

# Phylogenetic Evidence for Horizontal Transmission of *Wolbachia* in Host-Parasitoid Associations

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Endosymbiotic *Wolbachia* infect a number of arthropod species in which they can affect the reproductive system. While maternally transmitted, unlike mitochondria their molecular phylogeny does not parallel that of their hosts. This strongly suggests horizontal transmission among species, the mechanisms of which remain unknown. Such transfers require intimate between-species relationships, and thus host-parasite associations are outstandingly appropriate for study. Here, we demonstrate that hymenopteran parasitoids of frugivorous *Drosophila* species are especially susceptible to *Wolbachia* infection. Of the five common European species, four proved to be infected; furthermore, multiple infections are common, with one species being doubly infected and two triply infected (first report). Phylogenetic statuses of the *Wolbachia* infecting the different species of the community have been studied using the gene *wsp*, a highly variable gene recently described. This study reveals exciting similarities between the *Wolbachia* variants found in parasitoids and their hosts. These arguments strongly support the hypothesis of frequent natural *Wolbachia* transfers into other species and open a new field for genetic exchanges among species, especially in host-parasitoid associations.

## Introduction

The cytoplasmically inherited  $\alpha$ -proteobacterium *Wolbachia* is probably one of the most widespread symbionts of arthropods: it infects acari (Breeuwer and Jacobs 1996), and 15% of insect species could be infected (Werren, Windsor, and Guo 1995). Recently, it has also been evidenced in nematodes (Sironi et al. 1995). Its spreading in host populations is favored by its ability to modify the reproduction of its hosts in three ways. In most species, it induces cytoplasmic incompatibility in the form of a postzygotic reproductive isolation that occurs when infected males mate either with uninfected females or with females infected by another bacterial variant (Yen and Barr 1974; Hoffmann, Turelli, and Simmons 1986; Breeuwer and Werren 1990; O’Neill and Karr 1990). In some haplodiploid hymenopteran species, infected virgin females produce all-female progenies (Stouthamer, Luck, and Hamilton 1990; Zchori-Fein, Roush, and Hunter 1992). Finally, in isopods, males are genetically feminized when infected (Martin, Juchault, and Legrand 1973; Rigaud et al. 1991; Juchault, Rigaud, and Mocquard 1992). These modifications of the reproduction may interfere with the host’s population dynamics and could have consequences on speciation processes (Breeuwer and Werren 1990).

The ability of *Wolbachia* to invade host populations cannot account for the high number of arthropod species that are infected. Indeed, the reproduction alteration of the host can explain how *Wolbachia* invades new hosts but not how *Wolbachia* reaches these new

hosts. *Wolbachia*’s phylogeny has suggested possible explanations for this phenomenon. Studies based on 16S rDNA or *ftsZ* genes have placed *Wolbachia* in a monophyletic group that belongs to the  $\alpha$ -proteobacteria. *Wolbachia* that infect arthropods are composed of two subdivisions, named A and B, which diverged 50 MYA (O’Neill et al. 1992; Rousset et al. 1992; Moran and Bauman 1994; Werren, Zhang, and Guo 1995). *Wolbachia*’s phylogeny does not parallel that of its hosts, which diverged a much longer time ago. Thus, despite the lack of direct evidence, Werren and O’Neill (1997, p. 11) believe that the “widespread distribution in arthropods is clearly due to horizontal transmission.”

One of the main problems with the biology of *Wolbachia* is to attain an understanding of how these transfers can occur. Related pathogenic Rickettsia can

**Table 1**  
Infection Status and Type of *Wolbachia* in *Drosophila* Species and Their Parasitoids

	Species	Host Stage Attacked	Type of <i>Wolbachia</i>
Host . . . . .	<i>D. hydei</i>	—	None
	<i>D. immigrans</i>	—	None
	<i>D. melanogaster</i>	—	A
	<i>D. simulans</i>	—	A
	<i>D. subobscura</i>	—	None
Parasitoid . .	<i>Leptopilina bouvardi</i> (Figitidae)	Larvae	None
	<i>Leptopilina heterotoma</i> (Figitidae)	Larvae	A & A & A
	<i>Asobara tabida</i> (Braconidae)	Larvae	A & A & A
	<i>Pachycrepoideus dubius</i> (Pteromalidae)	Pupae	A
	<i>Trichopria</i> sp. (Diapriidae)	Pupae	A & B

NOTE.—A and B are the two clades of *Wolbachia*, and the number of letters is the number of variants. For each species, we checked at least 30 individuals for infection.

Key words: *Wolbachia*, horizontal transmission, *Drosophila* community, parasitoids, phylogeny.

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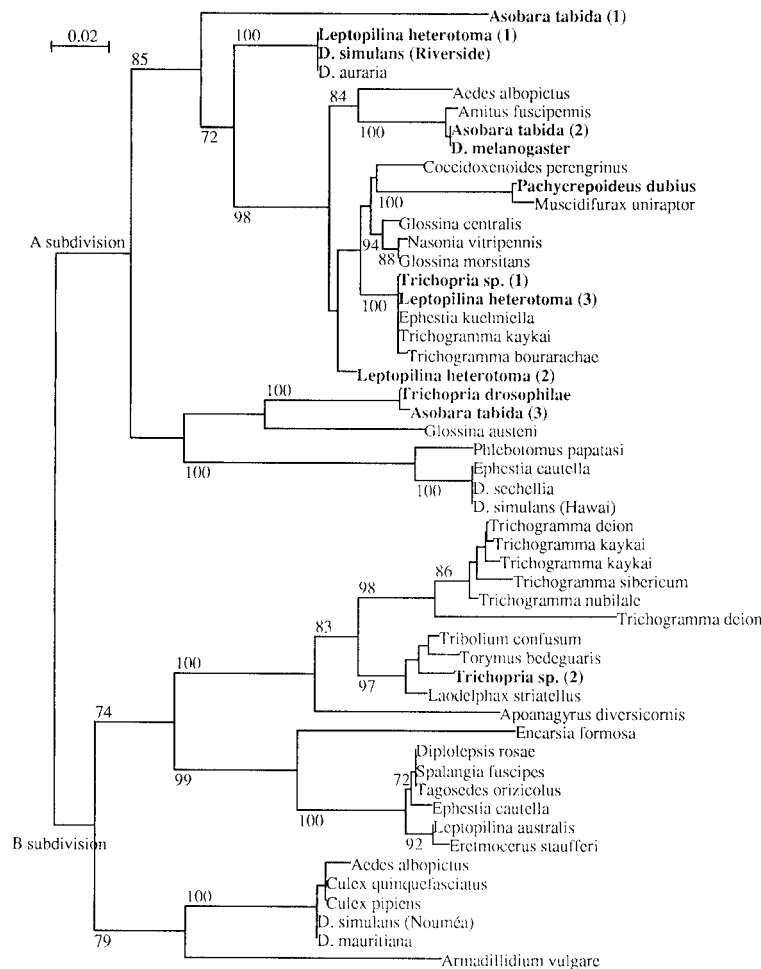


FIG. 1.—Phylogenetic tree of *Wolbachia* based on a 365-base sequence of the gene *wsp* using the neighbor-joining algorithm. *Wolbachia* are identified by the names of the host species from which they were isolated. Species of the *Drosophila* complex are in bold type. Numbers in brackets distinguish *Wolbachia* variants that are found in the same insect species. See *Materials and Methods* for details on the origin of the sequences.

be carried to their vertebrate hosts by arthropod vectors (Hackstadt 1996), demonstrating that transfer can occur between partners of different species. Host-parasitoid associations in which insect parasitoids develop at the expense of insect hosts before killing them are typical examples of intimate and long-lasting interactions between insect species (Godfray 1994) that may offer ideal conditions for transfers. However, experimental identification of horizontal transmission is difficult, since this is a rare event. One possible way to study natural transfers is through the phylogenetic comparison of *Wolbachia* infecting highly interacting species. If transfers have occurred, the *Wolbachia* present in these species must be very similar. Up to now, only two studies have investigated the phylogenetic relationships of *Wolbachia* in host-parasitoid communities (Schilthuisen and Stouthamer 1998; West et al. 1998), and none of them could show evidence of horizontal transmission. However, the authors of these studies used the *ftsZ* gene, which is not variable enough for clear-cut conclusions. Host and parasitoid share closely related *Wolbachia* in only one case (Werren, Zhang, and Guo 1995), but this remains an isolated example.

