Supplementary Materials

Wolbachia and host intrinsic reproductive barriers contribute additively to post-mating isolation in spider mites

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Box S1. Detailed information relating to the populations of _T. urticae_ used in this study.

All information concerning the three _T. urticae_ populations used in the experiments is provided in Table I. At the time of the experiments, all populations were fully infected with _Wolbachia_ [1, 2], and were controlled for the absence of other known bacterial reproductive manipulators (_Cardinium_, _Rickettsia_, _Spiroplasma_ and _Arsenophonus_) as described in [3]. Moreover, DNA extractions from pools of 100 females for each population, and subsequent PCR amplification and sequencing of a fragment of the nuclear ribosomal DNA _ITS2_ (internal transcribed spacer 2) region, mitochondrial DNA Cytochrome Oxidase subunit I (_COI_), and _Wolbachia wsp_ gene were performed using the same procedure and primers as in [3]. Finally, we performed a multilocus sequence typing for _Wolbachia_ (MLST; [4]) using primers and protocols described in [2].

We found that the two red populations (Ri1 and Ri2) are strictly identical based on _ITS2_ and _COI_ sequences, and carry the same _Wolbachia_ strain based on the _wsp_ gene and MLST profile. However, they differ from the green population (Gi) by 1 SNP in the _ITS2_ sequence and 21 SNPs in the _COI_ sequence (genetic distance of 0.056 with Kimura 2-parameter; rate of variation gamma distribution; shape parameter = 1). The _Wolbachia_ strain infecting the red (Ri1 and Ri2) and the green (Gi) populations also differ based on their MLST profile, with 1 SNP difference in fbpA and coxA sequences (Table I).

Table I. Spider mite populations and _Wolbachia_ infection.

Population		Ri1	Ri2	Gi
Host information	Original name	AMP	LOU	TOM
	Form	red	red	green
	Collection date	18/11/2013	03/10/2013	--/05/2010
	Host plant	_Datura stramonium_	_Solanum melongena_	_Solanum lycopersicum_
	Location	Aldeia da Mata Pequena	Lourinhã	Carregado
	Coordinates	38.534363, -9.191163	39.248145, -9.276321	39.078962, -8.993656
	Initial number	65 ♀	300 ♀	300 ♀
	ITS2[1]	GU565314	GU565314	AM408031
	COI[1]	MF428440	MF428440	HM486513
Wolbachia information	Isolate (id)[2]	Turt_B_wUrtAmp (1858)	Turt_B_wUrtAmp (1858)	Turt_B_wUrtTom (1857)
	Strain[2]	491	491	280
	gatB allele[2]	9	9	9
	coxA allele[2]	38	38	164
	hcpA allele[2]	143	143	143
	ftsZ allele[2]	23	23	23
	fbpA allele[2]	444	444	4
	wsp[1]	GU014541	GU014541	GU014541
	CI level	57%	30%	0%
References		[2, 3]	[2, 3]	[1, 5]; this study

[1] GenBank accession number matching with 100% coverage and identity at the nucleotide level.
[2] Allele number in the PubMLST _Wolbachia_ MLST database, available at http://www.pubmlst.org/wolbachia/

References

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5. Clemente SH, Rodrigues LR, Ponce R, Varela SAM, Magalhães S. Incomplete species recognition entails few costs in spider mites, despite first-male precedence. Behav Ecol Sociobiol. 2016;70:1161–1170.

Box S2. Detailed description of the minor differences in the experimental procedure used to perform the crosses of category 5 in Experiment 1.

Because simultaneously performing all possible crosses between 3 different populations, infected or not by *Wolbachia*, represents a very important work load for a single experimenter (*i.e.* a total of 36 types of crosses were performed), inter-population crosses using infected females and uninfected males (crosses of category 5; *cf*. Table 1) were performed *ca*. 23 months after the others.

These crosses were initially excluded because they do not inform on *Wolbachia*-induced CI (wCI), and because host incompatibility (HI) was already assessed by performing the crosses of category 3 (*i.e.* inter-population crosses using uninfected females and uninfected males; *cf.* Table 1). Nevertheless, we subsequently decided to perform them, to confirm that *Wolbachia* infection in females does not affect the pattern of HI (*cf.* Table 1).

