

AMERICAN UNIVERSITY OF BEIRUT

PHYLOGENETIC ANALYSIS OF HORIZONTAL TRANSFER  
OF *WOLBACHIA* AMONG INSECT HOSTS

by  
MARWA NABIL ABOUMOURAD

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MARWA NABIL ABOUMOURAD

Approved by:



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Dr. Heinrich Burggraf zu Dohna-Schlobitten, Assistant Professor Advisor  
Department of Biology



---

Dr. Khouzama Knio, Professor  
Department of Biology

Member of Committee



---

Dr. Zakaria Kambris, Assistant Professor  
Department of Biology

Member of Committee

Date of thesis defense: October 29, 2021

# AMERICAN UNIVERSITY OF BEIRUT

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# ABSTRACT OF THE THESIS OF

Marwa Nabil Aboumourad

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*Wolbachia* are highly prevalent endosymbiotic bacteria infecting more than two-thirds of arthropods. While *Wolbachia* are mainly transmitted vertically through the female germline, experiments and phylogenetic studies have suggested that horizontal transmission between hosts is not uncommon. However, there are no broad-scale surveys of *Wolbachia* horizontal transfer that describe patterns at a large scale, such as host order or continent. In this thesis, we aim to get a better understanding of horizontal transfer events and how they might occur in insects. Here, we report a comprehensive analysis of horizontal transfers in different host insect orders and continents using a large database containing nucleotide sequences of the genes *wsp* and *ftsZ*. We found heterogenous and unidirectional *Wolbachia* transition rates between insect orders and continents yet without any specific pattern of emergence and spread, based on both analysis of clades of high gene sequence similarity and best fitting models to the phylogenetic trees. Although each gene had a distinctive model of transfer and a distinctive phylogenetic signal, they exhibited alike indications within clades of similar sequences, suggesting that discrepancy in their phylogenetic histories of ancestry is more visible on a larger timescale. We were able to detect a signal of the host phylogeny and geography in the *Wolbachia* phylogeny of clades comprising highly similar sequences by Procrustean approach to co-phylogeny, however, host shifts between phylogenetically and geographically distant species was evident and frequent. Together, these results contribute to understanding the dynamics and patterns of *Wolbachia* host shifts. We anticipate this thesis to be a starting point for more sophisticated experiments, for example *Wolbachia* trans-infection experiments between insects of the orders that showed zero rates of transfer and analysis of the possible physiological and/or ecological factors that might prevent horizontal transfer.

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# CHAPTER I

## INTRODUCTION

*Wolbachia* are widespread microbial symbionts of arthropod hosts. *Wolbachia* are  $\alpha$ -proteobacteria belonging to the family *Anaplasmataceae* (formerly *Rickettsiaceae*) and order Rickettsiales (Dumler et al., 2001; O'Neill et al., 1997). The proportion of arthropod hosts harboring *Wolbachia* has been estimated at around 40% and as high as 66%, making it the most abundantly widespread endosymbiont among arthropods (Hilgenboecker et al., 2008; Zug & Hammerstein, 2012).

Despite the fact that all *Wolbachia* strains are labeled as a single species, *Wolbachia pipientis*, the remarkable genetic diversity of the genus necessitated subdividing it taxonomically into sixteen supergroups A-Q, except for G (Comandatore et al., 2015; Glowska et al., 2015; Lo et al., 2002). The latter was identified as a recombinant between A and B (Baldo & Werren, 2007). Another proposed supergroup, R (Wang et al., 2016), was later shown to be only a part of supergroup A (Gerth, 2016). Supergroups A and B are the most frequent among arthropods (Werren & Windsor, 2000).

The primary mode of transmission of *Wolbachia* is vertical, from mother to offspring, through the egg cytoplasm. A peculiar aspect of the association of insects with *Wolbachia* is that the latter manipulate their host's reproduction to facilitate their dispersal through the female germline, and this attribute has resulted in the global distribution of *Wolbachia* (Werren et al., 2008). Strains that alter their host's reproduction are often considered reproductive parasites. Alterations happen following two strategies, both of which lead to an increase in the number of infected female

insects and their descendants (Dale & Moran, 2006; Feldhaar, 2011; Stouthamer et al., 1999). The first strategy consists of shifting the sex ratio towards females via inducing parthenogenesis, male-killing, or feminization in genetic males. The second strategy involves decreasing the reproductive output of uninfected females via inducing sperm-egg incompatibility, which is referred to as cytoplasmic incompatibility (CI) and considered the most common *Wolbachia*-induced phenotype in insects and mites (Serbus & Sullivan, 2007).

This intimate relationship and tight interaction between *Wolbachia* and their hosts might give the impression that horizontal transfer is a rare event, and co-speciation of the hosts and *Wolbachia* is predominant. In that case, *Wolbachia* phylogeny topology would be expected to mirror that of their hosts. In other words, branches of a *Wolbachia* phylogeny can be rotated around ancestral nodes in a way that the association links do not intersect (Avino et al., 2019). Phylogenies of the nematode hosts and their associated *Wolbachia* show such congruence, indicating co-evolutionary stable associations (Bandi et al., 1998; Casiraghi et al., 2001). However, this is not the case in arthropod-associated *Wolbachia*, where closely related symbionts occur in phylogenetically distant lineages (Bright & Bulgheresi, 2010). The fact that *Wolbachia* phylogenies seldom match with host phylogenies suggests that *Wolbachia* has been transmitted between species frequently on evolutionary time scales (Pietri et al., 2016; Zhou et al., 1998). It is important to note, however, that incongruence not only implies host switching, but also other microevolutionary events such as extinction, incomplete lineage sorting, and independent speciation by parasites duplication; these processes are analogous to gene duplication, loss, allelic polymorphism, and lineage sorting in gene trees (Page, 1996, 2003; Page & Charleston, 1998; Paterson & Gray, 1997; Paterson et

al., 1999). Nevertheless, in addition to phylogenetic evidence, horizontal *Wolbachia* transfer has been documented in nature or lab - through microinjections (Boyle et al., 1993; Kageyama et al., 2008; Pigeault et al., 2014).

The means and routes of horizontal transmission in nature are not yet fully elucidated. Close ecological interactions appear to be essential to *Wolbachia*'s ability to cross species boundaries (Baldo et al., 2008; Pietri et al., 2016). Horizontal transfers mainly occur through host-parasitoid interactions, which have predominated *Wolbachia* horizontal transmission research (Huigens et al., 2004). Other routes occur through feeding on a contaminated resource or coprophagy (Baines, 1956; Brown & Lloyd, 2015), ingestion of particular leaf substrates in plant-feeding arthropods (Sintupachee et al., 2006), predator-prey interactions (Le Clec'h et al., 2013), exchange of salivary secretions (Roy et al., 2015), direct blood to blood contact (Rigaud & Juchault, 1995), through sharing the same habitat (Dyson et al., 2002; Stahlhut et al., 2010), through mating (Huigens et al., 2004; Moran & Dunbar, 2006) or during larval development inside an infected host (Ahmed et al., 2015).

Events of horizontal transmission could impact the evolutionary history of *Wolbachia* infections among insects and the co-evolution between host and symbiont (Kremer & Huigens, 2011). A number of studies have reported that *Wolbachia* strains move predominantly between closely related hosts, regardless of the underlying mechanism behind this result (Baldo et al., 2008; Zug & Hammerstein, 2012).

However, in some cases, the ecological relatedness of hosts is considered a more fundamental factor in *Wolbachia* strain distribution than genetic relatedness (Engelstädter & Hurst, 2009). Since genetic distance is generally negatively correlated with physiological similarity, *Wolbachia*'s transfer between more closely related host

species seems more probable than between distantly related host species where *Wolbachia* is less well adapted to the novel host's physiology, including the host's defense mechanisms (Gerth et al., 2013).

To date, knowledge of *Wolbachia*'s horizontal transfer on a broad scale is fragmentary, where most studies are limited to few host taxa. In this thesis, our ultimate goal is to get a broad understanding of horizontal transmission events in a wide range of insect hosts. We are interested in questions such as how congruent are host and *Wolbachia* trees? Does *Wolbachia* preferentially move among some orders? Is there unidirectional transmission between some orders? Do some patterns of horizontal transmission differ between genes? Is horizontal transfer more likely to occur among phylogenetically and geographically related species? Answering such questions will lead to a better understanding of *Wolbachia* dynamics, and specifically invasion dynamics. In addition, it will shed the light on recombination events associated with horizontal transfers, and generate new hypotheses on how *Wolbachia* transfer might occur.

To address these questions *Wolbachia* phylogenies based on two different genes were reconstructed, then *Wolbachia* transfer rates between different insect orders were estimated. Moreover, the ancestral node states for clades where the nucleotide sequences of *Wolbachia* are identical or highly similar were reconstructed. In addition, the phylogenetic signal of the insect host phylogeny and geography was investigated in the *Wolbachia* phylogeny by a permutation test, followed by matching *Wolbachia* trees to their hosts and applying a Procrustean statistical tool.

## CHAPTER II

### MATERIALS AND METHODS

#### A. Data Collection and Alignment

Sequences of the *Wolbachia* surface protein gene *wsp* and the cell division protein gene *ftsZ* were downloaded from the *Wolbachia* MLST database (<http://pubmlst.org/wolbachia/>) along with information on the *Wolbachia* strain, supergroup, host infection status, host taxonomic information, and more. The insect tree was obtained from a rooted species-level tree in Newick format, containing 49,358 species, 13,865 genera, 760 families of insects (Class Insecta) estimated by (Chesters, 2017) (<https://datadryad.org/stash/dataset/doi:10.5061/dryad.27114> on 13 October 2020).

The sequence data of the *wsp* gene in the nematode *Brugia malayi* was retrieved from GenBank Nucleotide (on November 10, 2020) (Accession: AY527202.1), and used as an outgroup in constructing the tree based on *wsp* gene. Similarly, the sequence data of *ftsZ* gene in *Anaplasma marginale* was retrieved from GenBank Nucleotide (on March 20, 2021) (Accession: MG807953), and used as an outgroup in constructing the tree based on *ftsZ* gene.