To date, horizontal transmission of *Wolbachia* in host-parasitoid associations thus appears poorly documented and rather speculative.

For our study, we used the Hymenoptera-*Drosophila* community, in which hosts and parasitoids interact strongly, and a recently described gene, *wsp* (*Wolbachia* outer surface protein) (Braig et al. 1998; Zhou, Rousset, and O'Neill 1998), the high variability of which now makes possible an accurate analysis of phylogenetic relationships among *Wolbachia* lineages, and which has never been used to for such a study. The results strongly suggest that parasitoids can acquire *Wolbachia* through horizontal transmission with high frequency.

## Materials and Methods

### Species and Strains

In southeast France, the community of frugivorous *Drosophila* comprises five main species, of which *D. melanogaster* and *D. simulans* are dominant. Among parasitoids, two species are specialists: *Leptopilina bouvardi* (Figitidae), restricted to *D. melanogaster* and *D. simulans*, and *Asobara tabida* (Braconidae), which

	1						
A.tabida (2)	ATAAGAAAGA	CAAGAGTGAT	TACAGTCCA-	-----	----TTAAAA	CCATCTTTTA	
D.melanogaster	ATAAGAAAGA	CAAGAGTGAT	TACAGTCCA-	-----	----TTAAAA	CCATCTTTTA	
A.fuscipennis	ATAAGAAAGA	CAAGAGTGAT	TACAGTCCA-	-----	----TNAAAA	CCATCTTTTA	
A.albopictus	ATAAGAAAGA	CAAGAGTGAT	TACAGTCCA-	-----	----TTAAAA	CCATCTTTTA	
M.uniraptor	ATAAGAAAGG	CAATAGTGAT	TACAGTCCA-	-----	----TTGAAA	GCGTCTTTTA	
P.dubius	ATAAGAAAGG	CAATAGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGTCTTTTA	
G.morsitans	ATAAGAAAGA	CAATGGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGCCTTTTA	
N.vitripennis	ATARGAAAGA	CAATGGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGCCTTTTA	
G.centralis	ATAAGAAAGA	CAATGGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGTCTTTTA	
C.peregrinus	ATAAGAAAGG	CAATAGTGAT	TACAGCCCG-	-----	----TTAAAA	GCGTCTTTTA	
Trichopria sp(1)	ATAAGAAAGA	CAATAGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGTCTTTTC	
L.heterotoma (3)	ATAAGAAAGA	CAATAGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGTCTTTTC	
E.kuehniella	ATAAGAAAGA	CAATAGTGAT	TACAGTCCA-	-----	----TTAAAA	GCGTCTTTTC	
T.bourarachae	ATAAGAAAGA	CAATAGTGAT	TACAGTCCA-	-----	----TNAAAA	GCGTCTTTTC	
T.kaykay	ATAAGAAAGA	CAATAGTGAT	TACAGTCCA-	-----	----TNAAAA	GCGTCTTTTC	
L.heterotoma (2)	ATAAGAAAGA	CAAGAGTGAT	TACAGTCCA-	-----	----TTAAAA	CCATCTTTTA	
D.auraria	ATAAAAAGGC	CACAGACATT	CATAATCCA-	-----	----TTAAAA	GCATCTTTTA	
D.sim(riverside)	ATAAAAAGGC	CACAGACATT	CATAATCCA-	-----	----TTAAAA	GCATCTTTTA	
L.heterotoma (1)	ATAAAAAGGC	CACAGACATT	CATAATCCA-	-----	----TTAAAA	GCATCTTTTA	
A.tabida (1)	TTAAAAAGGG	GACTGATGAT	GTTGATCCT-	-----	----TTAAAA	GCTTCTTTTA	
D.sim(Hawai)	GTGCTAAAA	GAAGACTGCG	GATACTGATA	CAACTACTGA	CCTTTATATA	GCTTCTTTTA	
D.sechellia	GTGCTAAAA	GAAGACTGCG	GATACTGATA	CAACTACTGA	CCTTTATATA	GCTTCTTTTA	
E.cautella	GTGCTAAAA	GAAGACTGCG	GATACTGATA	CAACTACTGA	CCTTTATATA	GCTTCTTTTA	
P.papatasi	GTGCT---AA	GAAGACTGCA	GATACTGCTA	CAAYTACTGA	CCTTTATATA	GCTTCTTTTA	
G.austeni	ATGTAGCAGG	CAAAGAAAAG	GATAGTCCC-	-----	----TTAAAA	GCATCTTTTA	
A.tabida (3)	ATGCAACAGG	CAAAGAAAAG	GATAGTCCC-	-----	----TTAAAA	GCATCTTTTA	
T.drosophilae	ATGCAACAGG	CAAAGAAAAG	GATAGTCCC-	-----	----TTAAAA	GCATCTTTTA	
A.vulgare	ATAAAAAAAA	TAACGACGCT	CAAGATCCT-	-----	----TTAAAA	GCATCTTTTA	
C.quinquefasciatus	ATAAAAAAAG	AACCGAAGTT	CATGATCCT-	-----	----TTAAAA	GCATCTTTTA	
C.piplens	ATAAAAAAAG	AACCGAAGTT	CATGATCCT-	-----	----TTAAAA	GCATCTTTTA	
D.mauritiana	ATAAAAAAAG	AACCGAAGTT	CATGATCCT-	-----	----TTAAAA	GCATCTTTTA	
D.sim(Nouméa)	ATAAAAAAAG	AACCGAAGTT	CATGATCCT-	-----	----TTAAAA	GCATCTTTTA	
A.albopictus	ATAAAAAAAG	AACCGAAGTT	CATGATCCT-	-----	----TTAAAA	GCATCTTTTA	
E.staufferi	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
L.australis	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
E.cautella	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	CCATCTTTTC	
S.fuscipes	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
D.rosae	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
T.orizicolus	ATAAATCAGG	TAAGGACAAC	AATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
E.formosa	ATAAATCAGG	TAAAGATAAG	GATAGTCCC-	-----	----TTAAAA	GCATCTTTTC	
Trichopria sp(2)	ATGCAACAGG	CAAAGAAAAG	GATAGTCCC-	-----	----TTAACA	AGATCTTTTA	
T.bedeguaris	ATGCAACAGG	TAAAGAAAAG	GATAGTCCC-	-----	----TTAACA	AGATCTTTTA	
L.striatellus	ATGCAACAGG	TAAAGAAAAG	GATAGTCCC-	-----	----TTAACA	AGATCTTTTA	
T.confusum	ATGTAACAGG	TAAAGAAAAA	GATAGTCCC-	-----	----TTAACA	AGATCTTTTA	
T.kaykai	ATGCAACAAG	TAAAGAGAAG	GATAGTCCC-	-----	----TTAAAA	AGATCTTTTA	
T.kaykai	ATGCAACAAG	CAAAGAGAAG	GATAGTCCC-	-----	----TTAAAA	AGATCTTTTA	
T.T.deion	ATGCAACAAG	TAAAGAGAAG	GATAGTCCC-	-----	----TTAAAA	AGATCTTTTA	
T.nubilale	ATGCAACAAG	TAAAGATAAG	GATAGTCCC-	-----	----TTAAAA	AGATCTTTTA	
T.sibericum	ATGTAACAAG	TAAAGGGGAG	GATAGTCCC-	-----	----TTAAAA	AGATCTTTTA	
T.deion	ATGCAGCAA	TAAAGACAAG	GATAGTACC-	-----	----TTAAAA	AGATCTTTTA	
A.diversicornis	TTTCACAAGA	TCAAATCCC	-----	-----	----TTAAAA	GCGTCTTTTA	

Fig. 1 (Continued)

infests frugivorous *Drosophila* except for *D. simulans*. Three other species are generalist: *Leptopilina heterotoma* (Figitidae), which can also infest fungivorous *Drosophila* species, and two other parasitoids, *Pachycrepoides dubius* (Pteromalidea) and *Trichopria* sp. (Diapriidae), that have larger and not well defined host spectra. The biologies of the different parasitoid species are described in Carton et al. (1986). All insects used in this study originate from a restricted geographic area near Antibes, France. Since their collection, they have been reared on a *Wolbachia*-free strain of *D. melanogaster*.