The general procedure used to perform these crosses was the same as for the other crosses (*cf.* Methods) with some minor differences. First, to reduce workload, the number of mated females used to create age cohorts was 2*100, instead of 3*100 (*cf.* Methods); and males were directly obtained from female age cohorts (*i.e.* no male age cohorts were prepared). Second, due to an overlap between different experiments performed simultaneously in our laboratory, the growth chamber used for the crosses of categories 1-4 was not available, and we had to use a different growth chamber, with no humidity control. As humidity affects development time in spider mites (Suzuki et al. 2012), female age cohorts for these crosses were created 11 days prior to the onset of the experiment (instead of 10 days; *cf.* Methods). Moreover, because humidity is also a main factor affecting egg hatchability in this species [6], it may explain the inflated embryonic mortality rates observed for this category of crosses (*cf.* Figure S1), and overall does not allow for quantitative comparison between the results obtained in these crosses and the previous ones.

Reference

Suzuki T, Ghazy NA, Amano H, Ohyama K. A high-performance humidity control system for tiny animals: Demonstration of its usefulness in testing egg hatchability of the two-spotted spider mite, *Tetranychus urticae*. Exp Appl Acarol. 2012;58:101–110.

Table S1. Overproduction of males, female embryonic mortality, juvenile mortality, and resulting hybrid production in all intra- and inter-population crosses using *Wolbachia*-infected and uninfected mites. Mean (± s.e.) relative proportion of F1 female embryonic mortality (FM$_{corr}$), F1 male overproduction (MD$_{corr}$), F1 juvenile mortality (JM$_{corr}$) and production of F1 adult females (FP) in the brood for each type of cross, as well as the total number of replicates excluding females that laid no eggs (N). The FM$_{corr}$ and JM$_{corr}$ indexes remove the basal (embryonic and juvenile, respectively) mortality estimated in control crosses. The MD$_{corr}$ index computes excess production of F1 males relative to the control crosses. Identical or absent superscripts indicate nonsignificant differences between crosses at the 5% level. Values revealing incompatibilities are highlighted by shaded background (green: MD-type; red: FM-type; orange: both types).

