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Supplementary materials

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Rapid evolution of *Wolbachia* incompatibility types

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Olivier Duron , Jennifer Bernard, Célestine M. Atyame, Emilie Dumas and Mylène Weill

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Abbreviations	<i>Culex pipiens</i> lines	wPip strains	Origins	References
Lv	LaVar	wPip(Lv)	France	(Duron et al. 2006a)
Is	Istanbul	wPip(Is)	Turkey	(Duron et al. 2006a)
Ko	Kol	wPip(Ko)	Crete	(Duron et al. 2006a)
Tn	Tunis	wPip(Tn)	Tunisia	(Duron et al. 2006a)
SI-TC	SlabTC	uninfected	derived from the Slab line (California)	(Duron et al. 2006a)

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6 **Table S1.** Description of the *Culex pipiens* lines and the wPip strains.

Genes	Putative products	Primers (5'-3')	Size (bp)	References
<i>Culex pipiens</i> (nuclear genome)				
<i>ace-2</i>	Acetylcholinesterase 2 (AChE2)	F1457-CAGGAGATGIGGAAATCCCAA B1246-TGGAGCCTCCTTTCACGGC	700	(Bourguet et al. 1998)
<i>Ester2</i>	Carboxylester hydrolase	Bdir1 530-CTCCAGATCAACCCCTTC MMI_R-CAGCTTCGGGTCGATCATCAT	1100	(Ben Cheikh et al. 2008)
<i>Culex pipiens</i> (mitochondrial genome)				
<i>cytB</i>	Cytochrome b	10366F-CTTTATTAGTAACGTAAAAAATTAC 11217R-ACTAAAGGATTAGCAGGAATGA	852	(Atyame et al. 2011a)
<i>Wolbachia</i> (wPip)				
<i>pk1</i>	Ankyrin domain protein	F-CCACTACATTGGCGTATAGA R-ACAGTAGAAACTACACTCCCTCCA	1,334-1,349	(Duron et al. 2007; Sinkins et al. 2005)
<i>ank2</i>	Ankyrin domain protein	F-CTTCTTCTGTGAGTGTACGT R2-TCCATATCGATCTACTGCGT	313-511	(Duron et al. 2007)
<i>vriC</i> (=GPI5)	Phage related probable secretory protein	F1-ACCATTACAGAACTTGAGGA R1-TAGACGTTCA TAGGCAACCA	1,511-1,538	(Atyame et al. 2011b; Duron et al. 2006b)
<i>WPa_679</i>	Guanylate kinase	F2-ACCTGACTCTGCAGTACTTGA R2-ACTGCTTCTCTCATAAAATTCA F-TATCCTCTCCTTCTGGAGCT R-CTTCCATTGAGGGAGGTAGT	360	This study
<i>Cardinium</i>				
16S rRNA	Small ribosomal subunit	ChF-TACTGTAAAGAATAAGCACCCGGC ChR-GTGGATCACTTAACGCTTTTCG	396	(Zchori-Fein & Perlman 2004)
<i>Arsenophonus</i>				
<i>yaеT</i>	Outer membrane protein assembly factor	yaеTf-GCATAACGGTTCAGACGGGTTTG yaеTr-GCCGAAACGCC TTCAGAAAAG	473	(Duron et al. 2010)
<i>Spiroplasma</i> (<i>ixodetis</i> group)				
16S rRNA	Small ribosomal subunit	Spixof-TTAGGGGCTCAACCCCTAACC Spixor-TCTGGCATTGCCAACTCTC	810	(Duron et al. 2008)
<i>Rickettsia</i>				
<i>gltA</i>	Citrate synthetase	RIC574IF-CATCCGGAGCTAATGGTTTTGC RCT1197R-CAITTCITTCATTTGCCATC	ca.450	(Davis et al. 1998)

9 **Table S2.** Genes and primers used for detection diagnosis tests.

Mosquito lines	n	Allelic profiles																			
		<i>Culex pipiens</i>					<i>Wolbachia</i> (wPip)														
		nuclear genes		mitochondrial gene			<i>pk1</i>		<i>ank2</i>		<i>vr1C</i>	<i>WPa_679</i>									
<i>ace-2</i>	<i>Ester2</i>	<i>cytb</i>																			
2005																					
Lv	10	a	a	pi7	c	e	b	a													
Is	10	a	b	pi12	d	c	d	a													
Ko	10	a	b	pi4	a	a	a	a													
Tn	10	a	b	pi4	a	a	a	a													
SI-TC	10	b	c	pi11	-	-	-	-													
2009 (original nuclear background)																					
Lv	12	a	a	pi7	c	e	b	a													
Is	12	a	b	pi12	d	c	d	a													
Ko	12	a	b	pi4	a	a	a	a													
Tn	12	a	b	pi4	a	a	a	a													
SI-TC	12	b	c	pi11	-	-	-	-													
2009 (SI-TC nuclear background)																					
SI ^{Lv}	10	b	c	pi7	c	e	b	a													
SI ^{Is}	10	b	c	pi12	d	c	d	a													
SI ^{Ko}	10	b	c	pi4	a	a	a	a													
SI ^{Tn}	10	b	c	pi4	a	a	a	a													

