@oranim.ac.il.
[4] CNR–Istituto per la Protezione delle Piante, UOS di Portici, Portici (NA), Italy.
[5] Agricultural Research Organization, Newe-Ya'ar Research Center, Ramat-Yishay, Israel.

0046-225X/14/0353–0362$04.00/0

of agricultural crops (Stansly and Naranjo 2010). It is currently recognized as a complex of at least 24 distinct genetic groups, many of which are equivalent to the "biotypes" of earlier works (De Barro et al. 2011). This species complex exhibits variation in a wide range of biological characteristics, including host range, virus transmission capacities, and insecticide resistance (Idris et al. 2001, Jiao et al. 2013, Su et al. 2013). The B species (=Middle East-Asia Minor 1) and Q species (=Mediterranean) are frequently sympatric; the B species is highly invasive and competitive, whereas the Q species is less invasive but resistant to several insecticides (Pascual and Callejas 2004, Horowitz et al. 2005, Dennehy et al. 2010).

B. tabaci harbors the obligate primary symbiont, *Portiera aleyrodidarum*, which produces amino acids and carotenoids lacking in the phloem (Santos-Garcia et al. 2012, Sloan and Moran 2012). Additionally, *B. tabaci* may be associated with various facultative bacterial secondary symbionts including *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Hemipteriphilus*, *Rickettsia*, and *Wolbachia*. The prevalence of such facultative symbionts is strongly correlated with whitefly genetic background: *Wolbachia*, for example, was found to infect many of the Q but not B populations (Chiel et al. 2007, Gueguen et al. 2010, Skaljac et al. 2010), with the exception of reports of *Wolbachia*-infected B populations in China (Ahmed et al. 2010). The function of secondary symbionts in *B. tabaci* is for the most part unknown, but available data suggest that they have variable effects on the fitness of their hosts (Kontsedalov et al. 2008, Himler et al. 2011, Xue et al. 2012).

Interspecific horizontal transmission of secondary symbionts ("horizontal transmission" in its evolutionary sense, that is, transmission that results in a stable heritable infection) has been inferred from phylogenetic data (Russell et al. 2003, Viljakainen et al. 2008, Kraaijeveld et al. 2011, Ahmed et al. 2013). Observations of interspecific horizontal transmission, however, are quite scarce. Transfection experiments by artificial means, mostly microinjection, have been variably successful, sometimes resulting in a stable heritable infection in the new host, and in other cases, infections are in very low titer and may be lost after several generations (Braig et al. 1994, Kang et al. 2003, Russell and Moran 2005, Kawai et al. 2009). By natural means, interspecific symbiont transmission has been documented between parasitoids developing in the same host (Huigens et al. 2004, Duron et al. 2010) and between hosts and the parasitoids developing on them (Heath et al. 1999, Chiel et al. 2009), but in all these cases, subsequent stable vertical transmission did not occur or diminished within 2–3 generations, suggesting that in nature, these events occur very rarely on an ecological timescale.

One of the potentially promising routes for horizontal transmission is between hosts and endoparasitoids that spend their immature stages bathed in and feeding on host contents. Phylogenetic analyses confirm this means of horizontal transmission (Vavre et al. 1999, Zchori-Fein et al. 2004). Parasitoids of the genera *Eretmocerus* and *Encarsia* feed and develop in *B. tabaci* nymphs and are potentially vulnerable to whitefly symbiont infections. Previously, we found that during development of *Er.* sp. nr. *emiratus* in *Rickettsia*-infected *B. tabaci* hosts, *Rickettsia* is initially found in the larval wasp gut, and later is found in the ovaries of female parasitoids, but fails to penetrate the oocytes (Chiel et al. 2009). Interestingly, in another parasitoid in the genus *Encarsia*, we found no evidence that *Rickettsia* ever escaped the gut, suggesting that the susceptibility to invasion may differ between lineages (Chiel et al. 2009). One difference between the genera is that *Eretmocerus* does not void gut contents before pupation, and may thus be more susceptible to invasion of symbionts into the hemocoel from the gut during metamorphosis.

Zindel et al. (2011) summarized the possible influence that symbiotic bacteria may have on every stage of a biological control program. These include the efficiency of mass rearing and possible protection of the pest host against natural enemies. Symbiont-induced thelytokous parthenogenesis (in which diploid females develop from unfertilized eggs) has been demonstrated in both of the common genera of whitefly parasitoids, *Encarsia* and *Eretmocerus*, with either *Cardinium* or *Wolbachia* as the causative agents (De Barro and Hart 2001; Zchori-Fein et al. 2001, 2004; Ardeh et al. 2005). CI caused by *Cardinium* or *Wolbachia* has also been documented in *Encarsia* (Hunter et al. 2003, White et al. 2009). Although *Wolbachia* has been reported from arrhenotokous *Eretmocerus* species before (Ahmed et al. 2010), to our knowledge, its phenotype in this economically important genus of parasitoids has not been determined.

In the current study, we investigated *Wolbachia* in two insect species: Q *B. tabaci* and the parasitoid *Er.* sp. nr. *emiratus*. We sequence-typed the *Wolbachia* in these two hosts, tested for horizontal transmission of *Wolbachia* between and within trophic levels, and studied the reproductive phenotype of *Wolbachia* in *Er.* sp. nr. *emiratus*.

Methods and Materials

Whiteflies. Two colonies of *B. tabaci* were used in this study: a Q colony (Q2 haplotype 45, Chu et al. 2012) that carried *Wolbachia* (W^+) and *Rickettsia*, and a B colony that carried only *Hamiltonella* (both are secondary symbionts; Table 1). Although the B species in the United States is now typically infected with *Rickettsia*, for these experiments, we used a laboratory line that was not infected (Himler et al. 2011). The infection status of each whitefly colony was verified by polymerase chain reaction (PCR), as described by Chiel et al. (2009). The presence/absence of *Wolbachia* was routinely monitored by diagnostic PCR, as described later in the text. Both colonies were maintained on cowpea plants (*Vigna unguiculata* variety 'California blackeye'), each colony in a separate climate-controlled walk-in chamber at 27 ± 1°C, ≈60% relative humidity (RH), and at a photoperiod of 16:8 (L:D) h.