#### *Wolbachia* Detection and Sequencing

For DNA extraction, adults were individually crushed in 150  $\mu$ l 5% chelex solution and kept for 2 h at 56°C. After 10 min at 95°C, samples were centrifuged. For PCR, 2  $\mu$ l of the supernatant was used. PCR reaction was done in a 25- $\mu$ l final volume reaction containing 200  $\mu$ M dNTP, 10 pM primers, 0.5 IU *Taq* DNA polymerase, and 2  $\mu$ l DNA solution. PCR conditions were 1 min at 95°C, followed by 35 cycles of 30 s at 95°C, 1 min at 55°C, and 1 min 30 s at 72°C. After the cycles, there was a 10-min elongation time at 72°C (Geneamp 2400, Perkin Elmer Cetus). We used either generalist primers of the

A.tabida (2)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
D.melanogaster	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
A.fuscipennis	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
A.albopictus	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
M.uniraptor	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
P.dubius	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
G.morsitans	TAGTTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
N.vitripennis	TAGTTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
G.centralis	TAGCTAGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
C.peregrinus	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
Trichopria sp(1)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
L.heterotoma (3)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
E.kuehniella	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
T.bourarachae	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
T.kaykay	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
L.heterotoma (2)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGAG
D.auraria	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGGC
D.sim(riverside)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGGC
L.heterotoma (1)	TAGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGGGTTGAT	GTTGAAGGGC
A.tabida (1)	TAGGAGGTGG	TGCCGCATTT	GGTTATAAAA	TGGACGACAT	TAGAGTTGAT	ATTGAAGGGC
D.sim(Hawai)	TGGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGAGTTGAT	GTTGAAGGGC
D.sechellia	TGGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGAGTTGAT	GTTGAAGGGC
E.cautella	TGGCTGGTGG	TGGTGCATTT	GGTTACAAAA	TGGACGACAT	CAGAGTTGAT	GTTGAAGGGC
P.papatasi	TGGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	CAGGGTTGAC	GTTGAAGGGC
G.austeni	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	CAGGGTTGAC	GTTGAAGGGC
A.tabida (3)	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	TAGAGTTGAT	GTTGAAGGGC
T.drosophilae	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	TAGAGTTGAT	GTTGAAGGGC
A.vulgare	TGGCTGGTAG	TGGTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAGGGAC
C.quinquefasciatus	TGGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGATAT	CAGGGTTGAT	GTTGAGGGAC
C.pipiens	TGGCTGGTGG	TGCTGCATTT	GGTTATAAAA	TGGACGATAT	CAGGGTTGAT	GTTGAGGGAC
D.mauritiana	TGGCTGGTGG	TGCTGCATTT	GGTTATAAAA	TGGACGATAT	CAGGGTTGAT	GTTGAGGGAC
D.sim(Nouméa)	TGGCTGGTGG	TGCTGCATTT	GGTTATAAAA	TGGACGATAT	CAGGGTTGAT	GTTGAGGGAC
A.albopictus	TGGCTGGTGG	TGCTGCATTT	GGTTATAAAA	TGGACGATAT	CAGGGTTGAT	GTTGAGGGAC
E.staufferi	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
L.australis	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
E.cautella	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
S.fuscipes	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
D.rosae	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
T.ORIZICOLUS	TAGCTGGAGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGGGTTGAT	GTTGAAGGAC
E.formosa	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGATGATAT	CAGAGTTGAT	GTTGAAGGAC
Trichopria sp(2)	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGATGACAT	TAGAGTTGAT	GTTGAAGGGC
T.bedeguaris	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGATGACAT	TAGAGTTGAT	GTTGAAGGGC
L.striatellus	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGATGACAT	TAGAGTTGAT	GTTGAAGGGC
T.confusum	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	TAGAGTTGAT	GTTGAAGGGC
T.kaykai	TAGCTGGTGG	TGGTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAAGGGC
T.kaykai	TAGCTGGTGG	TGTTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAAGGGC
T.T.deion	TAGCTGGTGG	TGTTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAAGGGC
T.nubilale	TAGCTGGTGG	TGTTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAAGGGC
T.sibericum	TAGCTGGKGG	TKTTGCATTT	GGTTATAAAA	TGGATGACAT	CAGAGTTGAT	GTTGAAGGGC
T.deion	TAGCTGGTGG	TGTTGCATTT	GGTTATAAAA	TGGATGACAT	TAGAGTTGAT	GTTGAAGGGC
A.diversicornis	TAGCTGGTAG	TGGTGCATTT	GGTTATAAAA	TGGACGACAT	TAGAGTTGAT	GTTGAAGGGC

FIG. 1 (Continued)

*wsp* gene or specific primers of subgroups of *Wolbachia* (Zhou, Rousset, and O'Neill 1998). According to *Wolbachia* variants, PCR products were sequenced either directly or after cloning in T-tailed vectors. Nucleotide sequences of *Wolbachia* are accessible in GenBank under accession numbers AF124852–AF124860.

#### Phylogenetic Analysis

The sequences from our data and other previously described sequences (Zhou, Rousset, and O'Neill 1998; Van Meer, Witteveldt, and Stouthamer 1999) were aligned using CLUSTAL W (Thompson, Higgins, and Gibson 1994), based on the alignment produced by Zhou,

Rousset, and O'Neill (1998). Because most species are coinfecting by different *Wolbachia* variants, we used specific primers which lead to shorter sequences than those used by Zhou, Rousset, and O'Neill (1998). Two different trees were made, either based on a restricted region (365 bases) and including all sequences or based on a larger region (479 bases excluding the third hypervariable region; Braig et al. 1998). This second tree does not contain the two variants infecting *Trichopria* sp. Trees were constructed by the neighbor-joining method using the Jukes and Kantor distance in the PHYLO-WIN program (Galtier, Gouy, and Gautier 1996). Bootstrapping was also done with PHYLO-WIN (500 replicates).