The datasets for both *wsp* and *ftsZ* were filtered using custom R scripts (R Core Team, 2015); only field isolates from orders Diptera, Lepidoptera, Hymenoptera, and Hemiptera were kept since lab strains may not be meaningful in studying horizontal transfer events. If there were sequences from the same host that were identical and from the same continent, only the longest sequence was kept. All sequences from the same host that differed from each other or came from different continents were kept. After

filtering the data comprised 316 *wsp* and 368 *ftsZ* sequences. The nucleotide sequences of each gene were aligned separately using MAFFT version 7 (<https://mafft.cbrc.jp/alignment/software/>)(Kato & Standley, 2013).

## **B. Tree Reconstruction Based on Bayesian Inference**

Phylogenetic trees were estimated from alignments of single loci *wsp* and *ftsZ*, using MrBayes v.3.2 (Ronquist et al., 2012), selecting a general time-reversible substitution model with a proportion of invariable sites and a gamma-shaped distribution of substitution rates across sites. Two Monte Carlo Markov Chains were run for 8 million generations with a sample frequency of 100 to generate samples from posterior distributions of trees and substitution rate parameters, given the alignment data.

## **C. Analysis of Clades with High Sequence Similarity**

The phylogenetic trees were searched for clades that have very similar gene sequences; the following condition was set: the clade must contain at least five descendants at least 90% of which have a maximum divergence less than 0.005 (less than 0.5% divergence), in addition, the clade must have a posterior probability greater than or equal to 0.85. The ancestral states were reconstructed for each clade fulfilling these conditions and the host orders and continents among the clade descendants were tabulated. The ancestral host order and continent of these clades were reconstructed using the “AddMRCA” command in the software package BayesTraits (Pagel et al., 2004) selecting the MultiState model and MCMC analysis.

#### **D. Estimating *Wolbachia* Transition Rates**

The phylogenetic trees obtained from MrBayes were used to estimate transition rates of *wsp* and *ftsZ* genes between the four different orders of hosts using the software package BayesTraits (Pagel et al., 2004) which estimates transition rates between character traits on a phylogenetic tree using Bayesian Monte Carlo Markov chains (MCMC). The host insect orders were treated as a discrete trait of *Wolbachia*.

There are twelve different transition rates along the phylogenetic tree which were estimated by MCMC with a reversible jump procedure that produces samples from the posterior distributions of parameters and different models. The model space is explored by setting some rates to zero and assigning the remaining rates to rate classes (Pagel & Meade, 2006). A uniform prior was used for the transition rates with a prior interval ranging from 0 to 200.

#### **E. Partition Homogeneity test**

To test whether *ftsZ* and *wsp* have a different underlying history of ancestry a partition homogeneity test was implemented in Rstudio (R Core Team, 2015). The concatenated alignment was divided into 2 partitions, one for *wsp* and the other for *ftsZ*, then the columns were randomly shuffled and a neighbor-joining tree based on a Kamura-2-parameter substitution model was reconstructed. Finally, the total lengths of the estimated trees were summed for each partition. We applied this procedure to the original partition and to 1000 random partitions.



## **F. Permutation Test for Horizontal *Wolbachia* transfer**

A permutation test was applied separately to *wsp* and *ftsZ* sequences to test whether strains with identical gene sequences are more likely than random to come from the same continent, host order or host genus. First, all pairs of *Wolbachia* strains from different host species with identical sequences were identified and among them the number of pairs of the same genus, same order, or same continent was counted. Then a null distribution was created by shuffling these pairs of host insects and counting the number of instances in which they are of the same genus, same order, or same continent. For each case these steps were iterated 10 000 times to evaluate the significance where the p-value is considered the proportion of the simulated values that is less than or equal to the original observed value. This permutation procedure was carried out in RStudio (R Core Team, 2015).

## **G. Co-phylogeny Estimation**

To create a co-phylogeny, insect species for which *Wolbachia* sequence data is not available were removed from the insect tree, and host species which have duplicates in the *Wolbachia* sequence data were duplicated on the insect tree by creating a new branch of negligible length. The co-phylogeny of the tree based on *Wolbachia* sequences and the insect host tree was plotted using ‘phytools’ package in RStudio (R Core Team, 2015), an association matrix was created, and the nodes were rotated to maximize the matching.

## **H. Procrustes Application to Cophylogenetic Analysis (PACo )**

To test whether the *Wolbachia* phylogeny is constrained by the host phylogeny the statistical tool PACo was applied using the model implemented in R by Hutchinson et al. (2017). PACo converts host and symbiont phylogenies to pairwise distance matrices, provides a residual sum of squares of the Procrustean fit that measures the congruency between two given phylogenies and uses a permutation approach to test its significance (Balbuena et al., 2013). The null hypothesis in this test is that the symbiont speciation is unrelated to host speciation. In addition to hypothesis testing, a residual bar chart for evaluation of the contribution of the individual host-parasite associations to the global fit were represented. The contributions of links are accessed by applying a jackknife procedure and calculating a goodness-of-fit statistic for each link (Balbuena et al., 2013).

## CHAPTER III

### RESULTS

#### A. Phylogenies Based on *wsp* and *ftsZ* and Analysis of Clades With High Sequence Similarity

The *wsp* gene tree contains eleven clades that are distinct and show high internal similarity (i.e. supported by posterior probabilities greater than 0.85 and 90% of the gene sequences of their descendants have a maximum divergence less than 0.05%) whereas there are only four such clades in the *ftsZ* tree (colored clades in Figures 1 and 2). Despite the high within-clade sequence similarity, some of these clades contain isolates from different host orders and continents (Tables 1 and 2). For example, clade C1 contains identical *wsp* sequences from four different continents: Asia, America, Europe, and Africa, and two orders: Diptera and Lepidoptera (Figure 1, Table 2).

Based on the ancestral node state reconstructions, there are two distinguishable patterns: 1) there is sequence similarity across diverse insect orders and diverse continents 2) there are different directions of transfer based on the likely ancestral state; however, there is no particularly dominant direction between different orders and different continents (Tables 1, 2, 3, and 4). In most of the clades, the most likely ancestral state corresponds to the highest percentage of the host order or continent in the clade, for example clades: C1, C2, C11, and others. Nevertheless, there are multiple clades where the descendants end up in other continents or in other host orders than the most likely ancestral node state, suggesting that even among sets of closely related sequences there is relatively frequent transfer across orders and continents. For *wsp*, the ancestral host order of clade C1 is Lepidoptera and the ancestral continent is Europe, which means that the *Wolbachia* must have spread from a lepidopteran species in

Europe to other continents and to the dipteran hosts *Culex pipiens*, *Culex modestis*, *Culex quinquefasciatus*, and *Aedes albopictus* (Table 1 and 2). Other horizontal transfers occurred from Lepidoptera to Hymenoptera and Diptera in clades C1, C3, C4, and C5 and from Hymenoptera to Diptera, and to Lepidoptera in clades C6 and C8, respectively (Table 1).

Based on the patterns and posterior probabilities of the reconstructed ancestral states, one can infer the direction of the host shift. For instance, the phylogenetic position of the dipteran *Rhagoletis cerasi* in a clade containing identical sequences from Lepidoptera hosts, and ancestral node reconstruction suggests that the ancestral state of this clade is Lepidoptera with a posterior probability of 0.91 (Table1, C5). Thus, the host shift likely occurred from a lepidopteran species to *Rhagoletis cerasi*.

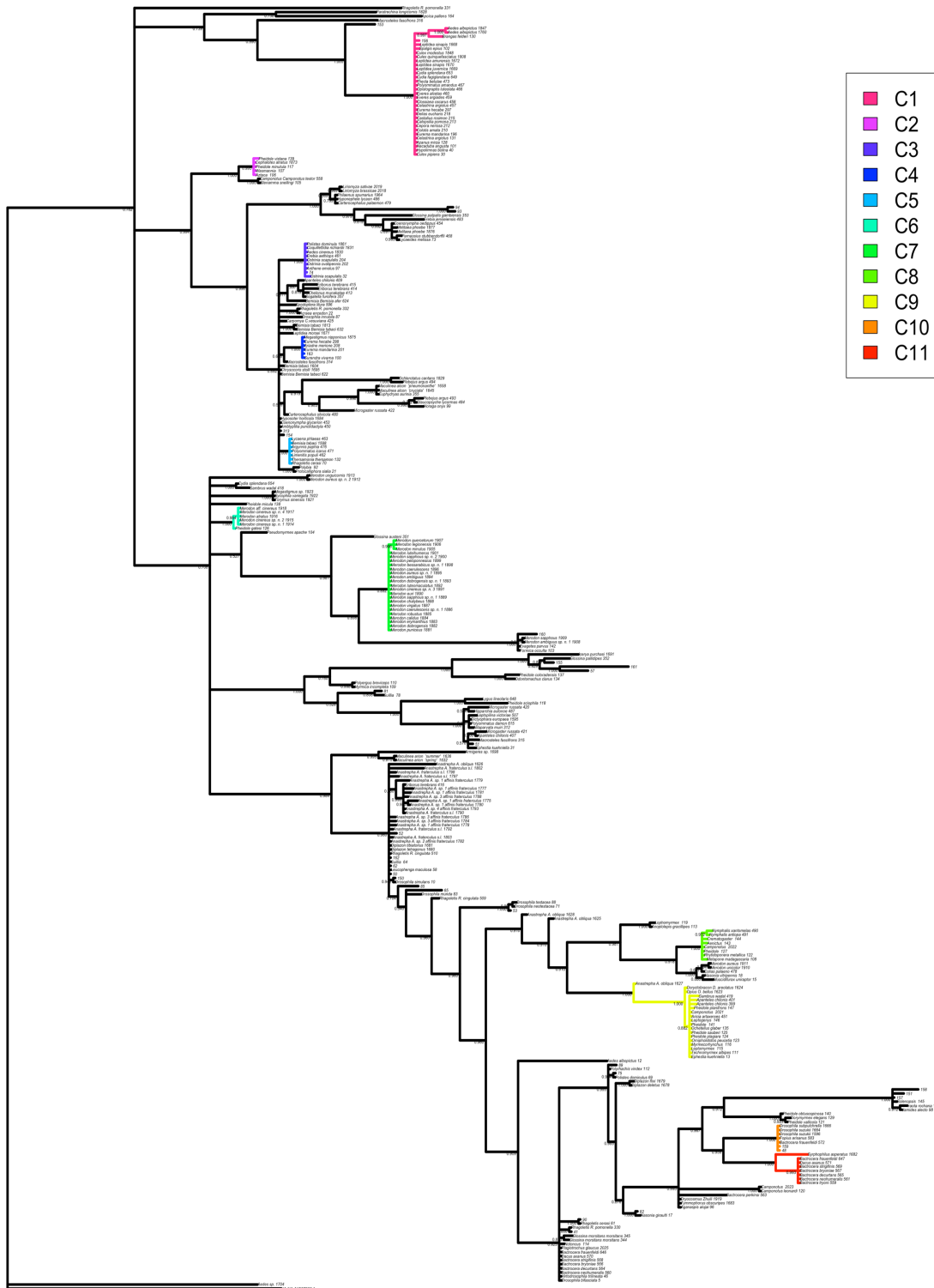


Figure 1 Bayesian MCMC inference phylogeny of *Wolbachia* based on *wsp* gene sequences. The tree was rooted with *wsp* from the host *Brugia malayi*. Posterior probabilities are shown at nodes.



