Table S1

Table S1: Primers, PCR cycling conditions, and references used for diagnostic PCR detection of endosymbionts, DNA quality control, and CO1 barcoding

Target Symbiont or DNA	Gene	Primer name	Primer sequence 5' to 3'	References	PCR cycling conditions
Rickettsia	16S	RKlongF RKlong R	ACGTGGGAATCTACCCATCA TAGCCTAGATGACCGCCTTC	this study	95°C for 2 m, followed by 35 cycles consisting of 92°C for 30 s, 60°C for 30 s, and 72°C for 30 s
Rickettsia	16S	RICS741F RICT1197R	CATCCGGAGCTAATGGTTTGC CATTTCTTTCCATTGTGCCATC	Davis et al. 1998	95°C for 2 m, followed by 35 cycles consisting of 92°C for 30 s, 60°C for 30 s, and 72°C for 30 s
Wolbachia	wsp	MFwspF MFwspR	TGGCTGGTGGTGGTGCGTTT CGCTACTCCAGCTTCTGCACCA	this study	95°C for 2 m, followed by 35 cycles consisting of 92°C for 30 s, 66°C for 30 s, and 72°C for 30 s
Wolbachia	wsp	wsp81F wsp691R	TGGTCCATAAGTGATGAAGAAACTAGCTA AAAAATTAAACGCTACTCCAGCTTCTGCAC	Zhou et al. 1998	95°C for 2 m, followed by 35 cycles consisting of 92°C for 30 s, 66°C for 30 s, and 72°C for 30 s
Cardinium	16S	ChF ChR	TACTGTAAGAATAAGCACCGGC GTGGATCACTTAACGCTTTCG	Zchori-Fein and Perlman 2004	94°C for 3 m, followed by 40 cycles consisting of 94°C for 30 s, 56°C for 45 s, and 72°C for 1 m
CO1	COI	lco1490 hco700	GGTCAACAAATCATAAAGATATTGG TCAGGGTGACCAAAAAATCA	Folmer et al. 1994	94°C for 3 m, followed by 35 cycles consisting of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 m
Eukaryote	18S rDNA	NSF 18 NSF 19	CTGGTTGATYCTGCCAGT TCTCAGGCTCCYTCTCCGG	Duron et al. 2008	94°C for 4 m, followed by 35 cycles consisting of 94°C for 1 m, 55°C for 1 m, and 72°C for 1 m

Table S1 References

Davis MJ, Ying Z, Brunner BR, Pantoja A, Ferwerda FH (1998). Rickettsial relative associated with papaya bunchy top disease. *Curr Microbiol* **36**: 80-84.

Duron O, Bouchon D, Boutin S, Bellamy L, Zhou LQ, Engelstädter J *et al* (2008). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol* **6**: 12.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Bio Biotech* **3**: 294.

Zchori-Fein E, Perlman SJ (2004). Distribution of the bacterial symbiont *Cardinium* in arthropods. *Mol Ecol* **13**: 2009-2016.

Zhou W, Rousset F, O'Neill S (1998). Phylogeny and PCR–based classification of *Wolbachia* strains using wsp gene sequences. *P Roy Soc Lond B Bio* **265**: 509-515.

Table S2a Distance matrix of genetic similarity (%) among *Rickettsia* strains in both *Mermessus* surveys as determined by sequencing 673bp of bacterial 16S rRNA

		M. fradeorum	*Mermessus* genus				
		R_1	R_2	R_3	R_4	R_5	R_6
M. fradeorum	R_1	100	95.9	99.5	99.1	99.3	98.9
Mermessus genus	R_2		100	95.8	95.6	95.9	95.6
	R_3			100	99.3	99.5	99.1
	R_4				100	99.1	98.8
	R_5					100	99.6
	R_6						100