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A.tabida (2)	TTTATTCATA	CCTAAACAAA	AATGATGTTA	AAGATGTAAC	ATTTGACCCA	GCAAATACTA
D.melanogaster	TTTATTCATA	CCTAAACAAA	AATGATGTTA	AAGATGTAAC	ATTTGACCCA	GCAAATACTA
A.fuscipennis	TTTATTCATA	CCTAAACAAA	AATGATGTTA	AAGATGTAAC	ATTTGACCCA	GCAAATACTA
A.albopictus	TTTATTCATA	CCTAAACAAA	AATGATGTTA	AAGATGTAGT	ATTTACCCCA	GCAGATACTA
M.uniraptor	TTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATGCTA
P.dubius	TTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATGCTA
G.morsitans	TTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATACTA
N.vitripennis	CTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATACTA
G.centralis	TTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATACTA
C.peregrinus	TTTATTCATA	CCTAAATAAA	AATGATGTTA	CAGATGCAAA	ATTTACGCCA	---GATACTG
Trichopria sp(1)	TTTATTCATA	CCTAAACAAA	AATAATGTTA	CAGATGCAAG	ATTTACGCCA	---GATACTA
L.heterotoma (3)	TTTATTCATA	CCTAAACAAA	AATAATGTTA	CAGATGCAAG	ATTTACGCCA	---GATACTA
E.kuehniella	TTTATTCATA	CCTAAACAAA	AATAATGTTA	CAGATGCAAG	ATTTACGCCA	---GATACTA
T.bourarachae	TTTATTCATA	CCTAAACAAA	AATAATGTTA	CAGATGCAAG	ATTTACGCCA	---GATACTA
T.kaykay	TTTATTCATA	CCTAAACAAA	AATAATGTTA	CAGATGCAAG	ATTTACGCCA	---GATACTA
L.heterotoma (2)	TTTATTCATA	CCTAAACAAA	AATGATGTTA	CAGATGCAGA	ATTTACGCCA	---GATACTA
D.auraria	TTTATTCACA	GCTAAACAAA	AATGATGTTA	CAGGTGCAGC	ATTTAACCCA	---GATACTG
D.sim(riverside)	TTTATTCACA	GCTAAACAAA	AATGATGTTA	CAGGTGCAGC	ATTTAACCCA	---GATACTG
L.heterotoma (1)	TTTATTCACA	GCTAAACAAA	AATGATGTTA	CAGGTGCAGC	ATTTAACCCA	---GATACTG
A.tabida (1)	TATATTCACA	GCTAAACAAG	AATGTGAACA	ATAATGAAGT	GCTTACTCCA	---GATACTG
D.sim(Hawai)	TTTATTCGCA	GCTAAGCAAG	GATACACTT-	-----GA	TGTAGCTCCT	ACTCCAGCAA
D.sechellia	TTTATTCGCA	GCTAAGCAAG	GATACACTT-	-----GA	TGTAGCTCCT	ACTCCAGCAA
E.cautella	TTTATTCGCA	GCTAAGCAAG	GATACACTT-	-----GA	TGTAGCTCCT	ACTCCAGCAA
P.papatasi	TTTATTCGCA	GCTAAGCAAG	GATGCACTT-	-----GC	TGTAGCTCCT	ACTCCAGCAA
G.austeni	TTTACTCACA	GTTGAATAAA	GATGCA----	-----GG	TGTAGCAGGT	ACT---ACAG
A.tabida (3)	TTTACTCATG	GTTGAATAAA	GATGCA----	-----GA	TGTAGTAGGT	GAT---ACAG
T.drosophilae	TTTACTCATG	GTTGAATAAA	GATGCA----	-----GA	TGTAGTAGGT	GAT---ACAG
A.vulgare	TTTACTCACA	ACTAAACAAA	AACGACGTTA	GTGGTGCAGC	ATTTACTCCA	GTA ACT---G
C.quinquefasciatus	TTTACTCACA	ACTAAACAAA	AACGACGTTA	GTGGTGCAC	ATTTACTCCA	ACA ACT---G
C.pipiens	TTTACTCACA	ACTAAACAAA	AACGACGTTA	GTGGTGCAC	ATTTACTCCA	ACA ACT---G
D.mauritiana	TTTACTCACA	ACTAAACAAA	AACGACGTTA	GTGGTGCAC	ATTTACTCCA	ACA ACT---G
D.sim(Nouméa)	TTTACTCACA	ACTAAACAAA	AACGACGTTA	GTGGTGCAC	ATTTACTCCA	ACA ACT---G
A.albopictus	TTTACTCACA	ACTAAACAAA	AACGACGTTG	GTGGTGCAC	ATTTGCTCCA	ACA ACT---G
E.staufferi	TTTACCCACG	ATTGAGTAAA	GATGCAGATG	TAGTAGGTAC	T---TCTCCA	GCA-----G
L.australis	TTTACTCAG	ATTGAGTAAA	GATGCAGATG	TAGTAGGTAC	T---TCTCCA	GCA-----G
E.cautella	TTTACTCACA	ATTGAGTAAA	GATGCAGATG	TAGTAGATAC	T---TCTCCA	GCA-----G
S.fuscipes	TTTACTCACA	ATTGAGTAAA	GATGCAGATG	TAGTAGATAC	T---TCTCCA	GCA-----G
D.rosae	TTTACTCACA	ATTGAGTAAA	GATGCAGATG	TAGTAGATAC	T---TCTCCA	GCA-----G
T.orizicolus	TTTACTCACA	ATTGAGTAAA	GATGCAGATG	TAGTAGATAC	T---TCTCCA	GCA-----G
E.formosa	TCTACTCACA	ATTGAGTAAA	GACGGAGATG	TAGCTGGTGA	T-----TCA	GCA-----A
Trichopria sp(2)	TTTACTCACA	ATTGGCTAAA	GATGCAGCTG	TAGTAAATAC	T---TCTGAA	ACAAAT---G
T.bedeguaris	TTTACTCACA	ATTGGCTAAA	GATACAGCTG	TAGTAAATAC	T---TCTGAA	ACAAAT---G
L.striatellus	TTTACTCACA	ATTAGCTAAA	GATACAGATG	TAGTAAATAC	T---TCTGAA	ACAAAT---G
T.confusum	TTTACTCACA	ATTGGCTAAA	GATACAGCTG	TAGTAAATAC	T---TCTGAA	ACAAAT---G
T.kaykai	TTTACTCAG	ATTGGCTAAA	AATAAAGCTG	TAATAGATGC	T---TCTGAA	GCAAAT---G
T.kaykai	TTTACTCAG	ATTGGCTAAA	AATAAAGCTG	TAATAGATGC	T---TCTGAA	GCAAAT---G
T.T.deion	TTTACTCAG	ATTGGCTAAA	AATAAAGCTG	TAATAGATGC	T---TCTGAA	GCAAAT---G
T.nubilale	TTTACTCAG	ATTAGCTAAA	AATAAAGCTG	TAATAGATGC	T---TCTGAA	GCAAAT---G
T.sibericum	TTTACTCAG	ATTGGCTAAA	AATAAAGCTG	TAATAGATGC	T---TCTGAA	GCAAAT---G
T.deion	TTTACTCAG	ATTGGCTAAA	AATGGAGCTG	TGATAGATGC	T---TCTGAA	GCAAGT---G
A.diversicornis	TTTACTCAG	ATTGGCTAAA	GATGACGCTG	TAGTA-----	-----TCTGAT	GCCAAT---G

Fig. 1 (Continued)

### Statistical Test of Horizontal Transmission

To test the possibilities for horizontal transmission, we developed the following statistical method. First, the phylogeny was simplified to eliminate possible cospeciation processes: the monophyletic group of *Wolbachia* that infect *Trichogramma* species was treated as a unique sequence, as were the closely related *Wolbachia* infecting two species of the same genus. Second, with respect to the tree topology, sequence names were randomly placed on the tree, and we recorded the number of wasp-host and wasp-wasp nearest neighbors (Jukes and Cantor distance <0.01). These two situations cor-

respond to the possible transfers of *Wolbachia* in the community. The process was repeated 10,000 times. The percentage of trees bearing at least the same number of transfers as that observed gives the bootstrap probability.

### Results

Table 1 shows *Wolbachia* infection in the different species present in the *Drosophila* community in southeast France. Although the infection statuses of *D. melanogaster* and *D. simulans* are well known, we also studied these two species to allow comparison within a complex of