Figure 2 Bayesian MCMC inference phylogeny of *Wolbachia* based on *ftsZ* gene sequences. The tree was rooted with *ftsZ* from the host *Anaplasma marginale*. Posterior probabilities are shown at nodes.

Percentage of Host Orders Within Clades							
Clade	Posterior Probability	Maximum Divergence	Diptera	Hymenoptera	Lepidoptera	Hemiptera	Ancestral Node of Highest Probability
C1	100%	1.20%	18.75%	0.00%	81.25%	0.00%	Lepidoptera: 98 %
C2	99.50%	0%	0.00%	100.00%	0.00%	0.00%	Hymenoptera: 98.4%
C3	100%	0.02%	11.11%	22.22%	66.66%	0.00%	Lepidoptera: 66.1 %
C4	100%	0.04%	16.66%	16.66%	66.66%	0.00%	Lepidoptera: 58.1 %
C5	100%	0%	14.28%	0.00%	85.71%	0.00%	Lepidoptera: 91.3 %
C6	100%	0.24%	83.33%	16.66%	0.00%	0.00%	Hymenoptera: 79.5%
C7	89.20%	0.00%	100.00%	0.00%	0.00%	0.00%	Diptera: 99.71%
C8	100%	0.74%	0.00%	75.00%	25.00%	0.00%	Hymenoptera: 96.1%
C9	100%	3.73%	15.78%	63.15%	21.05%	0.00%	Diptera: 87.2%
C10	100%	0%	85.71%	14.28%	0.00%	0.00%	Diptera: 93.4%
C11	100%	2.98%	88%	12.50%	0.00%	0.00%	Diptera: 43.2%

Table 1 Ancestral node states of orders in clades of similar *wsp* sequences. The posterior probability, maximum divergence between descendants, and percentage of each insect order within the clades is shown. In the last column, only the percentage of the most likely ancestral insect order is shown.

Percentage of Host Continents Within Clades								
Clade	Posterior Probability	Maximum Divergence	America	Australia	Africa	Asia	Europe	Ancestral Node Of Highest Probability
C1	100%	1.20%	15.62%	0.00%	3.12%	34.37%	46.87%	Europe: 51.8%
C2	99.50%	0%	100.00%	0.00%	0.00%	0.00%	0.00%	America: 96%
C3	100%	0.02%	22.22%	0.00%	0.00%	33.33%	33.33%	Europe: 89.5%
C4	100%	0.04%	16.66%	0.00%	0.00%	83.33%	0.00%	Asia: 95.6%
C5	100%	0%	0.00%	0.00%	0.00%	14.28%	85.71%	Europe: 96.7%
C6	100%	0.24%	0.00%	0.00%	0.00%	16.66%	83.33%	Asia: 60.19%
C7	89.20%	0.00%	0.00%	0.00%	0.00%	0.00%	100%	Europe: 99.46%
C8	100%	0.74%	25.00%	12.50%	12.50%	50.00%	0.00%	Europe: 50%
C9	100%	3.73%	21.05%	15.78%	5.26%	47.36%	5.26%	America: 88.41%
C10	100%	0%	28.57%	28.57%	0.00%	28.57%	0.14%	Australia: 54.2%
C11	100%	2.98%	0%	87.50%	0.00%	0.00%	12.50%	Australia: 67.42%

Table 2 Ancestral node states of continents in clades of similar *wsp* sequences. The posterior probability, maximum divergence between descendants, and percentage of each continent is shown. In the last column, only the percentage of the most likely ancestral insect continent is shown.

Percentage of Host Orders Within Clades							
Clade	Posterior Probability	Maximum Divergence	Diptera	Hymenoptera	Lepidoptera	Hemiptera	Ancestral State Of Highest Probability
C1	100.00%	0.00%	0.00%	25.00%	75.00%	0.00%	Lepidoptera: 0.97%
C2	100.00%	0.00%	16.66%	0.00%	50.00%	33.33%	Lepidoptera: 0.62%
C3	100.00%	0.00%	100.00%	0.00%	0.00%	0.00%	Diptera: 0.96%
C4	89.30%	0.00%	33.33%	50.00%	16.66%	0.00%	Hymenoptera: 0.54%

Table 3 Ancestral node states of orders in clades of similar *ftsZ* sequences. The posterior probability, maximum divergence between descendants, and percentage of each insect order within the clades is shown. In the last column, only the percentage of the most likely ancestral insect order is shown.

Percentage of host continents within each clade								
Clade	Posterior Probability	Maximum divergence	America	Australia	Africa	Asia	Europe	Ancestral Node Of Highest Probability
C1	100.00%	0.00%	12.50%	0.00%	0.00%	25.00%	37.50%	Europe: 0.88%
C2	100.00%	0.00%	16.66%	0.00%	0.00%	83.33%	0.00%	Asia: 0.98%
C3	100.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	Australia: 0.97%
C4	89.30%	0.00%	0.00%	33.33%	16.66%	16.66%	33.33%	Australia: 0.53%

Table 4 Ancestral node states of continents in clades of similar *ftsZ* sequences. The posterior probability, maximum divergence between descendants, and percentage of each continent is shown. In the last column, only the percentage of the most likely ancestral insect continent is shown.



## B. Unidirectional Transfer Between Specific Insect Orders

To investigate the transition rates of *Wolbachia* and whether there is a preference of transfer between certain orders, the MCMC with reversible jump analysis in BayesTraits was carried separately for each gene. The most favorable model of transfer of the *wsp* gene was supported by a posterior probability equal to 0.6. In this model, the rates of transfer from: Diptera to Lepidoptera ( $q_{DL}$ ) and to Hemiptera ( $q_{DP}$ ), Hymenoptera to Hemiptera ( $q_{HP}$ ), and Hemiptera to Diptera ( $q_{PD}$ ) are zero and the remaining rates are all equal ( $q_{HD} = q_{DH} = q_{LD} = q_{HL} = q_{LH} = q_{PH} = q_{PL} = q_{LP} = 26.6$ ) (Figure 3).

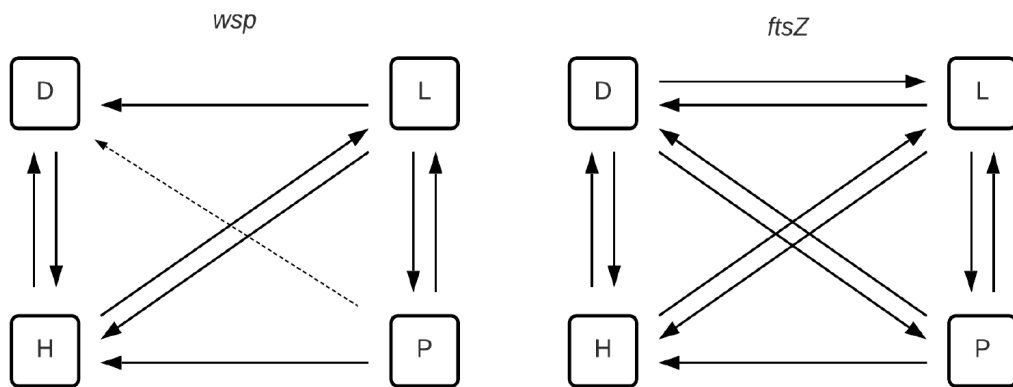


Figure 3 Rates of transfer of *wsp* and *ftsZ* between host orders. Missing arrows indicate a rate of transfer equal to zero. Same size arrows indicates equal rates of transfer. The dashed arrow indicates an additional rate from the second most favorable model. D: Diptera, H: Hymenoptera, L: Lepidoptera, P: Hemiptera.

The second most favorable model was supported by 0.4 posterior probability, and is analogous to the previous model except that it does not set  $q_{PD}$  to zero.

Combining these two models, the posterior probability for a model where  $q_{DL} = q_{DP} = q_{HP} = 0$  would have a posterior probability very close to 1.

The fact that the reciprocal transfer rates from Lepidoptera to Diptera ( $q_{LD}$ ), and Hemiptera to Hymenoptera ( $q_{PH}$ ) are non-zero, indicates unidirectional transfer in these

orders. In addition, the most supported model, suggests that horizontal transfer may be incompatible between Diptera and Hemiptera, as the rates of transition are zero in both directions ( $q_{DP} = q_{PD} = 0$ ).

On the other hand, analysis for *ftsZ* transition showed that rates of transfers between all orders are equal, except from Hymenoptera to Hemiptera ( $q_{HP}$ ) which is zero (Figure 3). Since both genes had a zero  $q_{HP}$  rate, this suggests that *Wolbachia* might not move horizontally between these two orders.

The discrepancies between *wsp* and *ftsZ* is also reflected by evidence that the two genes have significantly different underlying histories of ancestry ( $P < 0.001$ , partition homogeneity test, Figure 4).