Table 1. Insects, their symbionts, and hosts used in the study

Insect	Secondary symbiont	Host	Used for
B. tabaci (Q)	*Wolbachia* *Rickettsia*	Cowpea (*Vigna unguiculata)*	*Wolbachia* strain-typing Horizontal transmission experiments
B. tabaci (B)	*Hamiltonella*	Cowpea	Rearing parasitoids Hosts in CI experiments
Er. sp. nr. *emiratus*	*Wolbachia*	*B. tabaci* (B)	*Wolbachia* strain-typing Horizontal transmission experiments CI experiments
Er. sp. nr. *emiratus*	None (cured by antibiotic)	*B. tabaci* (B)	Horizontal transmission experiments CI experiments
Er. eremicus	None	*B. tabaci* (B)	Horizontal transmission experiments
En. pergandiella	*Cardinium*	*B. tabaci* (B)	Horizontal transmission experiments

Parasitoids. *Er.* sp. nr. *emiratus*, *Eretmocerus eremicus*, and *Encarsia pergandiella* were each reared separately on cowpea plants that were infested with B *B. tabaci* nymphs as hosts (Table 1) inside transparent ventilated plastic jars. The infection status of each parasitoid was verified by PCR, as described by Chiel et al. (2009) (Table 1). All parasitoid cultures were kept in a climate-controlled walk-in chamber (27 ± 1°C, ≈60% RH, and a photoperiod of 16:8 [L:D] h).

Whitefly Parasitoid Natural History. While both major genera of whitefly parasitoids are solitary endoparasitoids in the family Aphelinidae, their natural history differs. *Encarsia* species lay female eggs in the hemocoel of the whitefly nymph, and the larva develops directly within the host. In all but one known sexual *Encarsia* species, males develop as hyperparasitoids; adult female *Encarsia* deposit male eggs within late larval or pupal female parasitoids (conspecifics or heterospecifics) as they are developing within the whitefly (Gerling et al. 2001, Hunter and Woolley 2001). In contrast, both sexes of *Eretmocerus* are primary parasitoids, but eggs are laid under the whitefly nymph venter (between the host and the leaf). At hatching, the *Eretmocerus* first instar penetrates and develops within a cellular capsule inside the host larva (Gerling et al. 1990). Individuals of both genera pupate within the dry cuticular remains of the whitefly. The *En. pergandiella* and the *Er.* sp. nr. *eremicus* cultures used for these experiments are Nearctic, and were collected initially in Texas and Arizona, respectively. The *Er.* sp. nr. *emiratus* was one of several species introduced and established in the United States for biological control of *B. tabaci* in the mid-1990s and, like both the B and Q *B. tabaci* species, has a Palearctic origin.

Establishment of Symbiont-Free Parasitoid Colonies. *Wolbachia*-carrying *Er.* sp. nr. *emiratus* adults were fed on honey containing 50 mg/ml rifampicin for 48 h and were then released on cowpea plants bearing B *B. tabaci* nymphs for oviposition. This process was repeated for another generation, after which the infection status of the progeny was checked by PCR. The parasitoids were confirmed to be free of *Wolbachia* and subsequently were reared continuously on B whiteflies under the conditions described earlier.

PCR Analysis. To extract DNA, individual whiteflies or wasps were ground in a 3-μl droplet of proteinase K solution (20 mg/ml, Invitrogen, Grand Island, NY). The droplet was then transferred into a tube containing 50 μl of sterile 10% Chelex beads (Sigma-Aldrich, St. Louis, MO) in PCR water. The tubes were incubated at 37°C for 1 h, then at 96°C for 8 min, and then kept at −20°C until analysis. Two microliters of the DNA lysate were used as a template for PCR reactions. The presence of *Wolbachia* was determined using the *Wolbachia*-specific 16S rDNA primers, V1 and V6 (O'Neill et al. 1992). Screening for other *B. tabaci* symbionts was done using the primers and conditions described by Chiel et al. (2009). PCR products were visualized on 1.5% agarose gel using SYBR-Green (Cambrex Bio Science Rockland Inc.).

***Wolbachia* Characterization.** Characterization of the *Wolbachia* strains in *B. tabaci* and *Er. emiratus* was done by the MLST method (Jolley et al. 2004, Baldo et al. 2006). The gene fragments were amplified using the primers and conditions described in the MLST website (http://pubmlst.org/*Wolbachia*); the amplified DNA was then purified (QIAquick gel purification kit, Qiagen) and sent for direct sequencing at the University of Arizona's sequencing facility. The resulting sequences and allelic profiles were deposited in the *Wolbachia* MLST online database.

Each *Wolbachia* isolate from a single host population was assigned a sequence-type (ST) defined as the combination of five alleles identifying numbers for the five MLST loci (the allelic profile). Supergroup designation of STs is based on phylogenetic inference of the concatenated MLST data matrix (Baldo and Werren 2007). For all subsequent analyses, we included only one strain per ST per species. MLST sequences, which have either no indels or small indels (6–9 bp) in predictable locations, were aligned to *Wolbachia* MLST templates using ClustalW (Thompson et al. 1994). To assign our STs to a phylogenetic supergroup, a preliminary neighbor-joining analysis was carried out with PAUP* 4.0b10 (Swofford 2002) using all the sequences available in the *Wolbachia* database from the different supergroups, including those without host information. This analysis revealed that the two STs belonged to supergroup B. Hence, to infer phylogenetic relationships, maximum likelihood (ML) and Bayesian inference (BI) analyses were performed on a smaller concatenated MLST gene sequence alignment using all published STs belonging to supergroup B for which host information was available. A single outgroup strain belonging to supergroup A (from the

host *Drosophila melanogaster*) was included for rooting the trees.

Likelihood-ratio tests were performed using jModelTest2 (Darriba et al. 2012) to determine the model of evolution that best fit the concatenated MLST data. ML was carried out using RAxML 7.0.4 (Stamatakis 2006) after 1,000 multiple inferences on the original alignments by using the GTRGAMMAI nucleotide model as inferred by jModeltest2, starting from a random parsimonious tree, and default initial rearrangement settings and number of rate categories. Branch support was assessed by 1,000 bootstrap pseudoreplicates. BI was performed using MrBayes 3.2 (Ronquist et al. 2012). The likelihood model was set to the GTR + G + I. Two parallel runs of four simultaneous Monte Carlo Markov chains were run for 2 million generations, and trees sampled every 1,000 generations. The burn-in value was set at 25% of sampled topologies, and the phylogeny and posterior probabilities were estimated from a majority-rule consensus of the remaining trees.

The concatenated analysis can identify closely related strains and resolve major supergroups. However, it cannot be used to interpret more distant phylogenetic relationships within a supergroup, even when clades are highly supported, because of artifacts resulting from recombination among genes. To provide further insight into relatedness among *Wolbachia* strains, we performed a phylogenetic analysis using ClonalFrame, software specifically designed for multilocus data that accounts for both point mutation and homologous recombination (Didelot and Falush 2007). For the ClonalFrame analysis, we executed 1,000,000 MCMC iterations (500,000 burn-in iterations, and 500,000 postburn-in iterations), starting with a random tree, and using all options as default.