A. tabida (2)	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGAT	TAGTGAACGT	GTATTACGAT	ATAGCAATTG
D. melanogaster	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGAT	TAGTGAACGT	GTATTACGAT	ATAGCAATTG
A. fuscipennis	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGT	TAGTGAACGT	GTATTACGAT	ATAGCAATTG
A. albopictus	TTGCGAACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
M. uniraptor	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
P. dubius	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
G. morsitans	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
N. vitripennis	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
G. centralis	TTGCAGACAG	TTTAGCAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
C. peregrinus	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	ATAGCAGTTG
Trichopria sp(1)	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
L. heterotoma (3)	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
E. kuehniella	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
T. bourarachae	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
T. kaykay	TTGCAGACAG	TGTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
L. heterotoma (2)	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
D. auraria	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
D. sim (riverside)	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
L. heterotoma (1)	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
A. tabida (1)	TTGCGGACAG	CTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
D. sim (Hawai)	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
D. sechellia	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
E. cautella	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
P. papatasi	TTGCAGACAG	TTTAACAGCA	ATTTTCAGGGC	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
G. austeni	TTGCAGATAA	TTTAACAGCA	ATTTTCAGGAT	TAGTTAACGT	TTATTACGAT	ATAGCAATTG
A. tabida (3)	TTGCAGATAA	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	GTAGCAATTG
T. drosophilae	TTGCAGATAA	TTTAACAGCA	ATTTTCAGGAC	TAGTTAACGT	TTATTACGAT	GTAGCAATTG
A. vulgare	TTGCAGACAG	TGTGACAGCG	TTTTTCAGGAT	TAATTAATGT	TTATTATGAT	GTAGCAATCG
C. quinquefasciatus	TTGCAAACAG	TGTGGCAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
C. pipiens	TTGCAAACAG	TGTGGCAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
D. mauritiana	TTGCAAACAG	TGTGGCAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
D. sim (Nouméa)	TTGCAAACAG	TGTGGCAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
A. albopictus	TTGCAAACAG	TGTGGCAGTA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
E. staufferi	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
L. australis	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
E. cautella	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
S. fuscipes	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
D. rosae	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
T. orizicolus	TTGTAGAAAG	TTTAACAGCA	TTTTTCAGGAC	TAGTTAATGT	TTATTACGAT	ATAGCAATTG
E. formosa	TTGCAGAAAAG	TTTAACAGCA	TTTTTCAGGAT	TAGTTAACGT	TTATTACGAT	GTAGCAATTG
Trichopria sp(2)	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGGT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
T. bedeguaris	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGACTG
L. striatellus	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
T. confusum	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG
T. kaykai	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTATGAT	ATAGTGATTG
T. kaykai	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTATGAT	ATAGTGATTG
T. T. deion	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTATGAT	ATAGTGATTG
T. nubilale	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTATGAT	ATAGTGATTG
T. sibericum	TTGCAGACAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTATGAT	ATAGTGATTG
T. deion	TTGCAGAAAAG	TTTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGTGGTTG
A. diversicornis	TTGCAGATAG	TGTAACAGCA	TTTTTCAGGAT	TGGTTAACGT	TTATTACGAT	ATAGCGATTG

FIG. 1 (Continued)

sympatric species. Among the five hosts, only *D. melanogaster* and *D. simulans* proved to be infected, each with a single *Wolbachia* type, while among the five parasitoids, only *L. boulandi* proved to be uninfected. Moreover, double infection, where each individual wasp carries two different *Wolbachia* variants, occurred in *Trichopria* sp., and the first cases of triple infection were recorded in *L. heterotoma* and *A. tabida*. On the other hand, *P. dubius* carries a single *Wolbachia*. Finally, among 11 insect-*Wolbachia* associations, 9 involve parasitoid species. Since the four hymenopteran species belong to phylogenetically distant families, we can compare the results with a bi-

nomial distribution where  $P = 0.5$  (five *Drosophila* species and five parasitoid species). The observed distribution is highly biased ( $P = 0.003$ ), thus demonstrating that parasitoids are more susceptible to *Wolbachia* infection than are *Drosophila*.

A phylogenetic tree based on the partial sequence of the gene *wsp* (365 bases) evidences two subdivisions (A and B) with more than 20% divergence, in full agreement with Zhou, Rousset, and O'Neill (1998). The tree based on a larger sequence (479 bases) but without the *Trichopria* sp. variants gives the same topology, except for slight differences. The main difference is that the variant

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A. tabida (2)	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
D. melanogaster	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
A. fuscipennis	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
A. albopictus	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTCAGCACTC
M. uniraptor	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGCGTTGG	TACAGCGTAT	ATTAGCACAC
P. dubius	AAGATATGCC	TATCACTCCA	TATATTGGTG	TTGGCGTTGG	TGCAGCGTAT	ATTAGCACAC
G. morsitans	AAGATATGCC	TATCACTCCA	TATATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
N. vitripennis	AAGATATGCC	TATCACTCCA	TATATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
G. centralis	AAGATATGCC	TATCACTCCA	TATATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
C. peregrinus	AAGATATGCC	TATCACTCCA	TATATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
Trichopria sp(1)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
L. heterotoma (3)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
E. kuehniella	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
T. bourarachae	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
T. kaykai	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
L. heterotoma (2)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
D. auraria	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
D. sim(riverside)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
L. heterotoma (1)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
A. tabida (1)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
D. sim(Hawai)	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACAC
D. sechellia	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACAC
E. cautella	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACAC
P. papatasi	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAAC
G. austeni	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACAC
A. tabida (3)	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGCGTTGG	TGCAGCGTAT	ATTAGCACTC
T. drosophilae	AAGATATGCC	TATCACTCCA	TACATTGGTG	TTGGTGTGG	TGCAGCGTAT	ATTAGCACTC
A. vulgare	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	GTAAGCAATC
C. quinquefasciatus	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
C. pipiens	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
D. mauritiana	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
D. sim(Nouméa)	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
A. albopictus	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
E. staufferi	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
L. australis	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
E. cautella	AAGATATGCC	TATCACTCCA	TATGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
S. fuscipes	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
D. rosae	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
T. orizicolus	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCGTAT	GTAAGCAATC
E. formosa	AAGACATGCC	TGTCACTCCA	TATATTGGTG	TTGGTGTGG	CGCAGCATAT	GTAAGCAACC
Trichopria sp(2)	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. bedeguaris	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
L. striatellus	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. confusum	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. kaykai	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. kaykai	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. T. deion	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. nubilale	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. sibericum	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
T. deion	AAGATATGCC	TATTATTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC
A. diversicornis	AAGATATGCC	TATCACTCCA	TACGTTGGTG	TTGGTGTGG	TGCAGCATAT	ATCAGCAATC

FIG. 1 (Continued)

(variant 2) infecting *L. heterotoma* is in closer association with the *T. bourarachae* group even if it remains isolated (tree not shown). Thus, in order to use all *Drosophila*-parasitoid sequences, we used the tree based on 365 bases and all sequences for further analysis. Sequences of *Wolbachia* infecting *D. simulans* (type Riverside) and *D. melanogaster* are identical to those already described (Zhou, Rousset, and O'Neill 1998).

In five cases, there are striking similarities between *Wolbachia* of parasitoids and hosts (fig. 1). *Leptopilina heterotoma* bears one *Wolbachia* (variant 1) which is identical to that of *D. simulans* (Riverside type), and

they form an individualized subgroup compared with other *Wolbachia* of the A clade. This branch is highly supported, with a bootstrap score of 100. Similarly, *A. tabida* bears one *Wolbachia* (variant 2) closely related to that of *D. melanogaster* (no difference in the 365-base sequence used in the tree, four differences in the complete sequence [560 bases]; bootstrap score: 100). *Leptopilina heterotoma* (variant 3) and *Trichopria* sp. (variant 1) have identical bacteria which also form a separate highly supported branch with three other species that do not belong to the *Drosophila* complex (bootstrap score: 100). In this case, either the two parasitoids