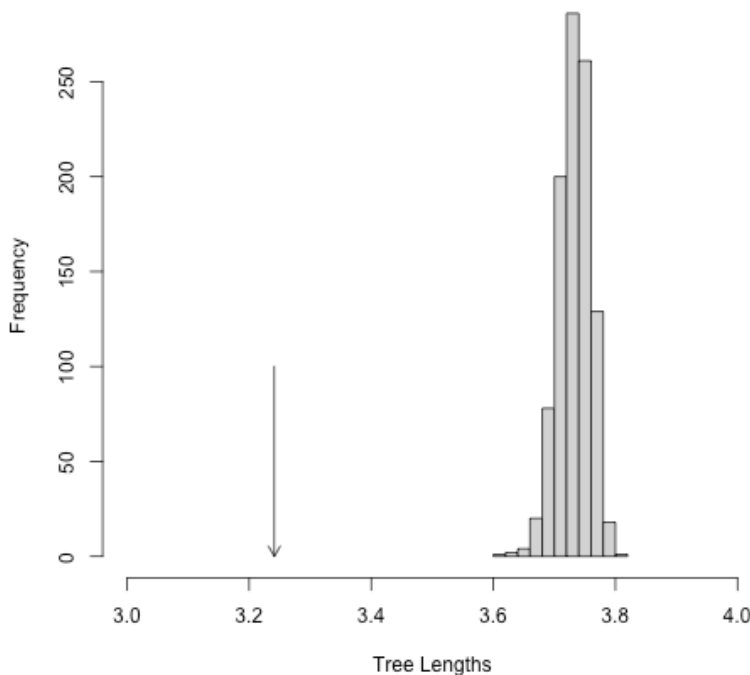


Figure 4 Sum of tree lengths for 1000 random partitions of the *Wolbachia* sequence data. Genes *wsp* and *ftsZ* have different underlying histories of ancestry. The arrow indicates the length of the original data.

Notably, the results of ancestral node reconstruction of highly similar *Wolbachia* gene sequences were consistent with these Bayesian transition rate models.

### C. Horizontal Transfer Is More likely Within Same Genus, Order, and Continent

For both the *wsp* and the *ftsZ* gene, the pairs of identical nucleotide sequences involved more often sequences from the same host genus, host order, or continent than what would be expected by chance (Figure 5). This indicates that events of *Wolbachia* horizontal transfer are more likely to occur within the same geographical region, and intra-ordinal and intraspecific transfer between phylogenetically related hosts is more favorable. Nevertheless, it is not impossible to see host shifts among phylogenetically and geographically distant species.

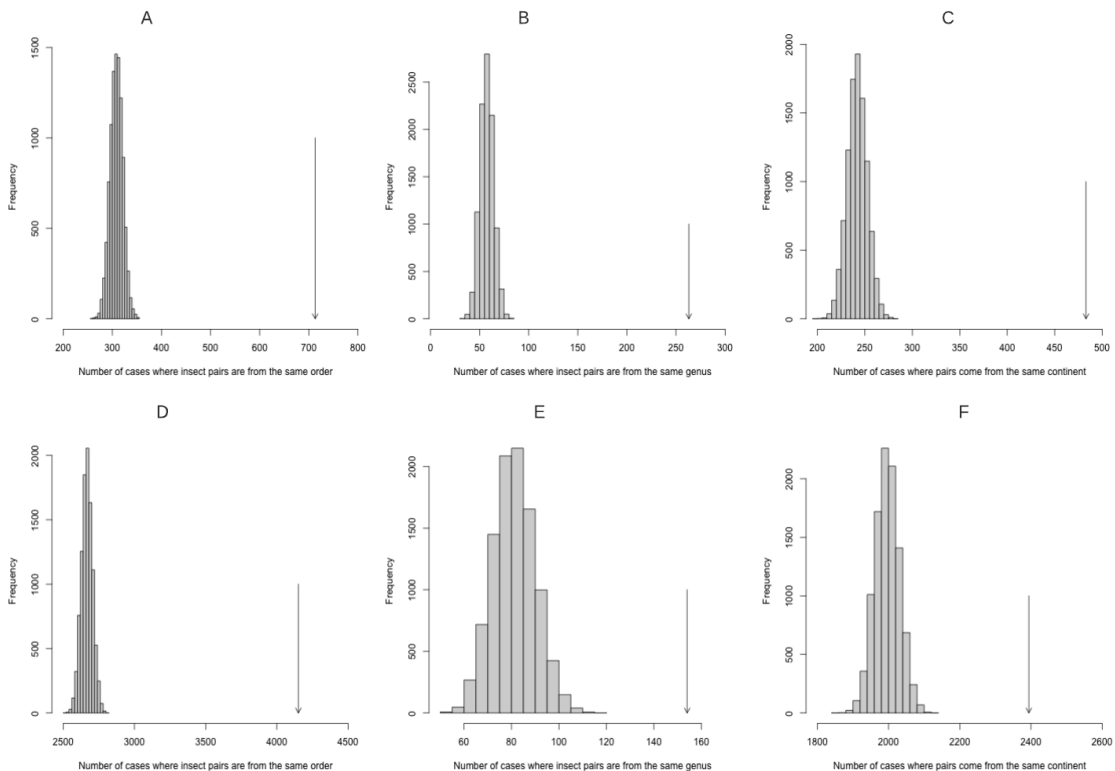


Figure 5 Histograms of frequency of insect pairs with identical gene sequences that have the same order (A and D), same genus (B and E), same continent (C and F). A, B, and C correspond to *wsp* gene and D, E, F correspond to *ftsZ* gene. The arrows indicate the observed value.

#### D. Co-phylogenetic Analysis

A total of 64 and 15 insect species, representing mostly the orders Diptera, Hymenoptera, Lepidoptera, and to a lower extent, Hemiptera (only 1 species: *Bemesia tabaci*) were co-plotted with their corresponding *wsp* (Figure 6) and *ftsZ* (Figure 7) sequences of their *Wolbachia* infections, respectively. On the scale of host order, there is an approximate phylogenetic correspondence indicating codivergence through evolutionary time. Any breaking of the matching clade pattern is an indication of horizontal transfer of *Wolbachia* from one species to another. Within Diptera, evolution of *Wolbachia* of *Drosophila* species displays an overall congruence with the evolution of their host (Figure 6). All insect species in the clade of *Drosophila* correspond to one clade on the *Wolbachia* tree, except for the *Wolbachia* of *Rhagoletis cerasi*, which is positioned with lepidopterans, indicating the occurrence of a horizontal transmission event in this species. There is also a clade of similar *Wolbachia* sequences which all associate to closely related insect species of the order Lepidoptera (butterflies and moths), except for three which associate with closely related species at a different part of the insect tree, namely *Aedes albopictus*, *Culex pipiens*, *Culex modestus*, and *Culex quinquefasciatus*, of order Diptera.

The *Wolbachia* phylogeny based on *ftsZ* contains three clades, one is entirely Diptera (blue), the other is entirely Lepidoptera (red) while the third is mixed and contains dipterans, lepidopterans and hymenopterans (Figure 7).

Co-plot of the subtrees of the two genes, which contain clades of similar sequences shows congruency (Figure 8). In other words, all sequences that occurred together in one clade according to *wsp*, also occurred together in one clade in the *ftsZ* tree.

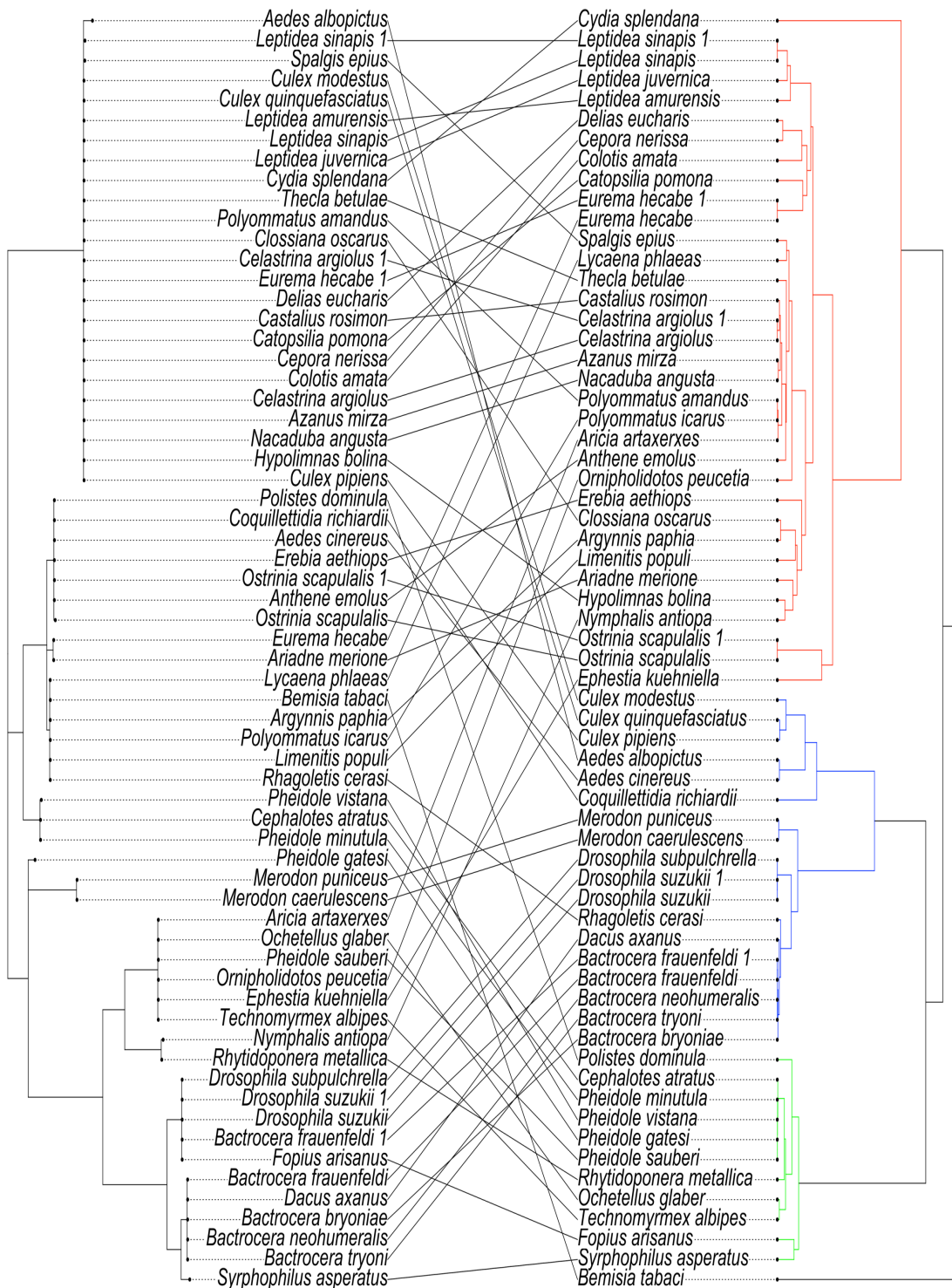


Figure 6 Tanglegram showing the associations between the host insects phylogeny (right) and their *Wolbachia* endosymbionts phylogeny based on *wsp* gene (left). Nodes have been rotated to maximize matching. Associated taxa are joined by solid lines. The colors red, blue, green, and black in the insect tree correspond to insect orders Lepidoptera, Diptera, and Hymenoptera, and Hemiptera respectively.