Testing for *Wolbachia* Phenotype in *Er. emiratus*. To determine whether *Wolbachia* in the parasitoid causes CI, two experiments were performed.

Full-Factorial Crosses Between W^+ and W^- *Er.* sp. nr. *emiratus*. If CI was the reproductive phenotype of *Wolbachia* in *Er.* sp. nr. *emiratus*, we would predict that among the four possible crosses of W^+ and W^- individuals, the W^+ male/W^- female cross would exhibit CI. Pupae of W^+ and W^- *Er.* sp. nr. *emiratus* were removed from leaves and placed individually in 1.2-ml glass vials. A droplet of honey was supplied, and the vials were plugged with cotton and placed in an incubator (27 ± 1°C, 65% RH and a photoperiod of 16:8 [L:D] h). On emergence, adults were sexed, randomly assigned to one of the four possible crosses, and allowed to mate for 48 h on a plant infested with B-species *B. tabaci* nymphs. After the mating period, female *Er.* sp. nr. *emiratus* were transferred individually to 30-mm cowpea leaf disks bearing 30–50 B-species *B. tabaci* nymphs (second and third instars). The leaf disks rested on 1% agar in 35-mm petri dishes and were closed with screen lids. The female wasps were retrieved after 24 h, preserved at −20°C, and the leaf disks were incubated until progeny emergence, at which point they were counted and the sex ratio determined. To ensure that the experimental females had indeed mated, we dissected and verified the presence of sperm in their spermathecae (a sperm storage organ of female insects) and excluded arenas that had contained unmated females from the data set. The numbers of progeny produced were analyzed using one-way analysis of variance and Bonferroni post hoc tests. Progeny sex ratios were analyzed as a logistic regression with binomial or quasibinomial errors in the statistical analysis package R (R Development Team 2010).

Comparison of Parasitism and Survivorship Rates in a Control and a Putative CI Cross. To distinguish potential differences in host parasitism rates or sex ratios among treatments from the characteristic effects of CI on larval survivorship, we performed another experiment in which parasitism rate could be distinguished from immature survival. W^+ and W^- *Er.* sp. nr. *emiratus* were prepared as in the first experiment, but here just two crosses were performed: a control cross (W^- females with W^- males) and the putative CI cross (W^- females with W^+ males). To verify mating, each pair was observed for up to 15 min or until the pair mated; only mated females were used for the experiment. Each mated *Er.* sp. nr. *emiratus* female was transferred to a cowpea leaf disk infested with 30–50 R^-/W^- *B. tabaci* nymphs as described earlier. The number and distribution of *B. tabaci* nymphs on each disk were mapped. After 24 h, females were removed from the leaf disks, and half of the surviving hosts in each arena were selected, flipped over, and the *Er. emiratus* eggs found (on the leaf or whitefly venter) were recorded. The remaining hosts were reared on the leaf disks until progeny emergence. The emergence sex ratio and survivorship of wasps and whiteflies were recorded. Wasp sex ratios and the proportion surviving were analyzed using logistic regressions with binomial or quasibinomial errors in R (R Development Team 2010).

Horizontal Transmission of *Wolbachia*. Several horizontal transmission routes were tested.

From *B. tabaci* to *Er.* sp. nr. *emiratus*. We asked if *Er.* sp. nr. *emiratus* that had been cured from their own *Wolbachia* (W^-) can acquire the symbiont when they develop in *Wolbachia*-infected whitefly hosts. We hypothesized that wasps that normally harbor *Wolbachia* may be more susceptible to invasion by *Wolbachia* than normally uninfected wasps. Thirty W^- *Er. emiratus* females and males were introduced onto a cowpea plant that was infested with *Wolbachia*-infected (W^+) Q *B. tabaci* nymphs. As a control, *Er.* sp. nr. *emiratus* were introduced onto a plant with *Wolbachia*-free (W^-) Q *B. tabaci* nymphs. This procedure was repeated for four consecutive generations, with 20 individuals being randomly tested by PCR in each generation for *Wolbachia* presence.

From *Er.* sp. nr. *emiratus* to *Er. eremicus*. Even though *Eretmocerus* spp. tend not to super-parasitize or multi-parasitize, they will when hosts are in short supply (Lo Verde et al. 2008). Thus, we exposed *Er. eremicus* (W^-) females to hosts that had already been exposed to *Er.* sp. nr. *emiratus* (W^+), to determine whether *Wolbachia* would be transmitted during mul-

Table 2. Allele and allelic profile no. of *Wolbachia* from *Er.* sp. nr. *emiratus* and *B. tabaci*, as they appear in the MLST database

Gene	Allele no. in MLST database	
	Er. sp. nr. *emiratus*	*B. tabaci* (Q)
gatB	105	105
coxA	14	88
fbpA	4	165*
ftsZ	73	7
hcpA	3	106
Allelic profile no.	161*	166*

Numbers followed by * are alleles and profiles that were first described in this study.

tiparasitism. Single mated *Er.* sp. nr. *emiratus* females (W^+) were introduced to one of 10 cowpea leaf disks infested with 20–30 B *B. tabaci* nymphs (W^-). After 24 h, the females were retrieved and one *Er. eremicus* female was introduced to each disk for an additional 24 h and then collected and preserved. A set of disks ($n = 5$) to which only *Er.* sp. nr. *emiratus* or only *Er. eremicus* were introduced served as a control. On emergence (after 12–14 d of incubation), five *Er. eremicus* female progeny from each disk were tested for *Wolbachia* presence by PCR.

From *Er.* sp. nr. *emiratus* to *En. pergandiella*. Similar to the previous experiment, the experiment was designed to test if *En. pergandiella* males acquire *Wolbachia* when they develop as hyperparasitoids of W^+ *Er. emiratus* larvae. To do that, mated *En. pergandiella* females (W^-) were introduced onto cowpea leaf disks ($n = 10$) infested with 20–30 prepupae and early stage pupae of W^+ *Er.* sp. nr. *emiratus*. As a control, *En. pergandiella* were introduced onto leaf disks ($n = 5$) bearing pupae and prepupae of W^- *Er. emiratus*. In both treatment and control, B *B. tabaci* nymphs served as hosts for *Er. emiratus*. After 24 h, the *En. pergandiella* females were retrieved, disks were incubated, and *En. pergandiella* males from each disk were tested by PCR for *Wolbachia* presence.