A.tabida (2)	CTTTGGAACC	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
D.melanogaster	CTTTGGAACC	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
A.fuscipennis	CTTTGGAACC	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
A.albopictus	CTTTGAAAAC	CGCTATA---	AATAATCAAA	ACAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
M.uniraptor	CTTTGGCAAC	TGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
P.dubius	CTTTGGCAAC	TGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
G.morsitans	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
N.vitripennis	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
G.centralis	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
C.peregrinus	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
Trichopria sp(1)	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
L.heterotoma (3)	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
E.kuehniella	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
T.bourarachae	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	GAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
T.kaykay	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
L.heterotoma (2)	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
D.auraria	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
D.sim (riverside)	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
L.heterotoma (1)	CTTTGAAAGA	CGCTGTG---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
A.tabida (1)	CTTTGAAAGA	CGCTGTG---	AATGGTCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
D.sim (Hawai)	CTTTGGCAAC	CGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
D.sechellia	CTTTGGCAAC	CGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
E.cautella	CTTTGGCAAC	CGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
P.papatasi	CTTTGGCAAC	TGCTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGCAA
G.austeni	CTTTGGCAAC	TGTTGTG---	AGTAGTCAAA	ATGGTAAATT	TGCTTTTGCT	GGTCAAGTAA
A.tabida (3)	CTTTGAAAAC	CCCTATA---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
T.drosophilae	CTTTGAAAAC	CCCTATA---	AATGATCAAA	AAAGTAAATT	TGGTTTTGCT	GGTCAAGTAA
A.vulgare	CTTTAGCAAC	AAAAGTTGCT	GGTGATAAAG	ACTCTGGATT	TGGTTTTGCT	TATCAAGCGA
C.quinquefasciatus	CTTCAGAAGC	TAGTGCAGTT	AAAGATCAAA	AR---GGATT	TGGTTTTGCT	TATCAAGCAA
C.pipiens	CTTCAGAAGC	TAGTGCAGTT	AAAGATCAAA	AR---GGATT	TGGTTTTGCT	TATCAAGCAA
D.mauritiana	CTTCAGAAGC	TAGTGCAGTT	AAAGATCAAA	AA---GAATT	TGGTTTTGCT	TATCAAGCAA
D.sim (Nouméa)	CTTCAGAAGC	TAGTGCAGTT	AAAGATCAAA	AA---GAATT	TGGTTTTGCT	TATCAAGCAA
A.albopictus	CTTCAGAAGC	TAGTGCAGTT	AAAGATCAAA	AA---GGATT	TGGTTTTGCT	TATCAAGCAA
E.staufferi	CTTTAGTAAC	AGAGGTTACT	GGTGATAAAA	AATTTGGATT	TGGTTTTGCT	TATCAAGCAA
L.australis	CTTTAGTAGC	AGAGGTTACT	GGTGATAAAA	AATTTGGATT	TGGTTTTGCT	TATCAAGCAA
E.cautella	CTTTAGTAAC	AGAGATTACT	GGTGATAAAA	AATCTGGATT	TGGTTTTGCT	TATCAAGCAA
S.fuscipes	CTTTAGTAAC	AGAGGTTACT	GGTGATAAAA	AATCTGGATT	TGGTTTTGCT	TATCAAGCAA
D.rosae	CTTTAGTAAC	AGAGGTTACT	GGTGATAAAA	AATCTGGATT	TGGTTTTGCT	TATCAAGCAA
T.ORIZICOLUS	CTTTAGTAAC	AGAGGTTACT	GGTGATAAAA	AATCTGGATT	TGGTTTTGCT	TATCAAGCAA
E.formosa	CTTTAGCGGC	AAAAGTTACT	GATGATAAAG	CCTCTGGATT	TGCTTTTGCT	TATCAAGCAA
Trichopria sp(2)	CTTCAAAAGT	TGATGCAGTT	AAAGATCAAA	AA---GGATT	TGGTTTTGCT	TATCAAGCAA
T.bedeguaris	CTTCAAAAAC	TGATGCAGTT	AAAGATCAAA	AA---GGATT	TGGTTTTGCT	TATCAAGCAA
L.striatellus	CTTCAAAAGC	TGGTGTAGTT	AAAGATCAAA	AA---GGATT	TGGTTTTGCT	TATCAAGCAA
T.confusum	CTTCAAAAGC	TGATGCAGTT	AAAGATCAAA	AA---GGATT	TGGTTTTGCT	TATCAAGCAA
T.kaykai	CTTCAAACGC	TGCTGACGTT	AAAGATCAAA	GG---AGATT	TGGTTTTGCT	TATCAAGCAA
T.kaykai	CTTCAAACGC	TGCTGACGTT	AAAGATCAAA	GG---AGATT	TGGTTTTGCT	TATCAAGCAA
T.T.deion	CTTCAAACGC	TGCTGACGTT	AAAGATCAAA	GG---AGATT	TGGTTTTGCT	TATCAAGCAA
T.nubilale	CTTCAAACGC	TGCTGACGTT	AAAGATCAAA	GG---AGATT	TGGTTTTGCT	TATCAAGCAA
T.sibericum	CTTCAAACGC	TGCTGACGTT	AAAAATCAAA	GG---AGGTT	TGGTTTTGCT	TATCAAGCAA
T.deion	CTTCAAAAGT	TGCTGAAGTT	AAAGATCAAA	GG---AGCTT	CGGTTTTGCT	TATCAAGCAA
A.diversicornis	CTTCAAGCGC	TGGTGAAGCT	AAAAAGCAA	GA---GGATT	TGGTTTTGCT	TATCAAGCAA

FIG. 1 (Continued)

could have inherited their *Wolbachia* from a common host, or horizontal transmission could have occurred from parasitoid to parasitoid through multi- or hyperparasitism (simultaneous infestation of a host and parasitism of a primary parasitoid, respectively). *Trichopria drosophilae* (variant previously described by Van Meer, Witteveldt, and Stouthamer 1999) and *A. tabida* (variant 3) also share closely related *Wolbachia* that form another subgroup (bootstrap score: 100). A fifth possible transfer is shown by the similarity of *Wolbachia* in *Muscidifurax uniraptor* and *P. dubius*. Since these two gen-

eralist wasps parasitize pupae of Diptera species, they could have caught *Wolbachia* from a so far untested common host (such as *Musca domestica*). Moreover, *P. dubius* is able to develop as a hyperparasitoid (Van Alphen and Thunissen 1983) and could have caught *Wolbachia* from a primary parasite as well. Among all possible horizontal transfers between the insects considered here, the bootstrap probability for getting such similarity is only 4%. The high frequency of observed similarity or identity of *Wolbachia* in parasitoids and hosts strongly reinforces the hypothesis of frequent horizontal



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A.tabida (2)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
D.melanogaster	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
A.fuscipennis	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
A.albopictus	AAGCTGGTGT	CAGCTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
M.uniraptor	GAGCTGGTGT	TAGTTACGAT	GTAACCCAG	AAGTCAAAC	TTACGCTGGA	GCTCGTATT
P.dubius	GAGCTGGTGT	TAGTTACGAT	GTAACCCAG	AAGTCAAAC	TTACGCTGGA	GCTCGTATT
G.morsitans	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
N.vitripennis	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
G.centralis	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
C.peregrinus	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
Trichopria sp(1)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAG-----	-----	-----
L.heterotoma (3)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
E.kuehniella	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
T.bourarachae	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
T.kaykai	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
L.heterotoma (2)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
D.auraria	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
D.sim(riverside)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
L.heterotoma (1)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
A.tabida (1)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
D.sim(Hawai)	GAGCTGGTGT	CAGCTATGAC	ATAACCCAG	AAATCAAAC	CTACGCTGGA	GCTCGTTATT
D.sechellia	GAGCTGGTGT	CAGCTATGAC	ATAACCCAG	AAATCAAAC	CTACGCTGGA	GCTCGTTATT
E.cautella	GAGCTGGTGT	CAGCTATGAC	ATAACCCAG	AAATCAAAC	CTACGCTGGA	GCTCGTTATT
P.papatasi	GAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAGTCAAAC	TTATGCTGGA	GCTCGTTATT
G.austeni	GAGCTGGTGT	TAGTTACGAT	GTAACCCAG	AAGTCAAAC	TTACGCTGGA	GCTCGTATT
A.tabida (3)	AAGCTGGTGT	TAGCTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGA	GCTCGTTATT
T.drosophilae	AAGCTGGTGT	TAGCTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGA	GCTCGTTATT
A.vulgare	AAGCTGGTGT	CAGTTATGAC	GTAACCCAG	AAATCAAAC	TTACGCTGGC	GCTCGTATT
C.quinquefasciatus	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTRGT	GCTCGTTATT
C.piplens	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTRGT	GCTCGTTATT
D.mauritiana	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
D.sim(Nouméa)	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
A.albopictus	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
E.staufferi	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
L.australis	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
E.cautella	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
S.fuscipes	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
D.rosae	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
T.orizicolus	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
E.formosa	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAGCT	TTATGCTGGT	GCTCGTTATT
Trichopria sp(2)	AAGCT-----	-----	-----	-----	-----	-----
T.bedeguaris	AAGCTGGTGT	TAGCTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
L.striatellus	AAGCTGGTGT	TAGCTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.confusum	AAGCTGGTGT	TAGCTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.kaykai	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.kaykai	AAGCNGNGC	TAGTTATGAN	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.T.deion	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.nubilale	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.sibericum	AAGCTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
T.deion	AAGGTGGTGT	TAGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGA	GCTCGTTACT
A.diversicornis	AAGCTGGTGT	TGTTATGAT	GTAACCCAG	AAATCAAAC	CTTTGCTGGT	GCCCATATT

↑  
565 bases

Fig. 1 (Continued)

transmission of *Wolbachia* in host-parasitoid associations.