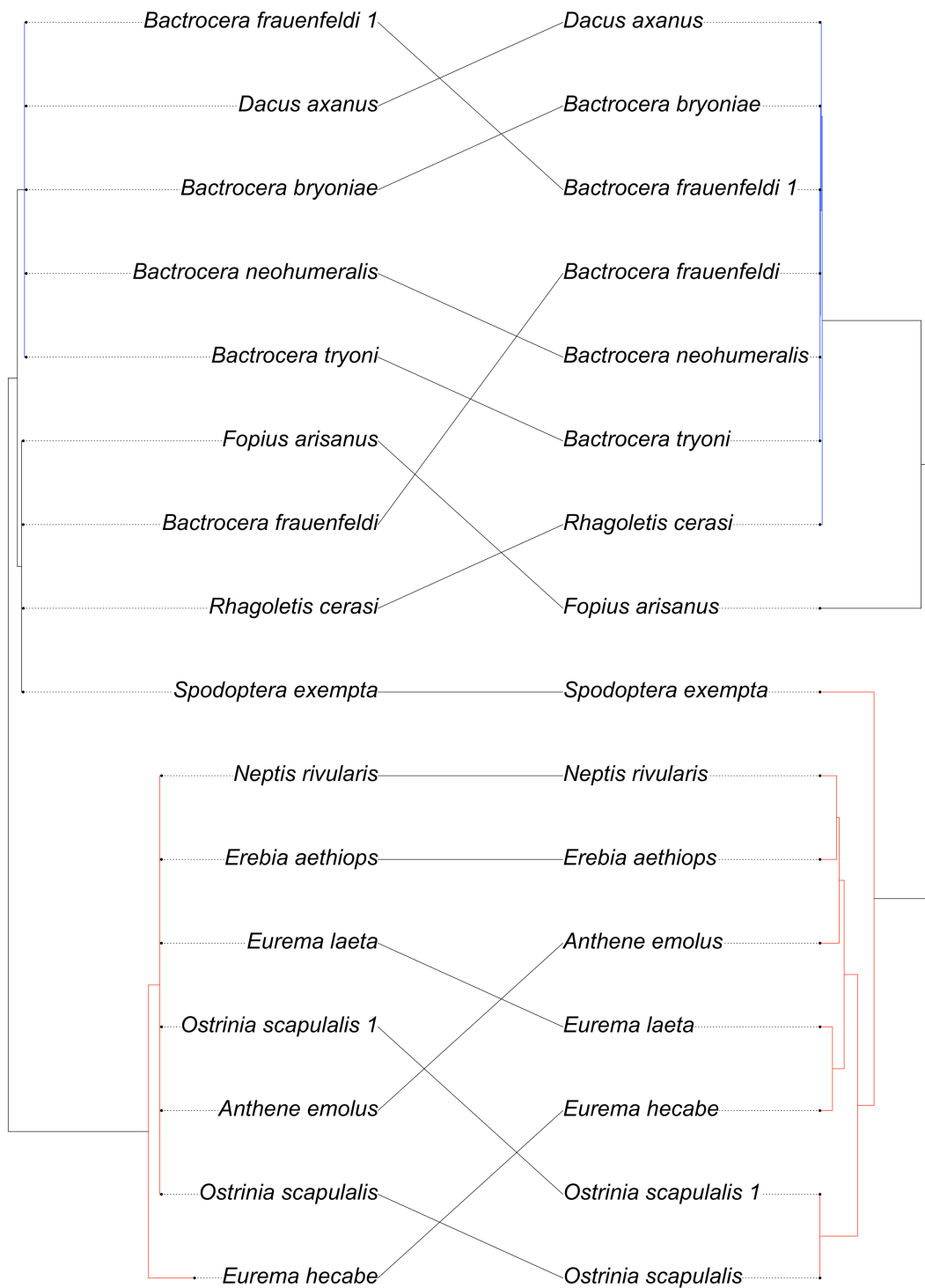


Figure 7 Cophylogeny of *ftsZ* (left) and insect hosts (right). Nodes have been rotated to maximize matching. Associated taxa are joined by solid lines. The major clades are colored blue and red, corresponding to orders Diptera and Lepidoptera, respectively.

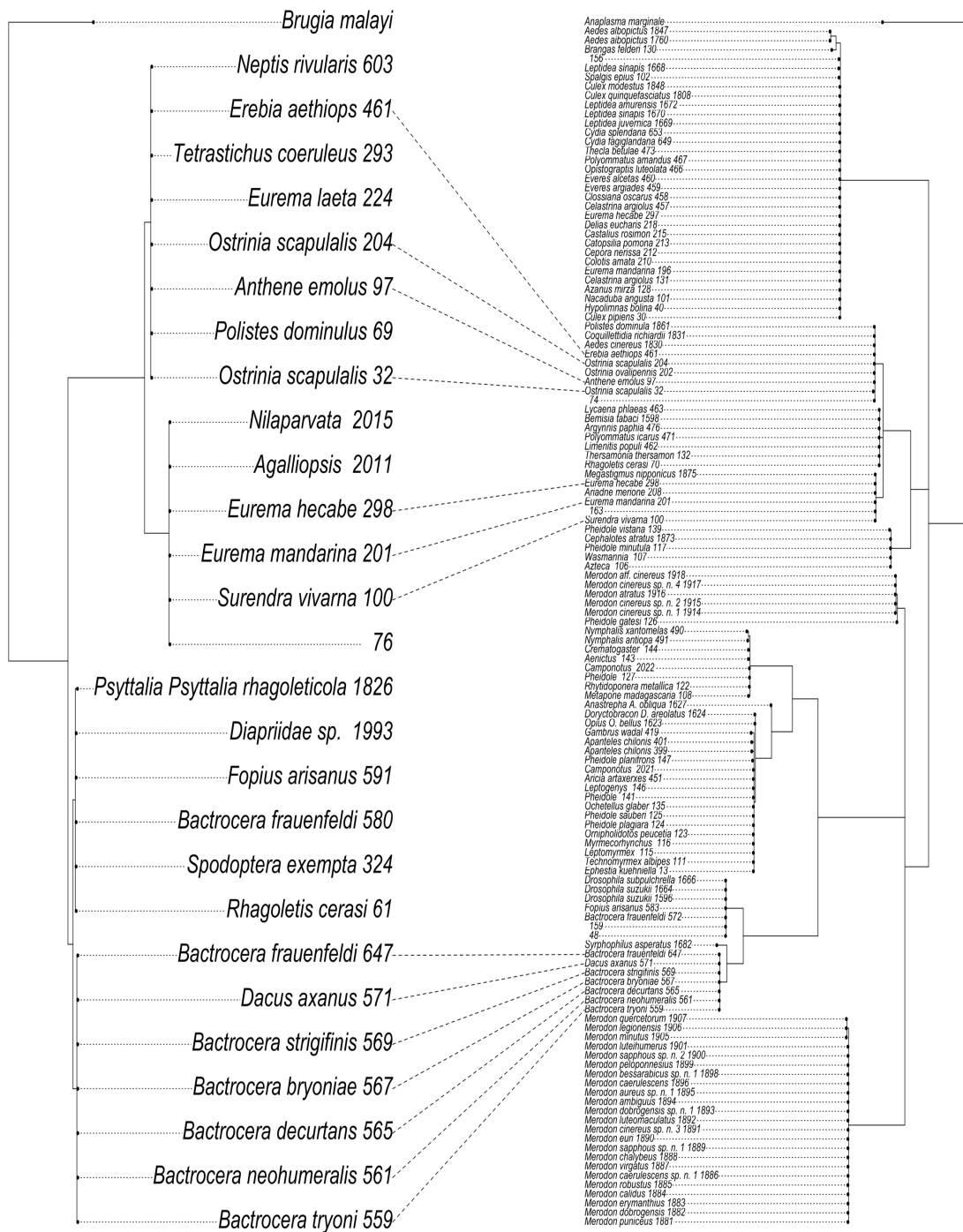


Figure 8 Cophylogeny of *wsp* (left) and *ftsZ* (right). Numbers next to taxa indicate unique IDs of *Wolbachia* isolates. Nodes haven been rotated to maximize matching. Identical taxa are joined by dashed lines.

### E. Congruency of *Wolbachia*-Insect Co-phylogenies

There is a significant signal of the host ancestry in the *wsp* and *ftsZ* trees ( $P \ll 0.0001$ , Procrustean fit test). This provides evidence that similarity between *Wolbachia* and their hosts has not arisen by chance, and although there are events of horizontal transfer they are not strong enough to mask the co-evolutionary signal. Mismatches which are equivalent to host switches are represented by links with high squared residuals between within *Wolbachia* and within host genetic distances on the other hand. Whereas links that contribute relatively little to squared residuals represent coevolutionary links equivalent to vertical transfer events. Despite the significant signal of the host phylogeny, 34% of the links have high residuals that denote horizontal transfers (22 links out of 64). For example, *Aedes albopictus*, *Fopius arisanus*, *Bemisia tabaci*, *Culex modestus*, *Culex pipiens*, *Pheidole vistana*, *Polistes dominula* and fifteen other host insects are involved in horizontal transfer events (Figure 10). On the other hand, 66% of the links range from low (44%) to average (14%) contribution to squared residuals, denoting co-evolutionary events.