Category	Cross (♀ x ♂)	MD$_{corr}$ (%)[1]	FM$_{corr}$ (%)[2]	JM$_{corr}$ (%)[3]	FP (%)[1]	N[4]
1	Ru1 x Ru1	14.47 ± 3.86[a]	4.01 ± 1.53[a]	4.40 ± 1.26	50.83 ± 3.41[a]	48
	Ru2 x Ru2	16.66 ± 5.08[a]	5.77 ± 2.17[a]	5.65 ± 2.51	44.75 ± 3.66[a]	47
	Gu x Gu	18.56 ± 4.61[a]	0.19 ± 0.19[a]	4.83 ± 1.21	46.15 ± 3.56[a]	49
	Ri1 x Ri1	6.97 ± 2.95[a]	2.59 ± 1.03[a]	4.49 ± 1.75	57.56 ± 2.68[a]	50
	Ri2 x Ri2	8.18 ± 3.18[a]	4.15 ± 1.29[a]	3.97 ± 1.27	52.27 ± 2.85[a]	43
	Gi x Gi	17.45 ± 4.38[a]	1.97 ± 0.60[a]	4.82 ± 1.88	46.39 ± 4.01[a]	49
2	Ru1 x Ri1	12.43 ± 3.78[a]	42.72 ± 4.07[c]	1.68 ± 0.56	23.45 ± 3.00[b]	49
	Ru2 x Ri2	21.52 ± 5.58[a]	30.88 ± 4.25[c]	1.73 ± 0.80	27.01 ± 3.29[b]	44
	Gu x Gi	12.64 ± 4.06[a]	1.29 ± 0.51[a]	2.87 ± 0.85	52.81 ± 3.43[a]	47
3	Ru1 x Ru2	19.97 ± 4.35[a]	3.78 ± 1.21[a]	5.40 ± 1.40	45.91 ± 3.39[a]	50
	Ru1 x Gu	17.30 ± 4.20[a]	4.84 ± 2.34[a]	1.54 ± 0.95	50.96 ± 3.48[a]	48
	Ru2 x Ru1	9.82 ± 3.66[a]	2.98 ± 1.39[a]	4.50 ± 1.35	50.82 ± 3.16[a]	41
	Ru2 x Gu	13.10 ± 4.50[a]	3.16 ± 1.05[a]	4.92 ± 2.16	51.19 ± 3.25[a]	44
	Gu x Ru1	53.94 ± 4.02[b]	10.51 ± 4.52[b]	5.71±1.33	12.71 ± 2.14[c]	47
	Gu x Ru2	54.13 ± 4.84[b]	2.90 ± 2.02[a]	7.46 ± 2.03	12.68 ± 2.26[c]	49
4	Ru1 x Ri2	17.14 ± 4.50[a]	33.82 ± 4.48[c]	2.84 ± 0.80	25.27 ± 3.09[b]	46
	Ru1 x Gi	6.78 ± 2.01[a]	2.77 ± 1.01[a]	3.71 ± 2.11	60.53 ± 2.30[a]	49
	Ru2 x Ri1	10.31 ± 3.96[a]	30.25 ± 4.40[c]	3.38 ± 0.87	27.01 ± 3.29[b]	44
	Ru2 x Gi	12.49 ± 3.69[a]	4.32 ± 1.53[a]	2.71 ± 1.13	49.42 ± 3.49[a]	45
	Gu x Ri1	46.36 ± 5.06[b]	33.31 ± 7.36[c]	2.62 ± 0.71	8.93 ± 1.99[d]	48
	Gu x Ri2	58.13 ± 4.49[b]	24.57 ± 5.43[c]	5.18 ± 1.46	7.46 ± 1.66[d]	48
	Ri1 x Ri2	16.84 ± 4.50[a]	2.73 ± 0.86[a]	3.43 ± 1.06	50.59 ± 3.56[a]	49
	Ri1 x Gi	5.59 ± 2.47[a]	5.08 ± 2.04[a]	3.41 ± 0.87	57.43 ± 2.61[a]	47
	Ri2 x Ri1	6.45 ± 2.66[a]	3.59 ± 1.33[a]	1.34 ± 0.56	57.51 ± 2.48[a]	48
	Ri2 x Gi	9.99 ± 3.24[a]	9.79 ± 2.81[b]	2.29 ± 0.71	48.53 ± 2.68[a]	49
	Gi x Ri1	53.73 ± 5.29[b]	30.56 ± 4.02[c]	2.87 ± 0.99	10.64 ± 1.89[d]	49
	Gi x Ri2	64.25 ± 4.09[b]	22.21 ± 5.86[c]	2.22 ± 0.69	7.90 ± 1.51[d]	49
5	Ri1 x Ru1	12.40 ± 4.22[A]	5.34 ± 1.35[A]	2.12 ± 0.69	52.25 ± 2.92[A]	49
	Ri2 x Ru2	12.46 ± 3.86[A]	5.42 ± 2.24[A]	1.85 ± 0.65	47.06 ± 3.82[B]	45
	Gi x Gu	30.30 ± 5.55[B]	2.02 ± 0.80[A]	2.97 ± 1.13	39.46 ± 4.28[AB]	50
	Ri1 x Ru2	9.42 ± 3.85[A]	3.96 ± 1.72[A]	3.31 ± 0.78	54.26 ± 3.24[A]	45
	Ri1 x Gu	8.95 ± 3.41[A]	2.16 ± 1.00[A]	1.79 ± 0.42	57.08 ± 2.55[A]	46
	Ri2 x Ru1	5.94 ± 3.04[A]	8.91 ± 2.49[A]	3.55 ± 1.06	52.56 ± 2.49[B]	46
	Ri2 x Gu	17.94 ± 4.03[B]	6.28 ± 2.16[A]	2.89±0.72	45.51 ± 3.15[B]	48
	Gi x Ru1	57.90 ± 4.25[C]	9.23 ± 3.42[AB]	2.63 ± 0.76	10.21 ± 1.81[C]	48
	Gi x Ru2	64.49 ± 4.66[C]	20.15 ± 4.42[B]	1.24 ± 0.42	9.17 ± 2.14[C]	46

[1] Includes all crosses for which at least one offspring reached adulthood.
[2] Includes all crosses that produced more than one female offspring.
[3] Includes all crosses for which at least one egg hatched.
[4] Includes all females that laid at least one egg.