Results

***Wolbachia* Characterization.** The allelic profiles of *Wolbachia* from *Er. emiratus* and Q2-species *B. tabaci* were found to be different from each other (Table 2), with the former being novel. Among the five genes analyzed, the *Wolbachia* of *B. tabaci* contains a novel allele in the *fbpA* gene, whereas *gatB*, *coxA*, and *hcpA* alleles were shared with *B. tabaci* from China (Bing et al., personal communication). The *gatB* allele in the *Er. emiratus Wolbachia* is identical to the *gatB* allele of the *B. tabaci Wolbachia*. The whitefly *Wolbachia ftsZ* allele was previously recorded from various hosts, including a parasitic wasp, butterflies, and other unspecified ones. All sequences were deposited in the MLST database and the allelic profiles were assigned the numbers 161 for *Er.* sp. nr. *emiratus* and 166 for *B. tabaci*.

ML and BI phylogenetic analyses for concatenated MLST loci revealed that, within supergroup B, the *Wolbachia* strains that infected *B. tabaci* and *Er.* sp. nr. *emiratus* appear to be distantly related (Fig. 1). The two trees had identical topologies, with overall higher nodal support in the Bayesian tree, although the lower part of both trees is poorly supported. Interestingly, the closest *Wolbachia* relative to the strain in the Q2 *B. tabaci* whiteflies of the current study was also found in a *B. tabaci* group whitefly, provisionally named Asia II7 (Bing et al., personal communication). This strain's ST (#178) shared three of the five alleles with the Q2 strain, *coxA*, *gatB*, and *hcpA*. The same phylogenetic pattern was retrieved by the ClonalFrame analysis (tree not shown). It is also interesting to note that the *Wolbachia* strains of *Er.* sp. nr. *emiratus* and *Encarsia formosa* are relatively distantly related (Fig. 1), even though the hosts are both in the chalcidoid family Aphelinidae and both attack *B. tabaci*.

***Er. emiratus* CI Experiments.** In the first experiment in which all four crosses were performed, the number of progeny was significantly lower in the putative CI ♀W−/♂W+ cross than in the other three possible crosses (Fig. 2B; $F_{3,82} = 26.9$, $P < 0.0001$). Similarly, the proportion of female progeny in the $♀W^-/♂W^+$ cross differed significantly among treatments (Fig. 2A; $F_{3,81} = 27.24$, $P < 0.0001$), and the proportion of females in the putative CI cross was significantly lower than in the $♀W^-/♂W^-$ cross ($F_{1,39} = 26.336$, $P < 0.0001$). These results are consistent with CI as the reproductive phenotype of *Wolbachia*.

In the second experiment, the percentage of attacked hosts (*B. tabaci* nymphs under which a wasp egg was observed) was similar in both crosses and not significantly different (Fig. 3). However, the number of wasp progeny was significantly higher in the $♀W^-/♂W^-$ cross than in the $♀W^-/♂W^+$ cross (15.7 ± 0.9 vs 9.2 ± 1, respectively; $t_{53} = 4.9$, $P < 0.0001$). Similarly, the proportion of wasps surviving to adulthood and the proportion of female progeny produced were significantly higher in the $♀W^-/♂W^-$ cross (Fig. 3; proportion surviving: $F_{1,53} = 29.29$, $P < 0.0001$; proportion of female progeny: $F_{1,53} = 25.31$, $P < 0.0001$). These results are also consistent with a CI phenotype.

Horizontal Transmission of *Wolbachia*. No evidence was found of *Wolbachia* transfer from *Er.* sp. nr. *emiratus* to either *Er.* sp. nr. *eremicus* females or *En. pergandiella* males. Similarly, *Wolbachia* was not found in progeny of cured *Er.* sp. nr. *emiratus* (W^-) that were reared on W^+ hosts (Q *B. tabaci* nymphs) for four consecutive generations.

Discussion

Taken together, the results of the current study show that: 1) *B. tabaci* and its parasitoid *Er.* sp. nr. *emiratus* carry different strains of *Wolbachia* belonging to supergroup B, 2) both *Wolbachia* strains in this system were not transmitted to any of the other species tested, and 3) *Wolbachia* causes CI in *Er.* sp. nr. *emiratus*.

In the *B. tabaci*–*Er.* sp. nr. *emiratus* host–parasitoid system, each species harbors its own distantly related strain of *Wolbachia* (Fig. 1; Table 2), but they share the same *gatB* allele that has so far been reported only from these two hosts, and their *ftsZ* allele is 99.8% similar. This