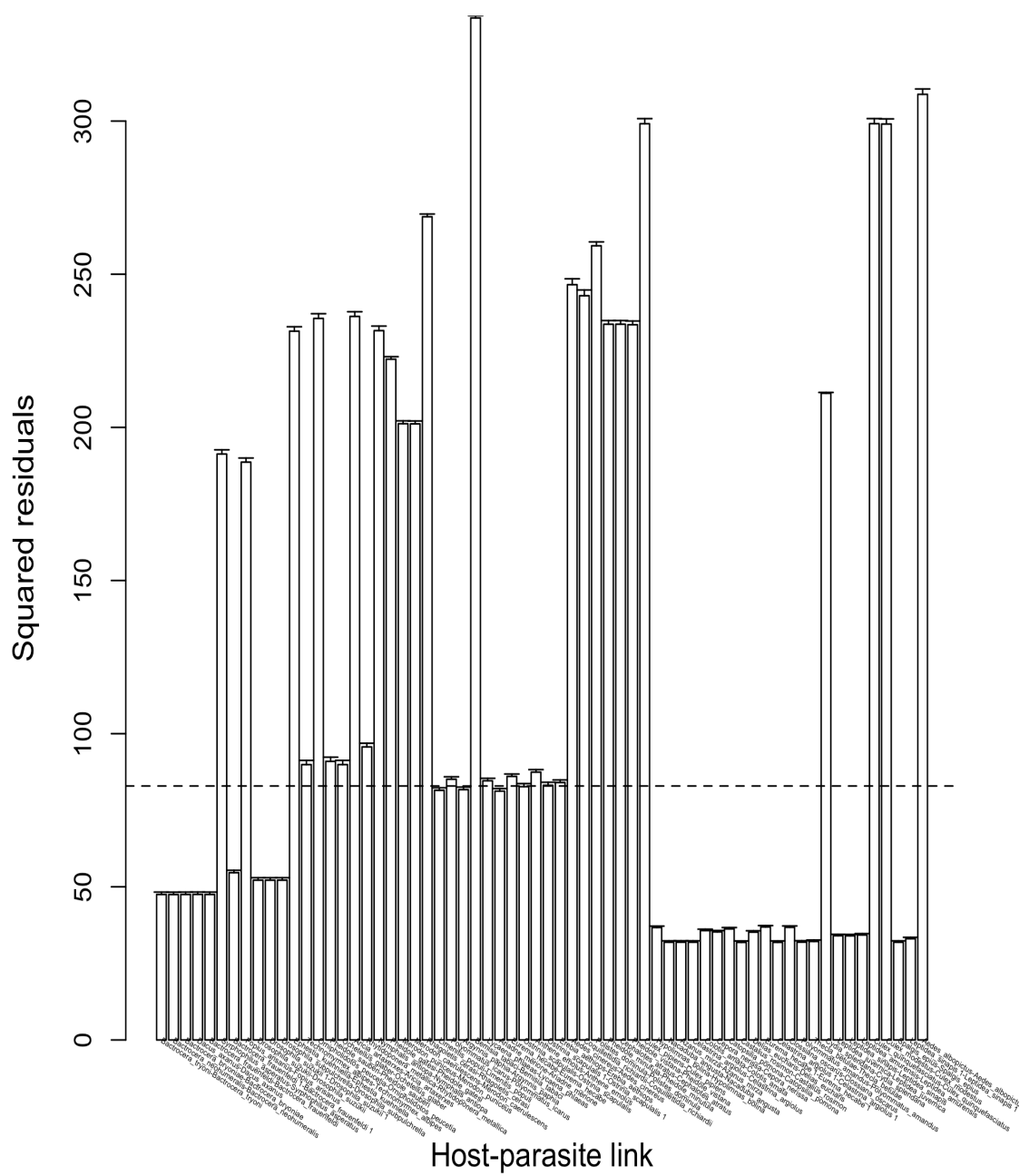


Figure 9 Contributions of individual host-parasite links to the Procrustean fit. Jackknifed squared residuals (bars) and upper 95% confidence intervals (error bars) resulting from applying PACo to *wsp* phylogeny.

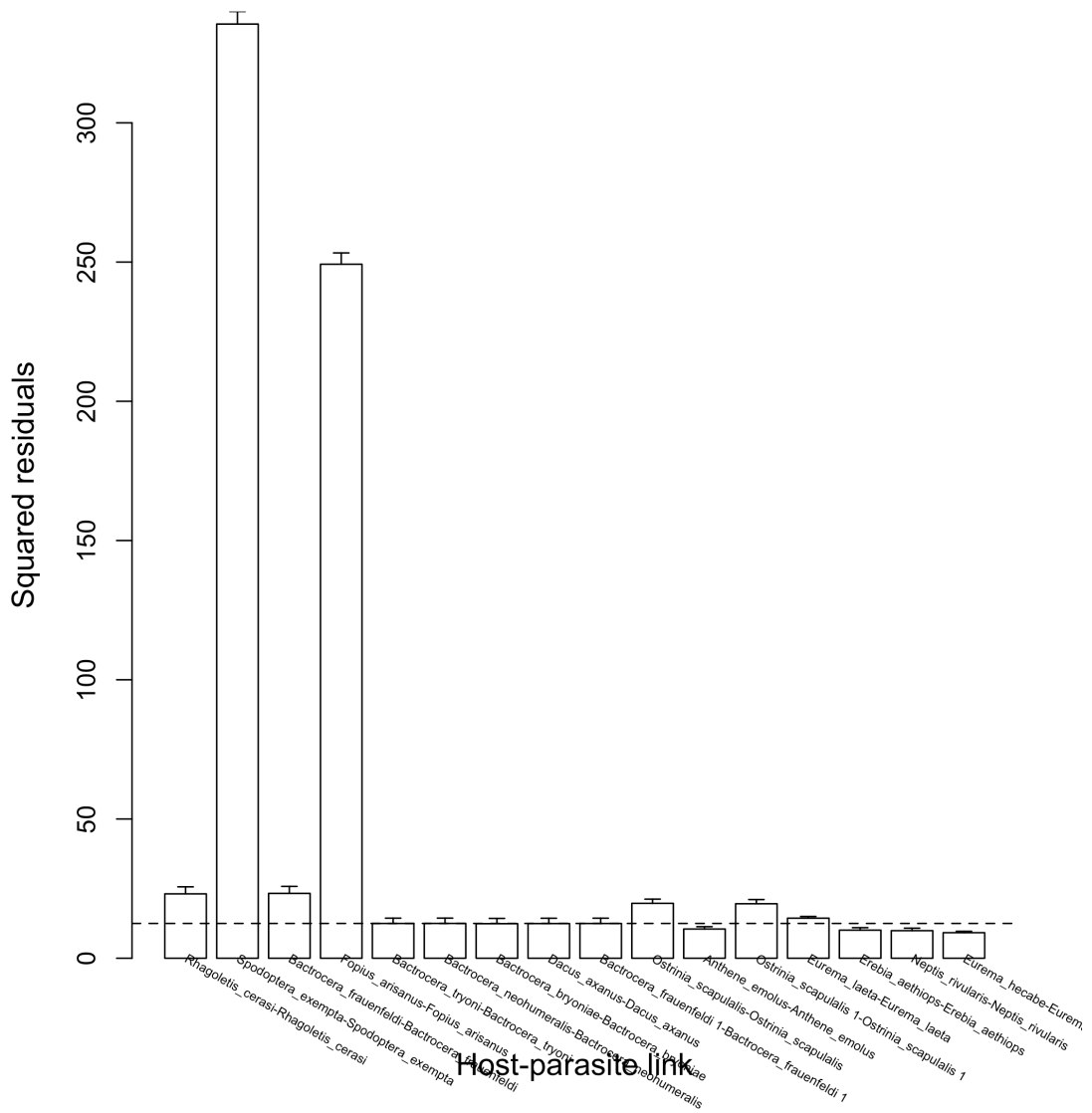


Figure 10 Contributions of individual host-parasite links to the Procrustean fit. Jackknifed squared residuals (bars) and upper 95% confidence intervals (error bars) resulting from applying PACo to *ftsZ* phylogeny.

## CHAPTER IV

### DISCUSSION

This study explored the patterns of *Wolbachia* horizontal transfers in arthropod hosts at the scale of host order and continent, based on separate loci *wsp* and *ftsZ*. According to Bayesian estimates, there is heterogeneity in a sense that some transitions do not occur between certain orders according to the best fitting model, and this also differs between the two genes. This discrepancy between the two genes points to conflicting underlying histories of ancestry, which was further verified by the partition homogeneity test. Yet when we look at clades with similar sequences we see the same patterns between both genes which could suggest that these different histories of ancestries are more visible on a larger timescale. Within the clades of highly similar *Wolbachia* sequences, there were no specific patterns of how the symbiont emerges and spreads among arthropods of different orders and continents. In such cases, widespread occurrence of closely related *Wolbachia* strains in unrelated hosts is due to horizontal transfers rather than inheritance from common ancestor. Lack of a clear and specific pattern of horizontal transfer could probably be attributed to highly diversified and unstable relations. Although, the geography and phylogeny signal of hosts was detected in the *Wolbachia* phylogeny, it is not uncommon to see host shifts between phylogenetically and geographically distant species.

Our phylogenetic analyses support the wide-ranging horizontal movement of *Wolbachia* strains between divergent host species. For example, *Wolbachia* of the Dipterans *Aedes albopictus*, *Culex pipiens*, *Culex modestus*, and *Culex quinquefasciatus* are more closely related to *Wolbachia* of Lepidoptera than to that of Dipterans and the ancestral state of this clade is Lepidoptera with a posterior probability equal to 98.3%

(Table 1, C1). Analysis of these clades with highly similar *Wolbachia* sequences generally revealed that the symbionts originate in various insect orders and continents as well, and there is no particular detected pattern of how they spread among arthropods worldwide. In most clades, it was more likely that the ancestral state was the same as the order of the insects that had the highest percentage in this clade. However, there were clades where the descendants end up in different orders and continents than the most likely ancestral node. Surprisingly, there are extreme cases such as in clade C9 in the *wsp* phylogeny, Diptera has the lowest percentage, but ancestral node reconstruction suggests that this clade emerged in Diptera and spread to other host orders, including Lepidoptera. According to the best fit model of the *wsp* phylogeny, there was evidence that there is no transfer from the order Diptera to Lepidoptera in a single state. Clade C9 has a particularly long branch in it and according to the model it allows for two transitions. Another striking clade is C10, which comprises only identical *wsp* sequences that are equally distributed in America, Australia, Asia, and some in Europe, and the most likely ancestral node is Australia, suggesting that this clade emerged from Australia and spread to the other continents. This clade contains invasive species and agricultural pests, *Drosophila suzukii*, and *Bactrocera frauenfeldi* (Armstrong & Ball, 2005; Bieńkowski & Orlova-Bienkowskaja, 2020; Nikolouli et al., 2018). The fact that this clade contains species with invasive capabilities could explain the spread of identical strains between different continents. Moreover, another interesting descendant of this clade is *Fopius arisanus*, a polyphagous egg-pupal parasitoid of tephritid fruit flies including most of the genus *Bactrocera* (Bautista et al., 1998; Rouse et al., 2005). Such host-parasitoid interactions imply close ecological connections that could explain why *wsp* sequences are identical in this clade.