Table S2. F2 offspring production and unviability. The production and mortality of F2 offspring stemming from F1 virgin females or from F1 males backcrossed with females from their maternal population are displayed for each type of F0 cross. The left part of table provides the number of F1 virgin females that laid at least one egg (#fertile), and the mean (± s.e.) daily oviposition per female over 4 days (#eggs), proportion of unhatched eggs (*i.e.* embryonic mortality; mEM_{corr}) and proportion of dead juveniles (*i.e.* juvenile mortality; mJM_{corr}), as well as the total number of F1 females tested (N). The right part of the table provides the number of F1 males that sired at least one daughter (#fertile; as only females are sired by males in haplodiploids), and the mean (± s.e.) proportion of females among adult F2 offspring (sex ratio; SR), proportion of unhatched eggs among F2 females (F2 female embryonic mortality, fEM_{corr}) and proportion of dead juveniles among F2 females (F2 female juvenile mortality; fJM_{corr}), as well as the total number of F1 males tested (N). The mEM_{corr}, fEM_{corr}, mJM_{corr} and fJM_{corr} indexes, which are estimates of unviability due to hybrid breakdown, remove the basal embryonic and juvenile mortality estimated in control crosses. na: not applicable. F0 crosses in which incompatibities were found at the F1 are highlighted by shaded background (green: MD-type; red: FM-type; orange: both types). Identical or absent superscripts indicate nonsignificant differences between crosses at the 5% level.

Parents of tested F1 (F0♀ x F0♂)	Offspring produced by virgin F1 females					Offspring produced by backcrossed F1 males				
	#fertile[1]	#eggs[2]	mEM_{corr} (%)[2]	mJM_{corr} (%)[3]	N[1]	#fertile[1]	fEM_{corr} (%)[4]	fJM_{corr} (%)[4]	SR (%)[4]	N[1]
Ru1 x Ru1	91	5.77 ± 0.24[a]	5.38 ± 1.76[a]	4.76 ± 1.30[ab]	96	41	8.33 ± 2.36[a]	10.62 ± 3.13[b]	66.60 ± 2.85[a]	49
Gu x Gu	97	5.63 ± 0.17[a]	2.86 ± 0.66[a]	3.51 ± 0.58[ab]	100	58	4.95 ± 1.32[b]	8.75 ± 2.04[b]	63.53 ± 2.35[b]	85
Ru1 x Ri1	81	6.95 ± 0.25[b]	11.00 ± 2.94[a]	2.09 ± 0.90[ab]	85	68	4.23 ± 1.54[a]	8.05 ± 1.93[b]	67.89 ± 1.36[a]	91
Gu x Gi	96	6.31 ± 0.21[b]	2.58 ± 0.89[a]	6.04 ± 1.57[ab]	100	58	8.18 ± 1.82[b]	5.08 ± 1.66[a]	64.57 ± 2.46[b]	84
Ru1 x Gu	2	0.63 ± 0.13	100.00 ± 0.00	-	100	72	3.56 ± 1.39[a]	7.12 ± 1.93[a]	68.53 ± 1.46[a]	84
Gu x Ru1	2	0.25 ± 0.00	100.00 ± 0.00	-	96	57	5.85 ± 1.36[b]	5.79 ± 1.63[b]	66.24 ± 2.11[b]	82
Ru1 x Gi	0	-	-	-	100	56	2.09 ± 0.65[a]	3.00 ± 1.03[a]	70.99 ± 1.41[a]	69
Gu x Ri1	0	-	-	-	71	45	7.87 ± 2.38[b]	12.07 ± 2.66[b]	54.81 ± 3.24[c]	82
Ri1 x Ru1	98	7.45 ± 0.22[c]	5.86 ± 1.33[ab]	2.66 ± 0.66[a]	100	35	7.27 ± 2.58	7.14 ± 2.55	69.34 ± 1.06[A]	42
Gi x Gu	95	6.61 ± 0.24[b]	4.86 ± 1.01[b]	5.30 ± 1.13[b]	100	26	2.36 ± 0.86	0.95 ± 0.52	69.53 ± 2.41[A]	46
Ri1 x Ri1	54	5.64 ± 0.31[a]	2.40 ± 1.46[a]	1.83 ± 0.63[a]	57	10	6.49 ± 5.28	4.67 ± 3.05	69.96 ± 2.90[A]	14
Gi x Gi	75	6.34 ± 0.30[b]	5.10 ± 1.53[b]	5.69 ± 1.22[b]	80	20	3.00 ± 1.12	5.54 ± 2.86	61.37 ± 4.54[B]	37
Ri1 x Gu	0	-	-	-	100	25	7.67 ± 3.00	1.10 ± 0.52	65.77 ± 3.54[A]	31
Gi x Ru1	0	-	-	-	100	33	9.64 ± 2.94	6.09 ± 2.77	66.53 ± 2.58[A]	46
Ri1 x Gi	1	1.25 ± na	100.00 ± na	-	100	31	2.64 ± 0.85	4.76 ± 2.18	71.64 ± 1.26[A]	33
Gi x Ri1	1	0.75 ± na	100.00 ± na	-	93	31	4.15 ± 1.18	4.41 ± 1.39	62.50 ± 2.53[A]	47