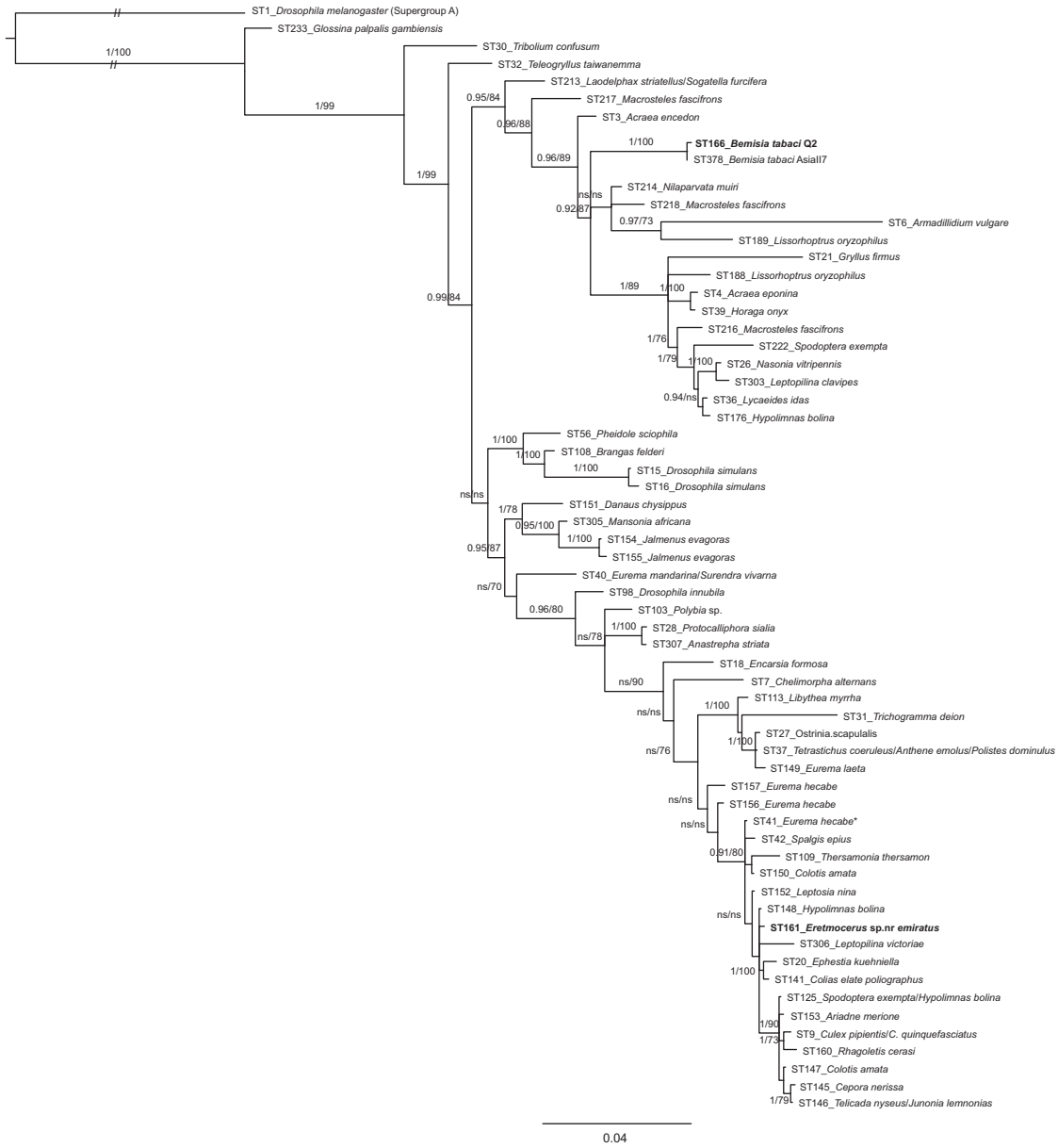

Fig. 1. Bayesian majority-rule consensus tree for *Wolbachia* MLST data with available host information. Posterior probabilities ≥0.90 and bootstrap values ≥70% relative to the ML tree (identical topology) are indicated above branches. Ns = not supported. *ST41 is shared with 10 more known hosts (see http://pubmlst.org/wolbachia/). Sequences generated in the course of this study are marked in bold.

may indicate lateral gene transfer or recombination of common ancestral strains followed by horizontal transmission. There are many recent examples in the literature of lateral gene transfer between co-occurring symbionts. In the symbiont *Cardinium* in *Encarsia* wasps, for example, 8% of genes characterized were likely horizontally acquired from other bacteria (Penz et al. 2012) Both whitefly (Q2) and wasp (*Er.* sp. nr. *emiratus*) are Palearctic species and may overlap in their original ranges. It should, however, be mentioned that our culture of *Er.* sp. nr. *emiratus* has been reared on *Wolbachia*-free B hosts, thus *Er.* sp. nr. *emiratus* were not acquainted with the Q2 *Wolbachia* before setting up the experiments. We acknowledge that horizontal transmission may well occur, but at a rate lower than our experimental design would detect.

The wide distribution of *Wolbachia* among host taxa and incongruence of the *Wolbachia* phylogeny with host phylogenies indicate frequent horizontal transmission among lineages (Raychoudhury et al. 2009, Zug et al. 2012, Ahmed et al. 2013). The fact that the allelic profile of *Wolbachia* from *Er.* sp. nr. *emiratus* in

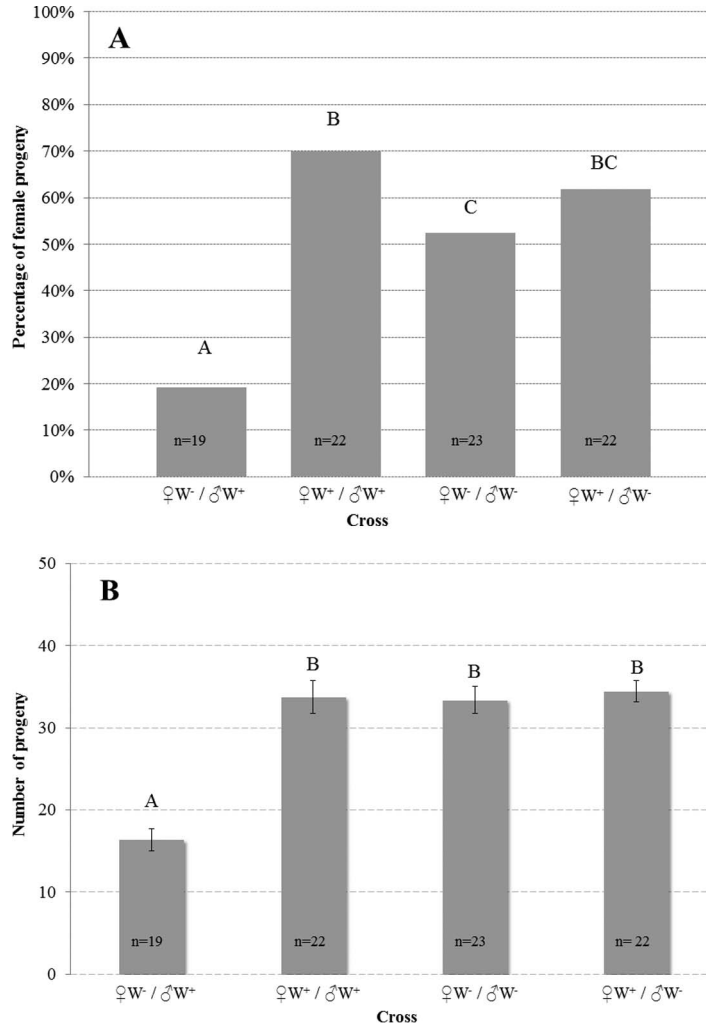

Fig. 2. Proportion of female progeny (A) and total number of progeny (B) in the four possible crosses between *Wolbachia*-infected (W^+) and *Wolbachia*-free (W^-) *Er.* sp. nr. *emiratus.*

our study is most related to a *Wolbachia* described from distantly related hosts supports this view.

We hypothesized that *Wolbachia* may transfer from the whitefly to the parasitoid in the current study because we 1) previously found that the symbiont *Rickettsia* moves from the whitefly host to *Er.* sp. nr. *emiratus* ovaries but does not invade the germ line (Chiel et al. 2009), 2) *Wolbachia* was shown to be present in the hemolymph of Q *B. tabaci* nymphs (Gottlieb et al. 2008), and 3) *Wolbachia* in *Drosophila* has been shown to navigate inside hosts to find the germ line (Frydman et al. 2006). However, our results do not support the hypothesis, as we found no evidence for horizontal transfer of *Wolbachia* between or within trophic levels in our experiments (herbivore to parasitoid, parasitoid to hyperparasitoid, or parasitoid to parasitoid). It is certainly possible that horizontal transfer of *Wolbachia* in our experiments took place at such a low frequency and/or titer that transmission was not detected. Nonetheless, our results are consistent with the scarcity of empirical horizontal transmission reports in the literature and underline the specificity of host–symbiont interactions, even when the recipient host has a recent history of infection with a related symbiont.