## Discussion

The higher infection frequency in parasitoids, the common occurrence of multiple infection in parasitoids and not in hosts, and the identity and/or similarity of host and parasitoid symbionts are good arguments that horizontal transfers occur from hosts to parasitoids. The higher infection frequency in parasitoids can be account-

ed for by different hypotheses. Infection in *Drosophila* could be less stable than that in parasitoids due to either higher exposure to natural antibiotics (even though both partners share the same environment) or intrinsic properties of Diptera and Hymenoptera. However, the better hypothesis is that parasitoids are more susceptible to horizontal transmission of *Wolbachia*. Indeed, parasitoids could catch symbionts from their host either at the time they develop as parasitic larva within or outside the host's body or when they consume the host. In contrast, transfer from parasitoids to hosts is quite unlikely,

A. tabida (2)	TCGGTTCTTA	TGGTGCTAAT	TTTGATGGAA	AAAAAACAGA	TCCTAAA---	-----GATTC
D. melanogaster	TCGGTTCTTA	TGGTGCTAAT	TTTGATGGAA	AAAAAACAGA	TCCTAAA---	-----AATTC
A. fuscipennis	TCGGTTCTTA	TGGTGCTAAT	TTTGATGGAA	AAAAAACAGA	TCCTAAA---	-----GATTC
A. albopictus	TCGGTTCTTT	TGGTGCTCAC	TTTGATAGCG	AAACTACTGG	TGCAGAT---	-----AACAA
M. uniraptor	TCGGTTCTTA	TGGTGCTAAC	TTTGATAAAA	CTGACAAAGA	CGGCAAAA---	-----
P. dubius	TCGGTTCTTA	TGGTGCTAAC	TTTGATAAAA	CTGACAAAGA	CGGCAAAA---	-----
G. morsitans	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GAT--
N. vitripennis	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GAT--
G. centralis	TCGGTTCTTT	TGGTGCTCAT	TTTGATAGA-	-----	-----	-----GAT--
C. peregrinus	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GAT--
Trichopria sp(1)	-----	-----	-----	-----	-----	-----
L. heterotoma (3)	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
E. kuehniella	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
T. bourarachae	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
T. kaykay	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
L. heterotoma (2)	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
D. auraria	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
D. sim(riverside)	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
L. heterotoma (1)	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
A. tabida (1)	TCGGTTCTTA	TGGTGCTAAT	TTCGATAAAA-	-----	-----	-----AG
D. sim(Hawai)	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
D. sechellia	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
E. cautella	TCGGTTCTTT	TGGTGCTCAT	TTTGATAAAA-	-----	-----	-----GATAC
P. papatasi	TCGGTTCTTA	TGGTGCTAAC	TTTGATAAAA-	-----	-----	-----
G. austeni	TCGGTTCTTA	TGGTGCTAAC	TTTGATAAAA	CTGACAAAGA	CGGCAAAA---	-----
A. tabida (3)	TCGGTTCTTA	TGGTGCTAAT	TTTGATGGAA	AAAAAACAGA	TCCTAAA---	-----GATTC
T. drosophilae	TCGGTTCTTA	TGGTGCTAAT	TTCGATGGAA	AAAAAACAGA	TCCTAAA---	-----GATTT
A. vulgare	TTGGTTCTTA	TGGCGCTAAC	TTTGATGGAA	CAAAACAAGA	TCCTGCACAT	CCTGATGATC
C. quinquefasciatus	TTGGTTCTTA	TGGTGCTAGT	TTTAATAAAG	AAGCAGTATC	A-----	-----
C. pipiens	TTGGTTCTTA	TGGTGCTAGT	TTTAATAAAG	AAGCAGTATC	A-----	-----
D. mauritiana	TTGGTTCTTA	TGGTGCTAGT	TTTAATAAAG	AAGCAGTATC	A-----	-----
D. sim(Nouméa)	TTGGTTCTTA	TGGTGCTAGT	TTTAATAAAG	AAGCAGTATC	A-----	-----
A. albopictus	TTGGTTCTTA	TGGTGCTAGT	TTTAATAAAG	AAACAGTATC	A-----	-----
E. staufferi	TTGGTTCTTA	TGGTGCTAAT	TTTGATCAGA	CAGCTAAAGA	T-----	-----
L. australis	TTGGTTCTTA	TGGTGCTAAT	TTTGATCAGA	CAGCTAAAGA	T-----	-----
E. cautella	TTGGTTCTTA	TGGTGCTAAT	TTTGATCAGA	CAGCTAAAGA	T-----	-----
S. fuscipes	TTGGTTCTTA	TGGTGCTAAT	TTTGATCAGA	CAGCTAAAGA	T-----	-----
D. rosae	TTGGTTCTTA	TGGTGCTAAT	TTTGATCAGA	CAGCTAAAGA	T-----	-----
T. orizicolus	TTGGTTCTTA	TGGTGCTAAA	TTTGATCAGA	CAACTAAAGA	T-----	-----
E. formosa	TTGGTTCTTA	TGGTGCTAAT	TTT---AAGA	TAGCTAAAGA	T-----	-----
Trichopria sp(2)	-----	-----	-----	-----	-----	-----
T. bedeguaris	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGA	T-----	-----
L. striatellus	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGA	T-----	-----
T. confusum	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CGGCTAAGGA	T-----	-----
T. kaykai	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGG	T-----	-----
T. kaykai	TCGGTTCTTA	TGGTGCTAGC	TTTGATAAAG	CAGCTAAGGG	T-----	-----
T. T. deion	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGG	T-----	-----
T. nubilale	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGG	T-----	-----
T. sibericum	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAGCTAAGGG	T-----	-----
T. deion	TCGGTTCTTA	TGGTGCTAGT	TTTGATAAAG	CAACTAAGGG	T-----	-----
A. diversicornis	TTGGTTCTTA	TGGTGCTAGG	TTTCATAAAA	TAAAAGAACC	A-----	-----

→ third hypervariable region

FIG. 1 (Continued)

since nearly no surviving hosts have been parasitized, and no parasitoid could develop in the absence of the host. Thus, parasitoids may be highly sensitive to *Wolbachia* infection but may be only a little involved in the transfer of *Wolbachia* to host species.

The common multi-infection found in parasitoids raises different questions. (1) Are these multi-infections stable? The triple infection is stable during generations, demonstrating a good transmission of the three variants. Moreover, all populations of *L. heterotoma* checked (10) are triply infected (results not shown). The same pattern

is observed in *A. tabida*. Thus, these multiple infections are both temporally and spatially stable. (2) How can multi-infection invade populations? *Wolbachia* is known to induce cytoplasmic incompatibility in *L. heterotoma* (Vavre et al. 1999), as well as in *A. tabida* (Werren, Zhang, and Guo 1995) and *Trichopria* sp. (results not shown). Under the hypothesis that all variants induce incompatibility and that mono-infected individuals are incompatible with multiply infected ones, multiply infected individuals are advantaged (Sinkins, Braig, and O'Neill 1995). However, it is uncertain whether all var-