[1] All tested individuals

[2] Includes all F1 females that laid at least one egg. Note, given that only a few F1 females resulting from inter-population crosses laid eggs, they were excluded from analyses.

[3] Includes all F1 females that laid at least one egg that hatched

[4] Includes all F1 males that sired at least one daughter

Table S3. Description of all statistical models used in the experiments. All response variables were analysed using the glmmTMB procedure, with the type of cross fit as a fixed explanatory variable and the experimental block as random explanatory variable, *i.e.* y ~ cross+(1|block). For the analyses of F1 female/male fertility, the number of days each female/male was alive over the 4-day oviposition period was added to the minimal models as it significantly improved their fit. The "sample size" column gives the number of individual crosses included in each analysis, and the "Family" column indicates the error structure used in each model (bb: betabinomial, zibb: zero-inflated betabinomial, b: binomial, n: log-linked gaussian). Models with (beta)binomial error structure require either a binary response variable (fertile or sterile), a concatenated response variable binding together the number of successes and failures for a given outcome (*e.g.* being a female or not, for female proportion and sex-ratio), or a proportion (bounded between 0 and 1, for all corrected variables). In the latter case, a "weights" argument was added in the model to account for the number of observations per replicate (*i.e.* the denominator). F1 female/male fertility: whether each cross produced at least 1 egg/daughter (1) or none (0); MD_{obs}: percentage of adult F1 males relative to total eggs, CCMD: mean percentage of adult F1 males relative to total eggs in control crosses, FM_{obs}: percentage of unhatched eggs relative to adult females, CCFM: mean percentage of unhatched eggs relative to females in control crosses, $(m/f)JM_{obs}$: percentage of dead juveniles relative to the total number of eggs (for JM_{obs} and mJM_{obs}) or to the total number of females (for mJM_{obs}), CC(m/f)JM: mean percentage of dead juveniles relative to the total number of eggs (for mJM_{obs}) or to the total number of females (for fJM_{obs}) in control crosses, $(m/f)EM_{obs}$: percentage of unhatched eggs relative to the total number of eggs (for mEM_{obs}) or to the total number of females (for fEM_{obs}), CC(m/f)EM: mean percentage of unhatched eggs relative to the total number of eggs (for CCmEM) or to the total number of females (for CCfEM) in control crosses, daughters/sons: total number of adult daughters/sons produced in each cross, eggs: total number of eggs laid by each female during the oviposition period, day: number of days during which each female used in the experiments was alive.