Table S3. Allelic profiles of *Culex pipiens* lines and wPip strains. The allelic profiles of mitochondrial and wPip genes were assessed by direct sequencing and the profiles of nuclear genes by PCR/RFLP tests. Nomenclatures for mitochondrial and wPip alleles are those used by Atyame et al. (2011a). SI-TC is a *Wolbachia*-uninfected line. n: number of individuals typed; dash: absence of PCR product.

15 **References**

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40 **Figure legends**

41 **Figure S1.** Hatching rates from crosses (A) between ♀Lv and ♂Tn in 2005 and 2009, and
42 between ♀SI^{wLv} and ♂SI^{wTn}; and (B) between ♀Is and ♂Ko in 2005 and 2009, and between
43 ♀SI^{wIs} and ♂SI^{wKo}. y axis indicates hatching rates. The number of clutches observed (n) is
44 indicated for each cross (2000-3600 eggs were examined per cross). a and b represent
45 statistical groups (Wilcoxon two sided-tests).

46

47 **Figure S2.** Crossing relationships of a *Wolbachia*-uninfected line (SI-TC) with four infected
48 lines (Lv, Is, Ko and Tn) of *Cx. pipiens* in 2005 and 2009. A, crosses of uninfected males with
49 infected and uninfected females; B, crosses of uninfected females with infected males.
50 Histograms give the distribution of hatching rates (HR) (x axis, HR; y axis, proportion of
51 clutches). The number of clutches observed (n) is indicated for each cross (a minimum of
52 1000 eggs was examined per cross). For each cross, no significant variation of HR was found
53 between 2005 and 2009 (Fisher exact tests; a refers to statistical group).

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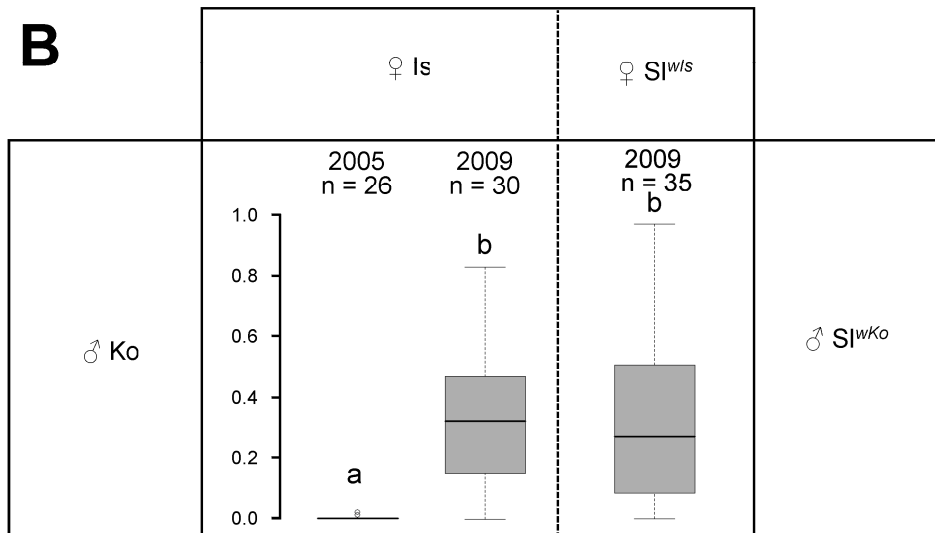
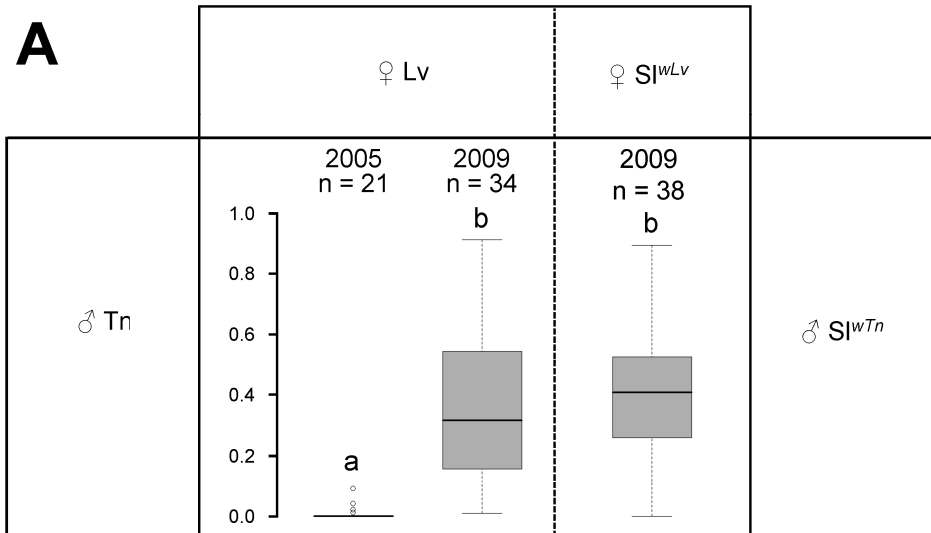
55 **Figure S3.** Hatching rates from crosses of (A) ♀Lv-sub12 and ♀Lv-sub10 with ♂Tn; and (B)
56 ♀Is-sub5 and ♀Is-sub2 with ♂Ko. The number of clutches observed (n) is indicated for each
57 cross (1500-4200 eggs were examined per cross). y axis indicates hatching rates. a and b
58 represent statistical groups (Wilcoxon two sided-tests).