This lack of a specific pattern of emergence and spread could either mean randomness of horizontal transfer events, or constraint by some biological and ecological traits yet to be investigated.

Congruency between the subtrees of closely related *wsp* sequences and closely related *ftsZ* sequences, indicates that *Wolbachia* spread horizontally at relatively short timescales and recombination between *ftsZ* and *wsp* occurs at a larger timescale.

Nonetheless, recombination might be a frequent event during horizontal transfer and this can be inferred from the fact that *ftsZ* can move across orders in directions where *wsp* movement does not take place. For example, a transfer from Diptera to Lepidoptera, or Hymenoptera to Hemiptera, occurs in *ftsZ* gene but not in *wsp* (Figure 3), and this suggests that a Diptera-adapted *wsp* gene undergoes recombination upon entry into a lepidopteran host. Whereas the bidirectional zero transition rates between Diptera and Hemiptera based on the *wsp* gene phylogeny only, indicate that horizontal transfer between these orders necessitates the recombination of *wsp* but not *ftsZ*. This is consistent with what is known about the *wsp* gene in being highly recombinant and *ftsZ* more conserved (Baldo et al., 2002). The estimated horizontal transfer rates of *Wolbachia* from Hymenoptera to Hemiptera are zero for both genes. This could be due to a failure in any of the steps of horizontal transfer. As reviewed by Sanaei et al. (2021), host shifting of *Wolbachia* could be summarized in four essential steps: physical transfer, overcoming immunity, successful vertical transfer, and spread within the host population. Many insects from the order Hymenoptera are parasitoids to hemipterans suggesting presence of ecological interactions between the two orders that can allow for physical transfer to be achieved. Accordingly, possible explanations why  $q_{HP} = 0$  could be that there is no evolved resistance mechanism of the Hymenoptera-adapted symbiont

against immunity of hemipteran species, or it may be that the transfer of *Wolbachia* occurs but the infection in hemipterans does not constitute a significant and negative selective pressure. Alternatively, the pattern could be attributed to characteristics of hemipterans in general such as being hemimetabolous. Trans-infection experimentations can shed light on this observed pattern and elucidate the different immune reactions or physiological responses of hemipteran hosts against *Wolbachia* strains from a hymenopteran source.

Notably, there are two likely explanations why the results differ between both genes. Either there is no strong phylogenetic signal of *ftsZ*, or *wsp* and *ftsZ* have different underlying histories of ancestry and thus different phylogenies. In order to tease these two reasons apart we implemented the partition homogeneity test. Because none of the random partitions yields pairs of trees that are as short as the original tree, we have good reason to conclude that the original partitions are nonrandom, suggesting that partitions for *wsp* and *ftsZ* have distinct phylogenetic signals. This would argue that the two partitions do not share the same phylogenetic history, which is also consistent with the analysis of Jiggins et al. (2001). Given the obvious conflict between these two data partitions, they should not be concatenated into a single data matrix.

Closely related host species are similar in several aspects, ranging from their intra- and extracellular environments to their immunity (Gilbert & Webb, 2007; Perlman & Jaenike, 2003). The concept of the ‘phylogenetic distance effect’ (PDE) would accordingly be expected, where the symbiont tends to switch more easily between close relatives (Baldo et al., 2008; Charleston & Robertson, 2002). Although this expectation was observed in our study (Figure 5), it was not always the case; numerous parts of the analysis indicate it is not uncommon to have *Wolbachia* with

identical sequences in two unrelated hosts from different geographical regions. Sequence similarity and horizontal transfers are therefore not strongly constrained; even if it is easier to spread to most related hosts, it is not impossible to spread to entirely different hosts within a short time. This is consistent with previous studies, which reported both presence (Haine et al., 2005; Russell et al., 2009) and lack of signals of a PDE (Ahmed et al., 2016; Gerth et al., 2013; Shoemaker et al., 2002) in different arthropods ranging from ants to butterflies to wasps, to *Drosophila* and more. Although there is evidence for a PDE, it is imprecise to exclusively refer host shifts to close relatedness as it could be stemming from ecological niche similarity and interactions such as common predators, parasitoids, and food sources.

While lack of exact geographic coordinates prevents us from presenting a more pinpointed and quantitative analysis of geographic transfers of *Wolbachia*, but there seems to be no evidence that host shifts are constrained by geography. For example, several clades contain closely related strains stemming from a single continent and spreading to other continents (Tables 2 and 4). Means of human activities might have driven this global spread, especially in the cases where insect hosts have invasive abilities, as discussed previously. Mobility of hosts might also play a role, especially in lepidopteran species which are able of migration and flights traversing thousands of miles (Straatman, 1963). More investigations are needed to understand the contributions of host mobility, geographic barriers, and phylogenetic distance effect to host shifts of *Wolbachia* endosymbionts.

In conclusion, *Wolbachia* horizontal transfers are occurring relatively frequently on an evolutionary timescale, without any detected pattern of transmission across geographical regions or host orders. On the insect order scale, host shifts could be

unidirectional and some genes are more constrained and host specific than others. Overall, there is no preferential movement across orders. While it might be more convenient for host shifts to occur between closely related species, it is not rare to observe such events between phylogenetically and geographically distant species.



## REFERENCES

- Ahmed, M. Z., Breinholt, J. W., & Kawahara, A. Y. (2016, 2016/05/27). Evidence for common horizontal transmission of Wolbachia among butterflies and moths. *BMC Evolutionary Biology*, *16*(1), 118. <https://doi.org/10.1186/s12862-016-0660-x>
- Ahmed, M. Z., Li, S.-J., Xue, X., Yin, X.-J., Ren, S.-X., Jiggins, F. M., Greeff, J. M., & Qiu, B.-L. (2015). The intracellular bacterium Wolbachia uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. *PLoS pathogens*, *10*(2), e1004672-e1004672. <https://doi.org/10.1371/journal.ppat.1004672>
- Armstrong, K. F., & Ball, S. L. (2005). DNA barcodes for biosecurity: invasive species identification. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1813-1823. <https://doi.org/doi:10.1098/rstb.2005.1713>
- Avino, M., Ng, G. T., He, Y., Renaud, M. S., Jones, B. R., & Poon, A. F. (2019). Tree shape-based approaches for the comparative study of cophylogeny. *Ecology and evolution*, *9*(12), 6756-6771.
- Baines, S. (1956). The Role of the Symbiotic Bacteria in the Nutrition of *Rhodnius Prolixus* (Hemiptera). *Journal of Experimental Biology*, *33*(3), 533-541. <https://jeb.biologists.org/content/jexbio/33/3/533.full.pdf>
- Balbuena, J. A., Míguez-Lozano, R., & Blasco-Costa, I. (2013). PACo: a novel procrustes application to cophylogenetic analysis. *PLoS One*, *8*(4), e61048. <https://doi.org/10.1371/journal.pone.0061048>
- Baldo, L., Ayoub, N. A., Hayashi, C. Y., Russell, J. A., Stahlhut, J. K., & Werren, J. H. (2008). Insight into the routes of Wolbachia invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by Wolbachia strain and mitochondrial DNA diversity. *Molecular ecology*, *17*(2), 557-569.
- Baldo, L., Bartos, J. D., Werren, J. H., Bazzocchi, C., Casiraghi, M., & Panelli, S. (2002, Dec). Different rates of nucleotide substitutions in Wolbachia endosymbionts of arthropods and nematodes: arms race or host shifts? *Parassitologia*, *44*(3-4), 179-187.
- Baldo, L., & Werren, J. H. (2007). Revisiting Wolbachia supergroup typing based on WSP: spurious lineages and discordance with MLST. *Current microbiology*, *55*(1), 81-87.
- Bandi, C., Anderson, T. J., Genchi, C., & Blaxter, M. L. (1998). Phylogeny of Wolbachia in filarial nematodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1413), 2407-2413.

- Bautista, R. C., Harris, E. J., & Lawrence, P. O. (1998). Biology and rearing of the fruit fly parasitoid *Biosteres arisanus*: clues to insectary propagation. *Entomologia experimentalis et applicata*, 89(1), 79-86.
- Bieńkowski, A. O., & Orlova-Bienkowskaja, M. J. (2020). Invasive Agricultural Pest *Drosophila suzukii* (Diptera, Drosophilidae) Appeared in the Russian Caucasus. *Insects*, 11(11), 826. <https://doi.org/10.3390/insects11110826>
- Boyle, L., O'Neill, S. L., Robertson, H. M., & Karr, T. L. (1993). Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science*, 260(5115), 1796-1799.
- Bright, M., & Bulgheresi, S. (2010). A complex journey: transmission of microbial symbionts. *Nature reviews. Microbiology*, 8(3), 218-230. <https://doi.org/10.1038/nrmicro2262>
- Brown, A. N., & Lloyd, V. K. (2015). Evidence for horizontal transfer of *Wolbachia* by a *Drosophila* mite. *Experimental and Applied Acarology*, 66(3), 301-311.
- Casiraghi, M., Anderson, T., Bandi, C., Bazzocchi, C., & Genchi, C. (2001). A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology*, 122(1), 93.
- Charleston, M. A., & Robertson, D. L. (2002). Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Systematic Biology*, 51(3), 528-535.
- Chesters, D. (2017, May 1). Construction of a Species-Level Tree of Life for the Insects and Utility in Taxonomic Profiling. *Syst Biol*, 66(3), 426-439. <https://doi.org/10.1093/sysbio/syw099>
- Comandatore, F., Cordaux, R., Bandi, C., Blaxter, M., Darby, A., Makepeace, B. L., Montagna, M., & Sasser, D. (2015). Supergroup C *Wolbachia*, mutualist symbionts of filarial nematodes, have a distinct genome structure. *Open biology*, 5(12), 150099-150099. <https://doi.org/10.1098/rsob.150099>
- Dale, C., & Moran, N. A. (2006, Aug 11). Molecular interactions between bacterial symbionts and their hosts. *Cell*, 126(3), 453-465. <https://doi.org/10.1016/j.cell.2006.07.014>
- Dumler, J. S., Barbet, A. F., Bekker, C., Dasch, G. A., Palmer, G. H., Ray, S. C., Rikihisa, Y., & Rurangirwa, F. R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *International journal of systematic and evolutionary microbiology*, 51(6), 2145-2165.