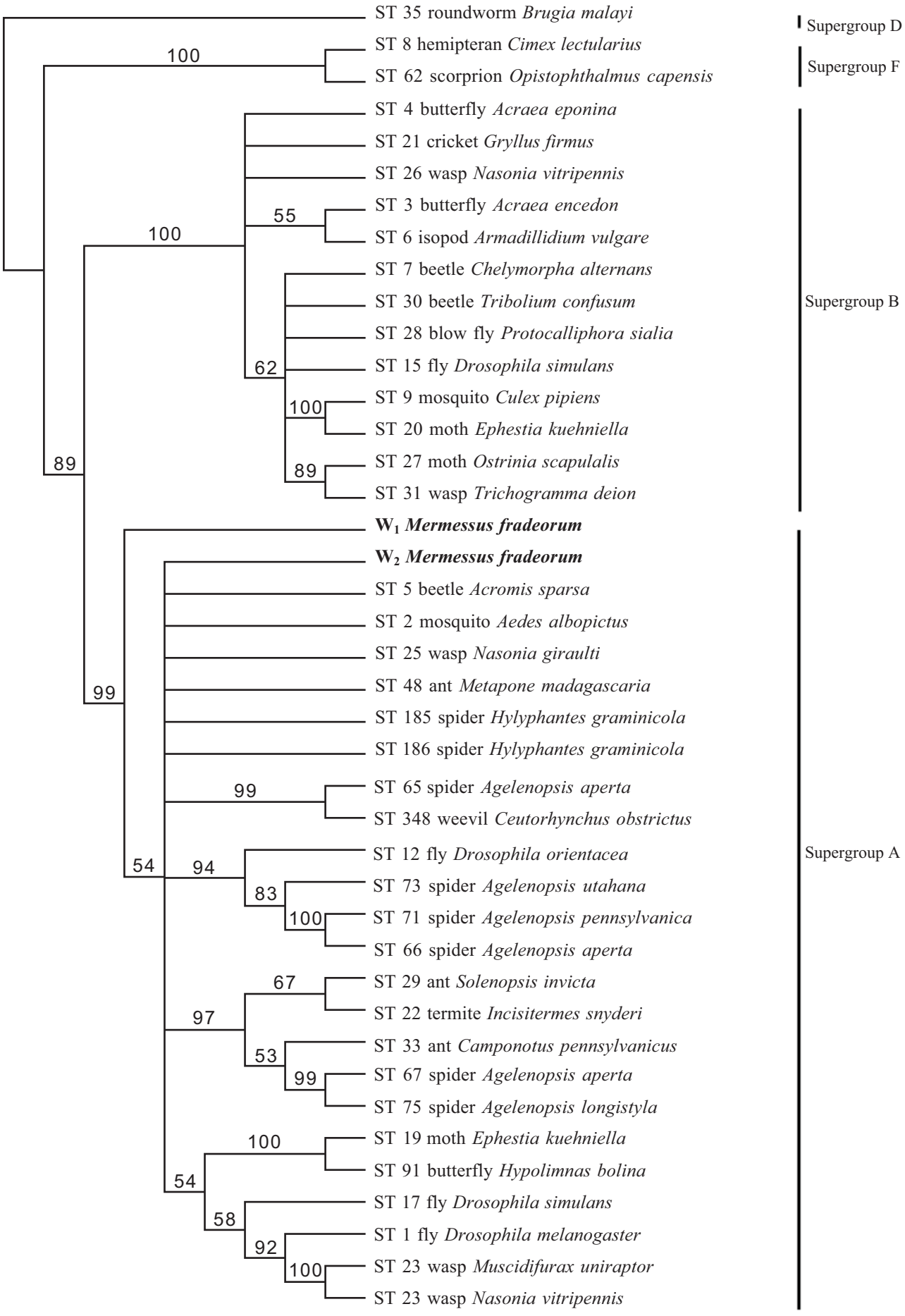
Table S2b Distance matrix of genetic similarity (%) among *Wolbachia* strains in both *Mermessus* surveys as determined by sequencing 561bp of the *Wolbachia wsp* gene

		M. fradeorum		*Mermessus* genus		
		W_1	W_2	W_3	W_4	W_5
M. fradeorum	W_1	100	91.2	90.5	77.8	86.1
	W_2		100	92.9	78.7	90.0
Mermessus genus	W_3			100	79.9	86.1
	W_4				100	75.9
	W_5					100

Table S2c Distance matrix of genetic similarity (%) among *Cardinium* strains in the *Mermessus* genus survey as determined by sequencing 333bp of bacterial 16S rRNA