59

60 **Figure S4.** Hatching rates from reciprocal crosses between (A) Lv-sub12 and Lv-sub10, and
61 (B) Is-sub5 and Is-sub2. The number of clutches observed (n) is indicated for each cross (800-
62 3800 eggs were examined per cross). y axis indicates hatching rates. a represents statistical
63 group (Wilcoxon two sided-tests).

64

65 **Figure S5.** Transgenerational survey of crosses (A) between ♀Lv-sub12 and ♂Tn males; (B)
66 between ♀Lv-sub10 and ♂Tn; (C) between ♀Is-sub5 and ♂Ko; and (D) between ♀Is-sub1
67 and ♂Ko. For each cross, no significant variation of hatching rates was observed over 10
68 generations but note in (C) the appearance of few compatible clutches (visualized by outliers).
69 The number of clutches observed (n) is indicated for each cross (1900-5400 eggs were
70 examined per cross). y axis indicates hatching rates. a represents statistical group for
71 transgenerational comparisons (Wilcoxon two sided-tests).



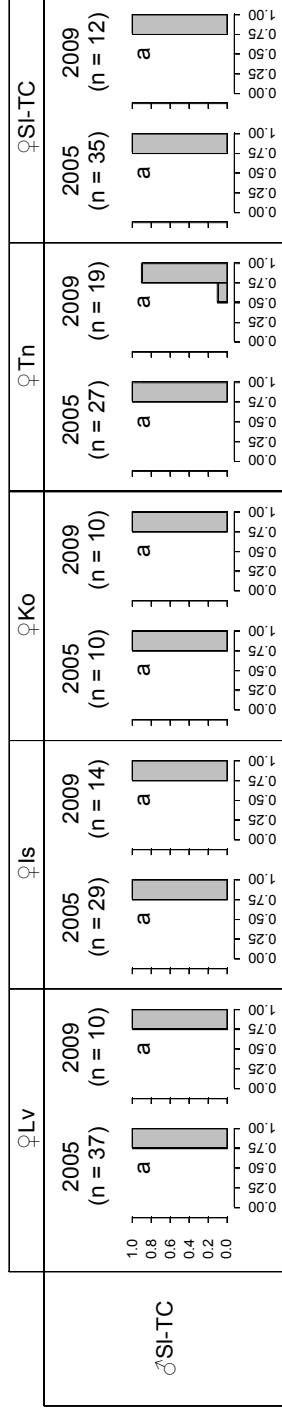
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Figure S1