- Dyson, E., Kamath, M., & Hurst, G. (2002). Wolbachia infection associated with all-female broods in *Hypolimnas bolina* (Lepidoptera: Nymphalidae): evidence for horizontal transmission of a butterfly male killer. *Heredity*, 88(3), 166-171.
- Engelstädter, J., & Hurst, G. D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution, and Systematics*, 40, 127-149.
- Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology*, 36(5), 533-543.  
<https://doi.org/10.1111/j.1365-2311.2011.01318.x>
- Gerth, M. (2016, 2016/09/01/). Classification of Wolbachia (Alphaproteobacteria, Rickettsiales): No evidence for a distinct supergroup in cave spiders. *Infection, Genetics and Evolution*, 43, 378-380.  
<https://doi.org/https://doi.org/10.1016/j.meegid.2016.05.034>
- Gerth, M., Röthe, J., & Bleidorn, C. (2013). Tracing horizontal Wolbachia movements among bees (*Anthophila*): a combined approach using multilocus sequence typing data and host phylogeny. *Molecular ecology*, 22(24), 6149-6162.
- Gilbert, G. S., & Webb, C. O. (2007). Phylogenetic signal in plant pathogen–host range. *Proceedings of the National Academy of Sciences*, 104(12), 4979-4983.
- Glowska, E., Dragun-Damian, A., Dabert, M., & Gerth, M. (2015). New Wolbachia supergroups detected in quill mites (Acari: Syringophilidae). *Infection, Genetics and Evolution*, 30, 140-146.
- Haine, E. R., Pickup, N. J., & Cook, J. M. (2005). Horizontal transmission of Wolbachia in a *Drosophila* community. *Ecological Entomology*, 30(4), 464-472.  
<https://doi.org/https://doi.org/10.1111/j.0307-6946.2005.00715.x>
- Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with Wolbachia? – a statistical analysis of current data. *FEMS Microbiology Letters*, 281(2), 215-220.  
<https://doi.org/10.1111/j.1574-6968.2008.01110.x>
- Huigens, M., De Almeida, R., Boons, P., Luck, R., & Stouthamer, R. (2004). Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing Wolbachia in *Trichogramma* wasps. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1538), 509-515.
- Hutchinson, M. C., Cagua, E. F., Balbuena, J. A., Stouffer, D. B., & Poisot, T. (2017). paco: implementing Procrustean Approach to Cophylogeny in R. *Methods in Ecology and Evolution*, 8(8), 932-940.  
<https://doi.org/https://doi.org/10.1111/2041-210X.12736>

- Jiggins, F. M., Schulenburg, J. H. G. v. d., Hurst, G. D. D., & Majerus, M. E. N. (2001). Recombination confounds interpretations of *Wolbachia* evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1474), 1423-1427. <https://doi.org/doi:10.1098/rspb.2001.1656>
- Kageyama, D., Narita, S., & Noda, H. (2008). Transfection of feminizing *Wolbachia* endosymbionts of the butterfly, *Eurema hecabe*, into the cell culture and various immature stages of the silkworm, *Bombyx mori*. *Microbial ecology*, 56(4), 733.
- Kremer, N., & Huigens, M. E. (2011). Vertical and horizontal transmission drive bacterial invasion. *Molecular ecology*, 20(17), 3496-3498.
- Le Clec'h, W., Chevalier, F. D., Genty, L., Bertaux, J., Bouchon, D., & Sicard, M. (2013). Cannibalism and predation as paths for horizontal passage of *Wolbachia* between terrestrial isopods. *PLoS One*, 8(4), e60232.
- Lo, N., Casiraghi, M., Salati, E., Bazzocchi, C., & Bandi, C. (2002). How many *Wolbachia* supergroups exist? *Molecular biology and evolution*, 19(3), 341-346.
- Moran, N. A., & Dunbar, H. E. (2006, Aug 22). Sexual acquisition of beneficial symbionts in aphids. *Proc Natl Acad Sci U S A*, 103(34), 12803-12806. <https://doi.org/10.1073/pnas.0605772103>
- Nikolouli, K., Colinet, H., Renault, D., Enriquez, T., Mouton, L., Gibert, P., Sassu, F., Cáceres, C., Stauffer, C., Pereira, R., & Bourtzis, K. (2018, 2018/03/01). Sterile insect technique and *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. *Journal of Pest Science*, 91(2), 489-503. <https://doi.org/10.1007/s10340-017-0944-y>
- O'Neill, S. L., Hoffmann, A., & Werren, J. (1997). *Influential passengers: inherited microorganisms and arthropod reproduction*. Oxford University Press.
- Page, R. D. (1996). Temporal congruence revisited: comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. *Systematic Biology*, 45(2), 151-167.
- Page, R. D. (2003). *Tangled trees: phylogeny, cospeciation, and coevolution*. University of Chicago Press.
- Page, R. D., & Charleston, M. A. (1998). Trees within trees: phylogeny and historical associations. *Trends in Ecology & Evolution*, 13(9), 356-359.
- Paterson, A. M., & Gray, R. D. (1997). Host-parasite co-speciation, host switching, and missing the boat. *Host-parasite evolution: General principles and avian models*, 236-250.

- Paterson, A. M., Palma, R. L., & Gray, R. D. (1999). How frequently do avian lice miss the boat? Implications for coevolutionary studies. *Systematic Biology*, *48*(1), 214-223.
- Perlman, S. J., & Jaenike, J. (2003). Infection success in novel hosts: an experimental and phylogenetic study of *Drosophila*-parasitic nematodes. *Evolution*, *57*(3), 544-557.
- Pietri, J. E., DeBruhl, H., & Sullivan, W. (2016). The rich somatic life of *Wolbachia*. *Microbiologyopen*, *5*(6), 923-936. <https://doi.org/10.1002/mbo3.390>
- Pigeault, R., Braquart-Varnier, C., Marcadé, I., Mappa, G., Mottin, E., & Sicard, M. (2014). Modulation of host immunity and reproduction by horizontally acquired *Wolbachia*. *Journal of insect physiology*, *70*, 125-133.
- Rigaud, T., & Juchault, P. (1995). Success and failure of horizontal transfers of feminizing *Wolbachia* endosymbionts in woodlice. *Journal of Evolutionary Biology*, *8*(2), 249-255.
- Rousse, P., Harris, E., & Quilici, S. (2005). *Fopius arisanus*, an egg-pupal parasitoid of Tephritidae. Overview. *Biocontrol News and Information*, *26*(2), 59-69.
- Roy, V., Girondot, M., & Harry, M. (2015). The distribution of *Wolbachia* in *Cubitermes* (Termitidae, Termitinae) castes and colonies: a modelling approach. *PLoS One*, *10*(2), e0116070.
- Russell, J. A., Goldman-Huertas, B., Moreau, C. S., Baldo, L., Stahlhut, J. K., Werren, J. H., & Pierce, N. E. (2009). Specialization and geographic isolation among *Wolbachia* symbionts from ants and lycaenid butterflies. *Evolution: International Journal of Organic Evolution*, *63*(3), 624-640.
- Sanaei, E., Charlat, S., & Engelstädter, J. (2021). *Wolbachia* host shifts: routes, mechanisms, constraints and evolutionary consequences. *Biological Reviews*, *96*(2), 433-453.
- Serbus, L. R., & Sullivan, W. (2007, Dec). A cellular basis for *Wolbachia* recruitment to the host germline. *PLoS Pathog*, *3*(12), e190. <https://doi.org/10.1371/journal.ppat.0030190>
- Shoemaker, D. D., Machado, C. A., Molbo, D., Werren, J. H., Windsor, D. M., & Herre, E. A. (2002, Nov 7). The distribution of *Wolbachia* in fig wasps: correlations with host phylogeny, ecology and population structure. *Proc Biol Sci*, *269*(1506), 2257-2267. <https://doi.org/10.1098/rspb.2002.2100>
- Sintupachee, S., Milne, J., Poonchaisri, S., Baimai, V., & Kittayapong, P. (2006). Closely related *Wolbachia* strains within the pumpkin arthropod community and the potential for horizontal transmission via the plant. *Microbial ecology*, *51*(3), 294-301.

- Stahlhut, J. K., Desjardins, C. A., Clark, M. E., Baldo, L., Russell, J. A., Werren, J. H., & Jaenike, J. (2010). The mushroom habitat as an ecological arena for global exchange of Wolbachia. *Molecular ecology*, 19(9), 1940-1952.
- Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). Wolbachia pipientis: microbial manipulator of arthropod reproduction. *Annu Rev Microbiol*, 53, 71-102.  
<https://doi.org/10.1146/annurev.micro.53.1.71>
- Straatman, R. (1963). Observations on migration in certain Australian Lepidoptera. *Tijdschrift voor Entomologie*, 106(4), 197-199.
- Wang, G.-H., Jia, L.-Y., Xiao, J.-H., & Huang, D.-W. (2016, 2016/07/01/). Discovery of a new Wolbachia supergroup in cave spider species and the lateral transfer of phage WO among distant hosts. *Infection, Genetics and Evolution*, 41, 1-7.  
<https://doi.org/https://doi.org/10.1016/j.meegid.2016.03.015>
- Werren, J. H., Baldo, L., & Clark, M. E. (2008, Oct). Wolbachia: master manipulators of invertebrate biology. *Nat Rev Microbiol*, 6(10), 741-751.  
<https://doi.org/10.1038/nrmicro1969>
- Werren, J. H., & Windsor, D. M. (2000). Wolbachia infection frequencies in insects: evidence of a global equilibrium? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1450), 1277-1285.
- Zhou, W., Rousset, F., & O'Neill, S. (1998). Phylogeny and PCR-based classification of Wolbachia strains using wsp gene sequences. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1395), 509-515.
- Zug, R., & Hammerstein, P. (2012). Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS One*, 7(6), e38544-e38544. <https://doi.org/10.1371/journal.pone.0038544>