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A.tabida (2)	AACCAGACAG	GTTACTGATG	CAGGCGCATA	CAAAGTTCTT	TACAGCAC
D.melanogaster	AACCAGACAG	GCTGCTGATG	CAGGCGCATA	CAAAGTTCTT	TACAGCAC
A.fuscipennis	AACCAGACGG	GTTACTGATG	CAGGCGCATA	CAAAGTTCTT	TACAGCAC
A.albopictus	AAAAGTAGTT	ACCAAAGATG	CA-----TA	CAAAGTTCTT	TACAGCAC
M.uniraptor	-----	-----	--GGGGA	CAAAGTTCTT	TACAGCAC
P.dubius	-----	-----	--GGGGA	CAAAGTTCTT	TACAGCAC
G.morsitans	-GCTGCTGCA	GGCAAAGACA	AAGGGGA	CAAAGTTCTT	TACAGCAC
N.vitripennis	-GCTGCTGCA	GGCAAAGACA	AAGGGGA	CAAAGTTCTT	TACAGCAC
G.centralis	-GCTGCTGCA	GGCAAAGACA	AAGGGGA	CAAAGTTCTT	TACAGCAC
C.peregrinus	-GCCGCTGCA	GGCAAAGACA	AAGGGGA	CAAAGTTCTT	TACAGCGC
Trichopria sp(1)	-----	-----	-----	-----	-----
L.heterotoma (3)	CGGTGGTGAG	---AAAGACA	AAGGAGGATA	TACAGTCCTT	TACAGCAC
E.kuehniella	CGTTGGTGAG	---AAAGACA	AAGGAGGACA	TACAGTCCTT	TACAGCAC
T.bourarachae	CGTTGGTGAG	---AAAGACA	AAGGAGGACA	TACAGTCCTT	TACAGCAC
T.kaykai	CGTTGGTGAG	---AAAGACA	AAGGAGGACA	TACAGTCCTT	TACAGCAC
L.heterotoma (2)	CGGTGGTGAG	---AAAGACA	AAGGAGGACA	TACAGTCCTT	TACAGCAC
D.auraria	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
D.sim(riverside)	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
L.heterotoma (1)	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
A.tabida (1)	CGGTGGTGAG	---AAAGACA	AAGGAGGACA	TACAGTCCTT	TACAGCAC
D.sim(Hawai)	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
D.sechellia	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
E.cautella	TGCTGCAGCA	AGCAAAGACA	AGGGGGA	CAAAGTTCTT	TACAGCAC
P.papatasi	---ACTGAC	AAAGATGGCA	AAGGGAA	CAAAGTTCTT	TACAGCAC
G.austeni	-----	-----	--GGGGA	CAAAGTTCTT	TACAGCAC
A.tabida (3)	AACCAAACAG	GTTACTGATG	CAGGCGCATA	CAAAGTTCTT	TACAGCAC
T.drosophilae	AACCAAACAG	GTTACTGATG	CAGGCGCATA	CAAAGTTCTT	TACAGCAC
A.vulgare	TAGAGAAAAA	GTTACTGATA	AAGGCGCACA	CAAAGTTTTT	TACAGTAC
C.quinquefasciatus	-----	-----GCTA	CTAAAGAGAT	CAATGTCCTT	TACAGCGC
C.pipiens	-----	-----GCTA	CTAAAGAGAT	CAATGTCCTT	TACAGCGC
D.mauritiana	-----	-----GCTA	CTAAAGAGAT	CAATGTCCTT	TACAGCGC
D.sim(Nouméa)	-----	-----GCTA	CTAAAGAGAT	CAATGTCCTT	TACAGCGC
A.albopictus	-----	-----GCTA	CTAAAGAGAT	CAACGTTCTT	TACAGCGC
E.staufferi	-----	-----G	ATGGCGGAAT	CAAAGTTCTT	TACAGCAC
L.australis	-----	-----GATG	GCGGAATCAA	A---GTTCTT	TACAGCAC
E.cautella	-----	-----GATG	GCGGAATCAA	A---GTTCTT	TACAGCAC
S.fuscipes	-----	-----GATG	GAGGAATCAA	A---GTTCTT	TACAGCAC
D.rosae	-----	-----GATG	GCGGAATCAA	A---GTTCTT	TACMGCAC
T.orizicolus	-----	-----GATG	GCGGAATCAA	G---GTTCTT	TACAGCAC
E.formosa	-----	-----GATG	CCAGAATCAA	A---GTTCTT	TACAGCAC
Trichopria sp(2)	-----	-----	-----	-----	-----
T.bedeguaris	-----	-----GATA	CTGGTATCAA	AAATGTTGTT	TACAGCAC
L.striatellus	-----	-----GATG	CTGGTATCAA	AAATGTTGTT	TACAGCAC
T.confusum	-----	-----GATG	CTGGTATCAA	AAATGTTCTT	TACAGCAC
T.kaykai	-----	-----GATG	ATGGTATCAA	AAATATTCTT	TACAACAC
T.kaykai	-----	-----GATG	ATGGTATCAA	AAATATTGTT	TACAACAC
T.T.deion	-----	-----GATG	ATGGTATCGA	AAATATTCTT	TACAACAC
T.nubilale	-----	-----GATG	ATGGTATCAA	AAATATTCTT	TACAACAC
T.sibericum	-----	-----GATG	ATGGTATCAA	AAATGTTCTT	TACAATAC
T.deion	-----	-----GATG	ATGGTATCAA	AAATGTTGTT	TACAACAC
A.diversicornis	-----	---GCAGATA	ATAAAGAGGT	CGGCCTCCTT	TACAACGC

excluded for calculating tree ←

Fig. 1 (Continued)

iants can induce incompatibility. Another hypothesis is that some variants are hitchhiked with the variants that induce cytoplasmic incompatibility. (3) How many variants can infect an individual? It seems obvious that the main factor that can constrain the number of variants within the same individual is the efficiency of maternal transmission, which logically depends on bacterial density. If a new variant reaches a triply infected host, it is unlikely that it can reach the abundance threshold for efficient transmission. Thus, the diversity of variants that can inhabit the same individual is probably limited.

There are several cases in which *Wolbachia* found in parasitoids do not correspond to those found in hosts. This can be interpreted in different ways. First, these *Wolbachia* could have been caught from some untested occasional host species (Carton et al. 1986). Second, these *Wolbachia* might have been transferred long ago and then have either diverged or been lost by the initial host. Symmetrically, we can wonder why some parasitoids do not bear *Wolbachia* caught from some of their usual infected hosts. For example, *L. heterotoma* usually develops on *D. melanogaster* (as well as on *D. simu-*



lans), but it does not bear the corresponding *Wolbachia*. We can propose either some specific incompatibility between the wasp's genome and the *D. melanogaster* *Wolbachia* variant or exclusion by the resident *Wolbachia* types. That *L. bouhardi* proved to be totally *Wolbachia*-free despite its high exposure to *Wolbachia* transfer from *D. melanogaster* and *D. simulans* raises another puzzling question, which can only be resolved by showing some kind of wasp-*Wolbachia* incompatibility.

To what extent can the process of horizontal transfer be generalized to other host-parasitoid associations? We should first note that absence of similarity is not a counterargument: occasional hosts may not have been checked for infection, and *Wolbachia* can be lost from the host. Moreover, the study of horizontal transmission may be easier for the A group. Among the nine variants detected in parasitoids, eight belong to the A clade, which includes only 50 of the 102 described *Wolbachia* (Werren, Windsor, and Guo 1995; Werren, Zhang, and Guo 1995; Braig et al. 1998; Zhou, Rousset, and O'Neill 1998; present data). The probability of getting at least eight variants in the A clade is only 0.003. This highly biased distribution of *Wolbachia* in parasitoids has been pointed out in other Hymenoptera, mostly parasitoids (West et al. 1998). Together with the higher differentiation within the B clade than within the A clade, this supports the idea of recent and rapid expansion of the A clade *Wolbachia* (Werren, Zhang, and Guo 1995), which could result from more frequent transfers and reduced probability of loss in this group. Thus, further studies should consider *Wolbachia* of the A and B groups separately.

The parasitoid way of life favors horizontal transmission of *Wolbachia* and, thus, high infection rates in parasitoids. Can this also play a role in parasitoid speciation? The differentiation of *Nasonia vitripennis* and *Nasonia giraulti* offers a good example of reproductive isolation between individuals carrying different *Wolbachia* (Bordenstein and Werren 1998). High occurrence of infection in parasitoids could thus account for a high rate of speciation in parasitoids (Godfray 1994).

Horizontal transmission of *Wolbachia* seems to be frequent in host-parasitoid associations. Can this phenomenon be involved in other between-species genetic exchanges? The case of transposable elements is of particular interest, since they are considered to have circulated among species (Kidwell 1992). We can thus wonder whether they used the same host-parasitoid route as *Wolbachia* and, even more, whether *Wolbachia* carried them while jumping across species.

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We would like to thank J. H. Werren and R. Grantham for helpful comments on the manuscript. The work was partly supported by an EC grant (AIRCT94-1433) and by Centre National de la Recherche Scientifique (UMR 5558 and FR 41).

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