Among the possible reproductive manipulations that are known to be caused by *Wolbachia*, CI is the one that does not necessarily bias the sex ratio. The *Er.* sp. nr. *emiratus* population did not show female-biased sex ratios, thus reducing the possibility that *Wolbachia* caused feminization, male-killing, or parthenogenesis, and leaving CI, an asymptomatic infection, or a mutualist infection (e.g., a nutritional or defensive symbiosis) as possible options. Our results are consistent with CI as the reproductive phenotype of *Wolbachia* in *Er.* sp. nr. *emiratus.* CI-inducing *Wolbachia* are common in parasitic Hymenoptera and have been studied extensively in genera such as *Nasonia* (Pteromalidae), *Trichogramma* (Trichogrammatidae), and *Leptopilina* (Figitidae) (Bordenstein and Werren 2007, Vavre et al. 2009). Within the guild of whitefly

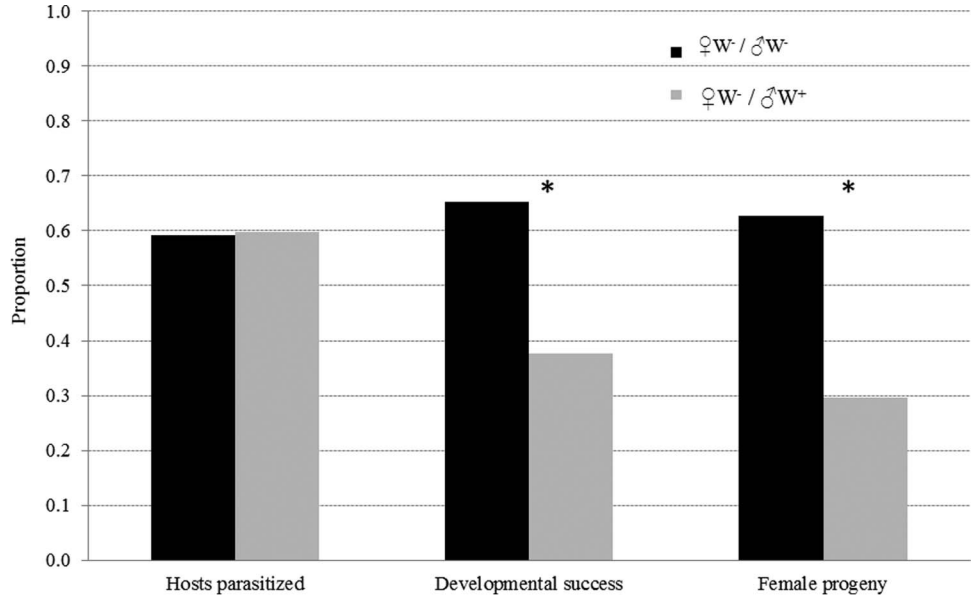

Fig. 3. Proportions of parasitized *B. tabaci* nymphs (as determined by detecting eggs laid under them), wasps that completed development successfully, and female progeny in two crosses of *Er. emiratus*: *Wolbachia*-free females with *Wolbachia*-infected males (gray bars, the predicted CI cross), and *Wolbachia*-free females with *Wolbachia*-free males (black bars, the control cross). The symbol * denotes a *P* value <0.0001.

aphelinid parasitoids, *Wolbachia* is known from several species of *Encarsia* and *Eretmocerus:* in *Encarsia inaron, Wolbachia* causes CI; in *En. formosa*, it causes parthenogenesis; and in *En. pergandiella*, CI is caused by another bacterial symbiont, *Cardinium* (Hunter et al. 2003, White et al. 2009). The current study adds a first species of *Eretmocerus*, *Er.* sp. nr. *emiratus*, to the list of parasitoids in which *Wolbachia* induces CI.

Microbial symbionts can influence the success of biological control programs. Symbionts may improve the mass production of such natural enemies and their performance in the field by increasing the fitness and/or the proportion of females in natural enemy populations (Stouthamer 1993, Zindel et al. 2011). In contrast, symbionts may also impair natural enemies' fitness, cause reproductive incompatibility among separate introductions, or protect the pest from pathogens and natural enemies, thereby compromising the success of biological control programs (Zindel et al. 2011). Vasquez et al. (2011), for example, showed that *Wolbachia* in *Aphytis melinus*, a common parasitoid of scale insects produced in several insectaries, is generally at high frequency, causes CI, and reduces wasp longevity and fecundity. Simple antibiotic treatment of introduced natural enemies, such as *Er.* sp. nr. *emiratus*, may be possible while they are in quarantine, but is not advisable without an assessment of the symbiont phenotype and its effects on agent fitness. Once releases have been made, however, there is no possibility of influencing the symbiont infection status of the established population. When multiple releases are performed, CI may affect the success of biological control programs if *Wolbachia*-free agents are released in areas with an established *Wolbachia*-infected population. Without a selective advantage, the introduced *Wolbachia*-free agents are predicted to decrease with time. Our results underline the importance of these cryptic players in biological control programs.

Acknowledgments

We thank Moshe Inbar, the University of Haifa, for the constructive advice. This research was supported by the following sources: grant no 2004416 from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel, to E.Z.-F. and M.S.H.; The National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant no 2006-35302-17165, to M.S.H. and E.Z.-F.; the United States–Israel Binational Agricultural Research and Development fund (BARD) Graduate Student Fellow award no GS-1-2007 to E.C.; BARD grant U.S.-4304-10R to M.S.H. and E.Z.-F.; NSF grant DEB-1020460 and USDA AFRI 2010-03752 to M.S.H.; and Marie Curie International Outgoing Fellowship (PIOF-GA-2012-327425) to M.G. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References Cited

Ahmed, M. Z., S. X. Ren, N. S. Mandour, J. M. Greeff, and B. L. Qiu. 2010. Prevalence of *Wolbachia* supergroups A and B in *Bemisia tabaci* (Hemiptera: Aleyrodidae) and some of its natural enemies. J. Econ. Entomol. 103: 1848–1859.

Ahmed, M. Z., P. J. De Barro, S. X. Ren, J. M. Greeff, and B. L. Qiu. 2013. Evidence for horizontal transmission of secondary endosymbionts in the *Bemisia tabaci* cryptic species complex. PLoS ONE 8: e53084. (doi:10.1371/journal.pone.0053084).

Ardeh, M. J., P. W. de Jong, and J. C. van Lenteren. 2005. Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp. Biol. Control 33: 74–80.

Baldo, L., and J. H. Werren. 2007. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. Curr. Microbiol. 55: 81–87.