	C_1	C_2	C_3
C_1	100	98.8	97.6
C_2		100	98.5
C_3			100

Figure S1


ST 35 roundworm Brugia malayi
ST 8 hemipteran Cimex lectularius
ST 62 scorprion Opistophthalmus capensis
ST 4 butterfly Acraea eponina
ST 21 cricket Gryllus firmus
ST 26 wasp Nasonia vitripennis
ST 3 butterfly Acraea encedon
ST 6 isopod Armadillidium vulgare
ST 7 beetle Chelymorpha alternans
ST 30 beetle Tribolium confusum
ST 28 blow fly Protocalliphora sialia
ST 15 fly Drosophila simulans
ST 9 mosquito Culex pipiens
ST 20 moth Ephestia kuehniella
ST 27 moth Ostrinia scapulalis
ST 31 wasp Trichogramma deion
W1 Mermessus fradeorum
W2 Mermessus fradeorum
ST 5 beetle Acromis sparsa
ST 2 mosquito Aedes albopictus
ST 25 wasp Nasonia giraulti
ST 48 ant Metapone madagascaria
ST 185 spider Hylyphantes graminicola
ST 186 spider Hylyphantes graminicola
ST 65 spider Agelenopsis aperta
ST 348 weevil Ceutorhynchus obstrictus
ST 12 fly Drosophila orientacea
ST 73 spider Agelenopsis utahana
ST 71 spider Agelenopsis pennsylvanica
ST 66 spider Agelenopsis aperta
ST 29 ant Solenopsis invicta
ST 22 termite Incisitermes snyderi
ST 33 ant Camponotus pennsylvanicus
ST 67 spider Agelenopsis aperta
ST 75 spider Agelenopsis longistyla
ST 19 moth Ephestia kuehniella
ST 91 butterfly Hypolimnas bolina
ST 17 fly Drosophila simulans
ST 1 fly Drosophila melanogaster
ST 23 wasp Muscidifurax uniraptor
ST 23 wasp Nasonia vitripennis
100
100
55
62
100
89
89
99
99
54
94
83
100
67
97
53
99
100
54
58
92
100
Supergroup D
Supergroup F
Supergroup B
Supergroup A

Supplementary File 4: Modeling Notes

We modified the Hoffman-Turelli model (Turelli, 1994; Turelli and Hoffman 1995) to incorporate an independent female-biasing symbiont in the population, in addition to a symbiont inducing cytoplasmic incompatibility (CI). For the purposes of this exercise, we assumed that female biasing (FB) was complete: FB symbionts are only found in females, and all offspring that carry this symbiont are female. The result is that there are three types of females in the population (females carrying the CI symbiont, females carrying the FB symbiont and uninfected females) and two types of males (males carrying the CI symbiont and uninfected males). Therefore, the proportion of CI -infected females and males in the population were not the same, and we kept track of females and males separately, similar to Vavre et al (2000). Note that the model is applicable to a broader set of female-biasing mechanisms than just feminization, based on the parameter values selected.

We used the following parameters:

1) F_{CI}= The proportional fecundity of CI-carrying females, relative to uninfected females
2) μ_{CI} = The proportional rate of failure to transmit the CI symbiont, resulting in uninfected offspring
3) H_U = The proportional offspring production of uninfected females when mated to CI males. Reflects incomplete penetrance of CI.
4) F_{FB}= The proportional fecundity of FB-carrying females, relative to uninfected females
5) μ_{FB} = The proportional rate of failure to transmit the FB symbiont, resulting in uninfected offspring
6) H_{FB} = The proportional offspring production of FB females when mated to CI males. Reflects incomplete penetrance of CI.
7) s = The proportion female offspring produced by the non-FB females. For FB females, s=1.

The first three parameters are those of the Turelli-Hoffman model, with subscripts added to specify that they refer to features of the CI-carrying or uninfected individuals. We added three parallel parameters for the FB carrying symbionts, allowing the FB symbiont to impose different fecundity costs, and have a different vertical transmission efficiency than the CI symbiont. We additionally allowed CI to have different penetrance in FB carrying individuals than uninfected individuals using the parameter H_{FB}. Finally, we used the parameter *s* to indicate the proportion of female offspring produced by the non-FB females. Because the ultimate value of interest, from the perspective of a maternally-inherited symbiont, is the proportion of the female population that is infected, *s* represents the cost of producing males by the non-FB mothers. We generated the following recursion equations to track the proportion of

females that carried CI, FB or were uninfected, and the proportion of males carrying CI versus uninfected.