	Variable of interest	Response variable	Model No.	Data subset	Sample size	Family	Effect of cross
Experiment 1 F0 crosses	Male overproduction (MD_{corr})	(MD_{obs}-CCMD)/(1-CCMD)	1.1	Crosses from categories 1-4	1263[1]	bb	χ^2_{26}=460.70, *p*<0.0001
			1.2	Crosses from category 5	422[1]	bb	χ^2_8=174.26, *p*<0.0001
	Female mortality (FM_{corr})	(FM_{obs}-CCFM)/(1-CCFM)	1.3	Crosses from categories 1-4	918[2]	bb	χ^2_{26}=506.20, *p*<0.0001
			1.4	Crosses from category 5	325[2]	bb	χ^2_8=35.85, *p*<0.0001
	Juvenile mortality (JM_{corr})	(JM_{obs}-CCJM)/(1-CCJM)	1.5	Crosses from categories 1-4	1266[3]	bb	χ^2_{26}=34.49, *p*=0.12
			1.6	Crosses from category 5	422[3]	bb	χ^2_8=9.13, *p*=0.33
	Female proportion (FP)	cbind(daughters,eggs-daughters)	1.7	Crosses from categories 1-4	1263[1]	zibb	χ^2_{26}=966.45, *p*<0.0001
			1.8	Crosses from category 5	422[1]	zibb	χ^2_8=278.23, *p*<0.0001
Experiment 2 F1 ♀	F1 female fertility	Proportion of fertile F1 females	2.1	Complete dataset	1478[4]	b	χ^2_{15}=214.26, *p*<0.0001
	F1 female daily oviposition	eggs/day	2.2	F1 females from intra-population crosses	687[5]	n	χ^2_7=55.65, *p*<0.0001
	F2 male embryonic mortality (mEM_{corr})	(mEM_{obs}-CCmEM)/(1-CCmEM)	2.3	F1 females from intra-population crosses	687[5]	bb	χ^2_7=23.33, *p*=0.001
	F2 male juvenile mortality (mJM_{corr})	(mJM_{obs}-CCmJM)/(1-CCmJM)	2.4	F1 females from intra-population crosses	681[3]	bb	χ^2_7=18.57, *p*=0.01
F1 ♂	F1 male fertility	Proportion of fertile F1 males	2.5.1	Uninfected F1 males	588[5]	b	χ^2_7=25.58, *p*=0.0006
			2.5.2	Infected F1 males	276[5]	b	χ^2_7=15.23, *p*=0.03
	F2 female embryonic mortality (fEM_{corr})	(fEM_{obs}-CCfEM)/(1-CCfEM)	2.6.1	Uninfected F1 males	455[2]	bb	χ^2_7=26.31, *p*=0.0004
			2.6.2	Infected F1 males	211[2]	bb	χ^2_7=5.58, *p*=0.59
	F2 female juvenile mortality (fJM_{corr})	(JM_{obs}-CCfJM)/(1-CCfJM)	2.7.1	Uninfected F1 males	455[2]	bb	χ^2_7=22.64, *p*=0.002
			2.7.2	Infected F1 males	211[2]	zibb	χ^2_7=11.68, *p*=0.11
	F2 offspring sex ratio (SR)	cbind(sons, daughters)	2.8.1	Uninfected F1 males	455[2]	bb	χ^2_7=42.10, *p*<0.0001
			2.8.2	Infected F1 males	211[2]	bb	χ^2_7=15.19, *p*=0.03

[1] Includes all crosses that produced at least one adult offspring.
[2] Includes all crosses that produced more than one female offspring.
[3] Includes all crosses that produced at least one hatched egg.
[4] Includes all crosses performed in the experiment.
[5] Includes all crosses that produced at least one egg.