Baldo, L., J.C.D. Hotopp, K. A. Jolley, S. R. Bordenstein, S. A. Biber, R. R. Choudhury, C. Hayashi, M.C.J. Maiden, H. Tettelin, and J. H. Werren. 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis.* Appl. Environ. Microbiol. 72: 7098–7110.

Bordenstein, S. R., and J. H. Werren. 2007. Bidirectional incompatibility among divergent *Wolbachia* and incompatibility level differences among closely related *Wolbachia* in *Nasonia.* Heredity 99: 278–287.

Braig, H. R., H. Guzman, R. B. Tesh, and S. L. Oneill. 1994. Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. Nature 367: 453–455.

Brownlie, J. C., B. N. Cass, M. Riegler, J. J. Witsenburg, I. Iturbe-Ormaetxe, E. A. McGraw, and S. L. O'Neill. 2009. Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis,* during periods of nutritional stress. PLoS Pathog 5: e1000368. (doi: 10.1371/journal.ppat.1000368).

Chiel, E., Y. Gottlieb, E. Zchori-Fein, N. Mozes-Daube, N. Katzir, M. Inbar, and M. Ghanim. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci.* Bull. Entomol. Res. 97: 407–413.

Chiel, E., E. Zchori-Fein, M. Inbar, Y. Gottlieb, T. Adachi-Hagimori, S. E. Kelly, M. K. Asplen, and M. S. Hunter. 2009. Almost there: transmission routes of bacterial symbionts between trophic levels. PLoS ONE 4: e4767. (doi: 10.1371/journal.pone.0004767).

Chu, D., X. S. Hu, C. S. Gao, H. Y. Zhao, R. L. Nichols, and X. C. Li. 2012. Use of mitochondrial cytochrome oxidase I polymerase chain reaction-restriction fragment length polymorphism for identifying subclades of *Bemisia tabaci* Mediterranean group. J. Econ. Entomol. 105: 242–251.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9: 772 (doi:10.1038/nmeth. 2109).

De Barro, P. J., and P. J. Hart. 2001. Antibiotic curing of parthenogenesis in *Eretmocerus mundus* (Australian parthenogenic form). Entomol. Exp. Appl. 99: 225–230.

De Barro, P. J., S. S. Liu, L. M. Boykin, and A. B. Dinsdale. 2011. *Bemisia tabaci:* a statement of species status. Annu. Rev. Entomol. 56: 1–19.

Dennehy, T. J., B. A. Degain, V. S. Harpold, M. Zaborac, S. Morin, J. A. Fabrick, R. L. Nichols, J. K. Brown, F. J. Byrne, and X. C. Li. 2010. Extraordinary resistance to insecticides reveals exotic Q biotype of *Bemisia tabaci* in the New World. J. Econ. Entomol. 103: 2174–2186.

Didelot, X., and D. Falush. 2007. Inference of bacterial microevolution using multilocus sequence data. Genetics 175: 1251–1266.

Duron, O., T. E. Wilkes, and G.D.D. Hurst. 2010. Interspecific transmission of a male-killing bacterium on an ecological timescale. Ecol. Lett. 13: 1139–1148.

Frydman, H. M., J. M. Li, D. N. Robson, and E. Wieschaus. 2006. Somatic stem cell niche tropism in *Wolbachia.* Nature 441: 509–512.

Gerling, D., T. Orion, and Y. Delarea. 1990. *Eretmocerus* penetration and immature development–a novel approach to overcome host immunity. Arch. Insect. Biochem. Physiol. 13: 247–253.

Gerling, D., O. Alomar, and J. Arno. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Prot. 20: 779–799.

Gottlieb, Y., M. Ghanim, G. Gueguen, S. Kontsedalov, F. Vavre, F. Fleury, and E. Zchori-Fein. 2008. Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. FASEB J. 22: 2591–2599.

Gueguen, G., F. Vavre, O. Gnankine, M. Peterschmitt, D. Charif, E. Chiel, Y. Gottlieb, M. Ghanim, E. Zchori-Fein, and F. Fleury. 2010. Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. Mol. Ecol. 19: 4365–4378.

Heath, B. D., R.D.J. Butcher, W.G.F. Whitfield, and S. F. Hubbard. 1999. Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. Curr. Biol. 9: 313–316.

Hedges, L. M., J. C. Brownlie, S. L. O'Neill, and K. N. Johnson. 2008. *Wolbachia* and virus protection in insects. Science 322: 702.

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. H. Werren. 2008. How many species are infected with *Wolbachia*? A statistical analysis of current data. FEMS Microbiol. Lett. 281: 215–220.

Himler, A. G., T. Adachi-Hagimori, J. E. Bergen, A. Kozuch, S. E. Kelly, B. E. Tabashnik, E. Chiel, V. E. Duckworth, T. J. Dennehy, E. Zchori-Fein, et al. 2011. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. Science 332: 254–256.

Horowitz, A. R., S. Kontsedalov, V. Khasdan, and I. Ishaaya. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Arch. Insect Biochem. Physiol. 58: 216–225.

Hosokawa, T., R. Koga, Y. Kikuchi, X. Y. Meng, and T. Fukatsu. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. U.S.A. 107: 769–774.

Huigens, M. E., R. P. de Almeida, P.A.H. Boons, R. F. Luck, and R. Stouthamer. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. Proc. R. Soc. Lond. B Biol. Sci. 271: 509–515.

Hunter, M. S., and J. B. Woolley. 2001. Evolution and behavioral ecology of heteronomous aphelinid parasitoids. Annu. Rev. Entomol. 46: 251–290.

Hunter, M. S., S. J. Perlman, and S. E. Kelly. 2003. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella.* Proc. R. Soc. Lond. B Biol. Sci. 270: 2185–2190.

Idris, A. M., S. E. Smith, and J. K. Brown. 2001. Ingestion, transmission, and persistence of Chino del tomate virus (CdTv), a New World begomovirus, by Old and New World biotypes of the whitefly vector *Bemisia tabaci.* Ann. Appl. Biol. 139: 145–154.

Jiao, X. G., W. Xie, S. L. Wang, Q. J. Wu, H. P. Pan, B. M. Liu, and Y. J. Zhang. 2013. Differences in host selection and performance between B and Q putative species of *Bemisia tabaci* on three host plants. Entomol. Exp. Appl. 147: 1–8.

Jolley, K. A., M. S. Chan, and M.C.J. Maiden. 2004. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics 5: 86 (doi:10.1186/1471-2105-5-86).

Kang, L., X. Ma, L. Cai, S. Liao, L. Sun, H. Zhu, X. Chen, D. Shen, S. Zhao, and C. Li. 2003. Superinfection of *Laodelphax striatellus* with *Wolbachia* from *Drosophila simulans.* Heredity 90: 71–76.