$$f_{CI,t+1} = \frac{f_{CI,t}F_{CI}(1-\mu_{CI})s}{f_{CI,t}F_{CI}s\left(1+\mu_{CI}\left(m_{U,t}+\ m_{CI,t}H_U-1\right)\right)+f_{FB}F_{FB}\left[m_{U,t}\left(1+\mu_{FB}(s-1)\right)+\ m_{CI,t}H_{FB}\left(1+\mu_{FB}(s-1)\right)\right]+f_{U,t}s(m_{U,t}+m_{CI,t}H_U)}$$

$$f_{FB,t+1} = \frac{f_{FB,t}m_{U,t}F_{FB}(1-\mu_{FB})+f_{FB,t}m_{CI,t}F_{FB}H_{FB}(1-\mu_{FB})}{f_{CI,t}F_{CI}s\left(1+\mu_{CI}\left(m_{U,t}+\ m_{CI,t}H_U-1\right)\right)+f_{FB}F_{FB}\left[m_{U,t}\left(1+\mu_{FB}(s-1)\right)+\ m_{CI,t}H_{FB}\left(1+\mu_{FB}(s-1)\right)\right]+f_{U,t}s(m_{U,t}+m_{CI,t}H_U)}$$

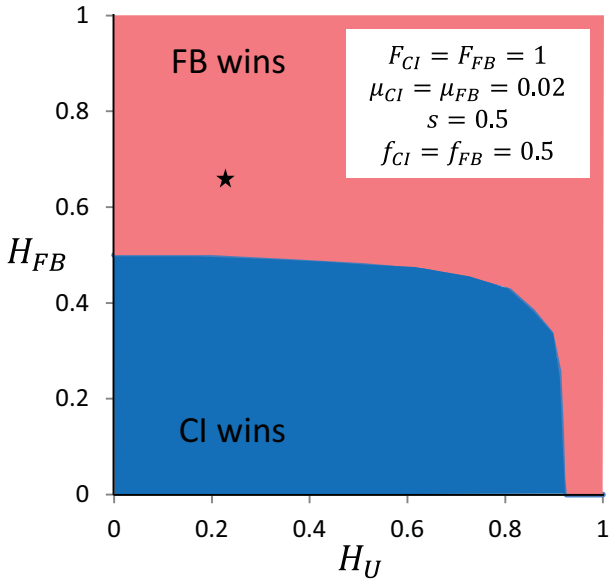
$$f_{U,t+1} = 1 - f_{CI,t+1} - f_{FB,t+1}$$

$$m_{CI,t+1} = \frac{f_{CI,t}F_{CI}(1-\mu_{CI})}{f_{CI,t}F_{CI}\left(1+\mu_{CI}\left(m_{U,t}+\ m_{CI,t}H_U-1\right)\right)+f_{FB}F_{FB}\mu_{FB}\left(m_{U,t}+\ m_{CI,t}H_{FB}\right)+f_{U,t}(m_{U,t}+m_{CI,t}H_U)}$$

$$m_{U,t+1} = 1 - m_{CI,t+1}$$

We find that when both manipulators have minimal fecundity costs and high vertical transmission, as we observed in our laboratory assays, the expectation is that one or the other symbiont is driven out of the population, whereas the other reaches near fixation. Which manipulation dominates depends on the level of CI penetrance, particularly in female biasing symbiont (H_{FB}). If H_{FB} is low and CI penetrance in FB carrying-females is strong, the CI symbiont will dominate, whereas if H_{FB} is high and CI penetrance in FB carrying females is weak, the FB symbiont will dominate. This result is robust across a large range of H_U values; when H_U is also very high, the CI symbiont would not be sustained in the population even in the absence of the FB symbiont.

Supplementary Excel file 5, which allows exploration of the model, is included.



(The star represents our point estimates of H_U and H_{FB} based on laboratory studies).

Supplementary File 4 and 5 References

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Turelli M, Hoffmann AA (1995). Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* **140**: 1319-1338.

Vavre F, Fleury F, Varaldi J, Fouillet P, Bouletreau M (2000). Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. *Evolution* **54**: 191-200.