Figure S1. Summary of the development of *T. urticae* eggs resulting from inter-population crosses between infected females and uninfected males (cross category 5). Bar plots represent mean ± s.e. relative proportions of unhatched eggs (i.e. embryonic mortality), dead juveniles (i.e. juvenile mortality), adult daughters and sons for each type of cross. Mothers are displayed on the bottom level of the x-axis and fathers on the top level.
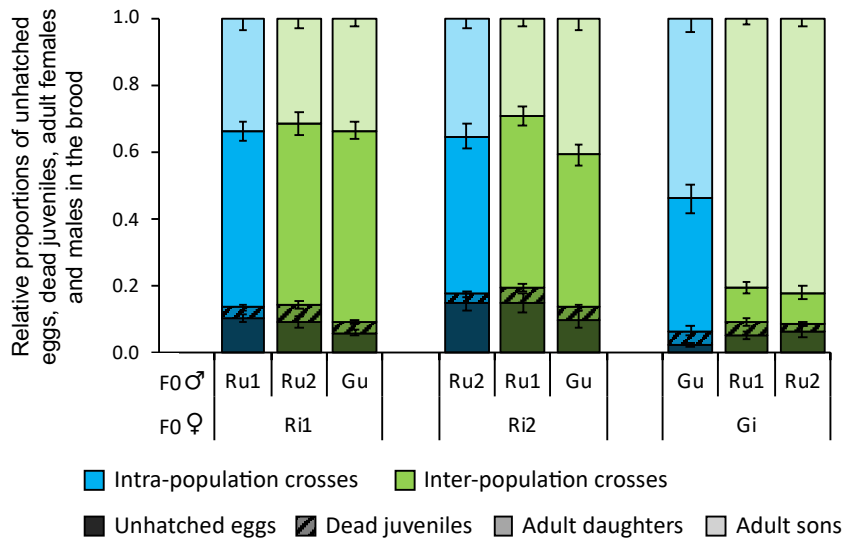
Figure S2. Overproduction of males, female embryonic mortality, and resulting hybrid production in intra- and inter-population crosses using *Wolbachia*-infected and uninfected mites (cross category 5). (a) Boxplot of the proportion of males produced in all crosses relative to that in control crosses (MD_{corr}). **(b)** Boxplot of the proportion of unhatched eggs relative to females, accounting for the basal level of this proportion observed in control crosses (FM_{corr}). **(c)** Proportion of F1 adult females (*i.e.* hybrids) in the brood. Mothers are displayed on the bottom level of the x-axis and fathers on the top level. Identical or absent superscripts indicate nonsignificant differences at the 5% level among crosses.
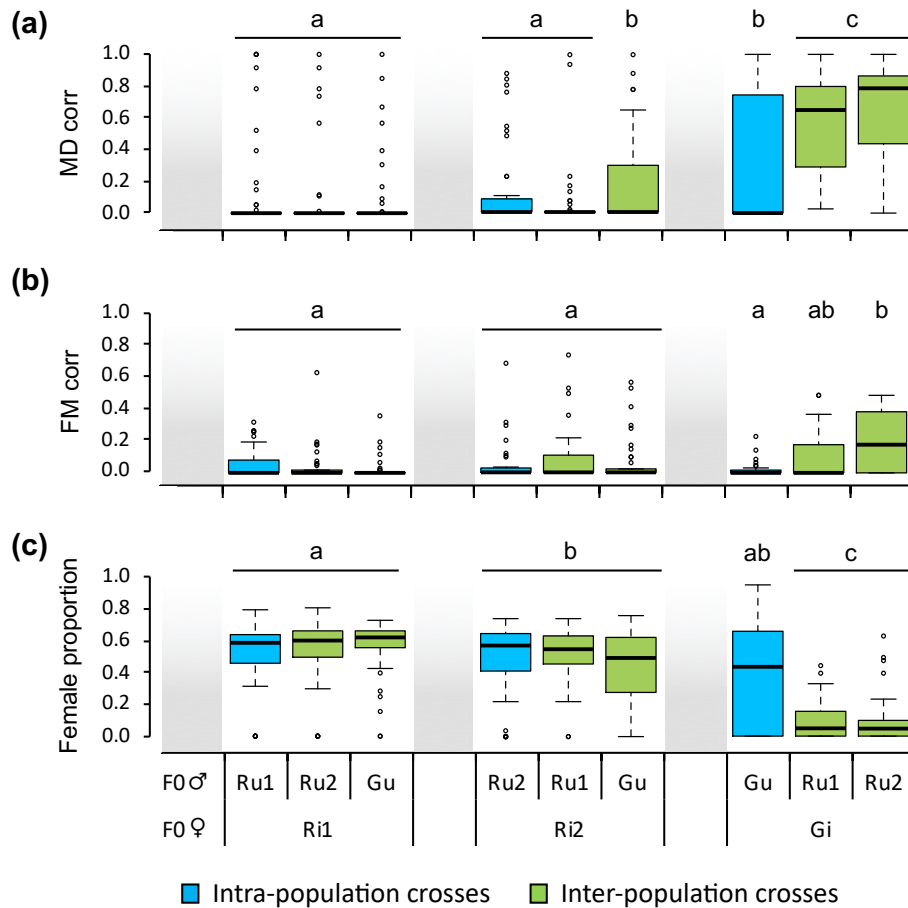
*Note, in **(b)** some differences in the level of FM_{corr} were found among crosses despite males not carrying Wolbachia (model 1.4, $\chi^2_8=35.85$, $p<0.0001$). This effect can be attributed to an overestimation of the FM_{corr} parameter when very few daughters were produced due to MD-type incompatibilities, i.e. Gi ♀xRu1 ♂ and Gi ♀xRu2 ♂ crosses. In **(a)** and **(c)**, for an unknown reason, a higher variance was found in the crosses Gi ♀xGu ♂ and Ri2 ♀xGu ♂ than in other crosses not affected by MD-type incompatibilities. Further experiments on mating behaviour are needed to test whether this effect results from a higher proportion of non-mated females in these crosses.*