Kawai, S., Y. Matsumoto, T. Gotoh, and H. Noda. 2009. Transinfection of *Wolbachia* in planthoppers: nymphal injection of cultured *Wolbachia* and infection dynamics. Environ. Entomol. 38: 1626–1633.

Kontsedalov, S., E. Zchori-Fein, E. Chiel, Y. Gottlieb, M. Inbar, and M. Ghanim. 2008. The presence of *Rickettsia*

is associated with increased susceptibility of *Bemisia tabaci* (Homoptera : Aleyrodidae) to insecticides. Pest Manag. Sci. 64: 789–792.

Kraaijeveld, K., P. Franco, P. De Knijff, R. Stouthamer, and J.J.M. Van Alphen. 2011. Clonal genetic variation in a *Wolbachia*-infected asexual wasp: horizontal transmission or historical sex? Mol. Ecol. 20: 3644–3652.

Lo Verde, V., J. C. van Lenteren, H. M. Smid, P. W. de Jong, C.I.C. Carrillo, and V. Caleca. 2008. External and internal elimination of supernumerary larvae in the whitefly parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). Biol. Control 46: 287–292.

O'Neill, S. L., R. Giordano, A. M. Colbert, T. L. Karr, and H. M. Robertson. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. U.S.A. 89: 2699–2702.

Pascual, S., and C. Callejas. 2004. Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera : Aleyrodidae) from Spain. Bull. Entomol. Res. 94: 369–375.

Penz, T. S., S. E. Schmitz-Esser, S. E. Kelly, B. N. Cass, A. Muller, T. Woyke, S. A. Malfatti, M. S. Hunter, and M. Horn. 2012. Comparative genomics suggests an independent evolution of cytoplasmic incompatibility in *Cardinium hertigii.* PLoS Genet. 8: e1003012.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (ISBN: 3–900051-07–0; http://www.R-project.org/).

Raychoudhury, R., L. Baldo, D. Oliveira, and J. H. Werren. 2009. Modes of acquisition of *Wolbachia:* horizontal transfer, hybrid introgression, and codivergence in the *Nasonia* species complex. Evolution 63: 165–183.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61: 539–542.

Russell, J. A., and N. A. Moran. 2005. Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. Appl. Environ. Microbiol. 71: 7987–7994.

Russell, J. A., A. Latorre, B. Sabater-Munoz, A. Moya, and N. A. Moran. 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol. 12: 1061–1075.

Santos-Garcia, D., P. A. Farnier, F. Beitia, E. Zchori-Fein, F. Vavre, L. Mouton, A. Moya, A. Latorre, and F. J. Silva. 2012. Complete genome sequence of "Candidatus *Portiera aleyrodidarum*" BT-QVLC, an obligate symbiont that supplies amino acids and carotenoids to *Bemisia tabaci.* J. Bacteriol. 194: 6654–6655.

Saridaki, A., and K. Bourtzis. 2010. *Wolbachia:* more than just a bug in insects genitals. Curr. Opin. Microbiol. 13: 67–72.

Skaljac, M., K. Zanic, S. G. Ban, S. Kontsedalov, and M. Ghanim. 2010. Co-infection and localization of secondary symbionts in two whitefly species. BMC Microbiol. 10: 142 (doi:10.1186/1471-2180-10-142).

Sloan, D. B., and N. A. Moran. 2012. Endosymbiotic bacteria as a source of carotenoids in whiteflies. Biol. Lett. 8: 986–989.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Stansly, P. A., and S. E. Naranjo. 2010. *Bemisia:* bionomics and management of a global pest, pp. 528. Springer, Amsterdam, The Netherlands.

Stouthamer, R. 1993. The use of sexual versus asexual wasps in biological control. Entomophaga 38: 3–6.

Su, Q., H. P. Pan, B. M. Liu, D. Chu, W. Xie, Q. J. Wu, S. L. Wang, B. Y. Xu, and Y. J. Zhang. 2013. Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus. Sci. Rep. 3: 1367 (doi:10.1038/srep01367).

Swofford, D. L. 2002. PAUP 4.0 b10: phylogenetic analysis using parsimony. Sinauer, Sunderland, MA.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Vasquez, C. J., R. Stouthamer, G. Jeong, and J. G. Morse. 2011. Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). Biol. Control 58: 192–198.

Vavre, F., F. Fleury, D. Lepetit, P. Fouillet, and M. Boulétreau. 1999. Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. Mol. Biol. Evol. 16: 1711–1723.

Vavre, F., L. Mouton, and B. A. Pannebakker. 2009. *Drosophila*-parasitoid communities as model systems for host-*Wolbachia* interactions, pp. 299–331. *In* G. Prevost (ed.), Advances in Parasitology, Parasitoids of *Drosophila*, vol. 70. Elsevier, Philadelphia, PA.

Viljakainen, L., M. Reuter, and P. Pamilo. 2008. *Wolbachia* transmission dynamics in *Formica* wood ants. BMC Evol. Biol. 8.

Werren, J. H., L. Baldo, and M. E. Clark. 2008. *Wolbachia:* master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6: 741–751.

White, J. A., S. E. Kelly, S. J. Perlman, and M. S. Hunter. 2009. Cytoplasmic incompatibility in the parasitic wasp *Encarsia inaron:* disentangling the roles of *Cardinium* and *Wolbachia* symbionts. Heredity 102: 483–489.

Xue, X., S. J. Li, M. Z. Ahmed, P. J. De Barro, S. X. Ren, and B. L. Qiu. 2012. Inactivation of *Wolbachia* reveals its biological roles in whitefly host. PLoS ONE 7: e48148. (doi:10.1371/journal.pone.0048148).

Zchori-Fein, E., Y. Gottlieb, S. E. Kelly, J. K. Brown, J. M. Wilson, T. L. Karr, and M. S. Hunter. 2001. A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. Proc. Natl. Acad. Sci. U.S.A. 98: 12555–12560.

Zchori-Fein, E., S. J. Perlman, S. E. Kelly, N. Katzir, and M. S. Hunter. 2004. Characterization of a 'Bacteroidetes' symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of '*Candidatus Cardinium* hertigii'. Int. J. Syst. Evol. Microbiol. 54: 961–968.

Zindel, R., Y. Gottlieb, and A. Aebi. 2011. Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. J. Appl. Ecol. 48: 864–872.

Zug, R., and P. Hammerstein. 2012. Still a host of hosts for *Wolbachia:* analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE 7: e38544. (doi:10.1371/journal.pone.0038544).

Received 11 October 2013; accepted 21 January 2014.