Figure S3. Viability of F2 offspring stemming from F1 virgin females. Boxplots of (a) F2 embryonic mortality estimated using the mEM_{corr} index, and (b) F2 juvenile mortality estimated using the mJM_{corr} index, which accounts for the basal level of mortality (observed in control crosses). The x-axis displays the parents of each tested F1 female. Mothers are displayed on the bottom level of the x-axis and fathers on the top level. Identical or absent superscripts indicate nonsignificant differences at the 5% level among crosses. *Note that no data are displayed for inter-population crosses because all but six F1 females obtained from these crosses were sterile, and none of the few F2 eggs laid by those 6 hybrid females hatched (cf. Table S2).*
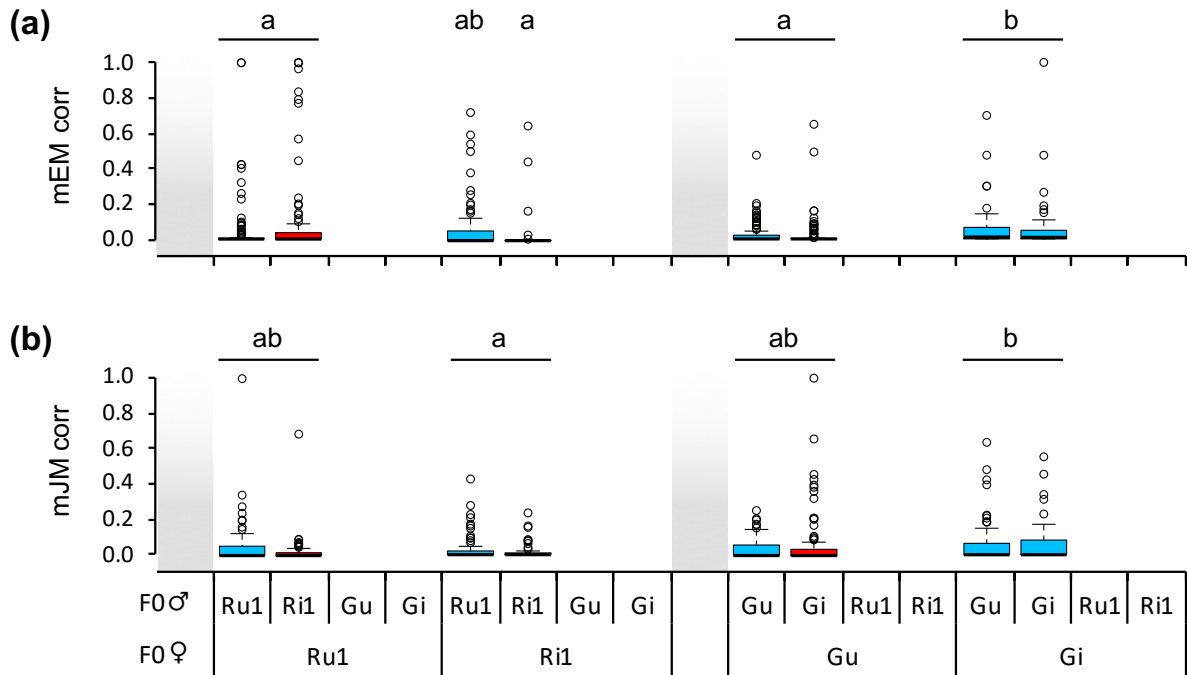
Figure S4. Viability of F2 offspring stemming from F1 males backcrossed with females from their maternal population. Boxplots of (a) F2 embryonic mortality estimated using the fEM_{corr} index, and (b) F2 juvenile mortality estimated using the fJM_{corr} index, which accounts for the basal level of mortality (observed in control crosses). The x-axis displays the cross that produced each tested F1 male. F1 males were mated with females from the same population as their mother. Mothers of F1 males are displayed on the bottom level of the x-axis and fathers of F1 males on the top level. Identical or absent superscripts indicate nonsignificant differences at the 5% level among crosses. *Note that crosses using F1 males stemming from uninfected mothers were analysed separately from those using F1 males stemming from infected mothers, as they were performed at different times than all other crosses.*