A



B

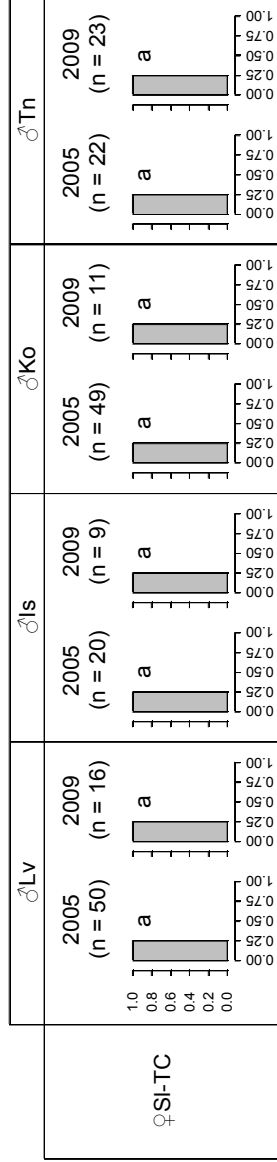


Figure S2

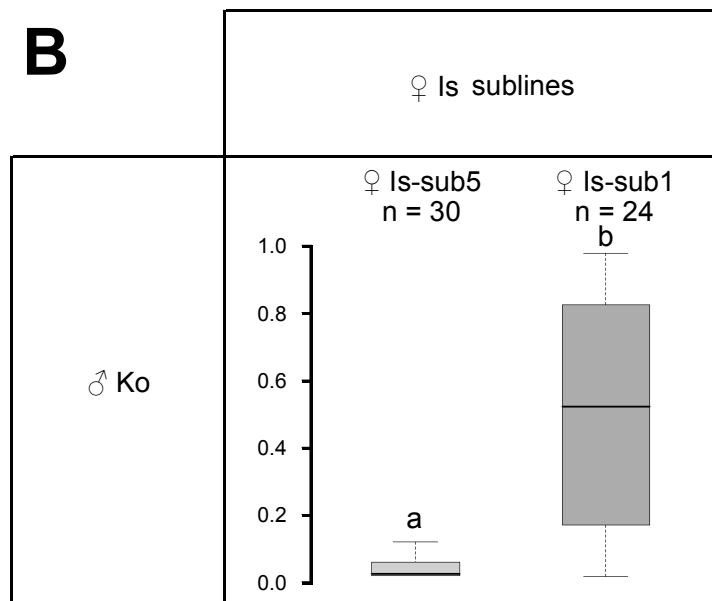
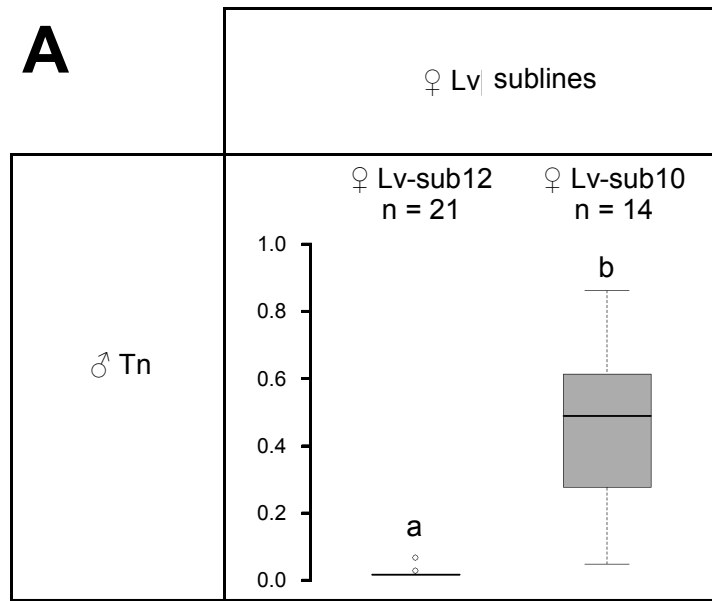


Figure S3

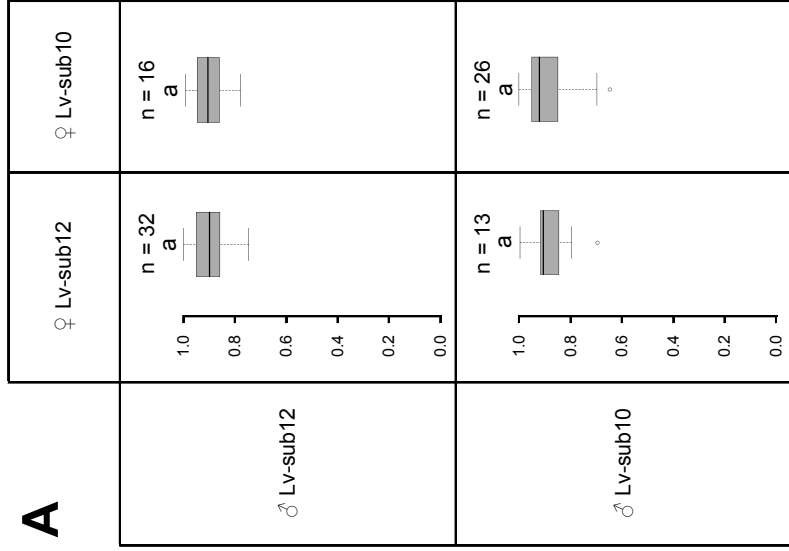
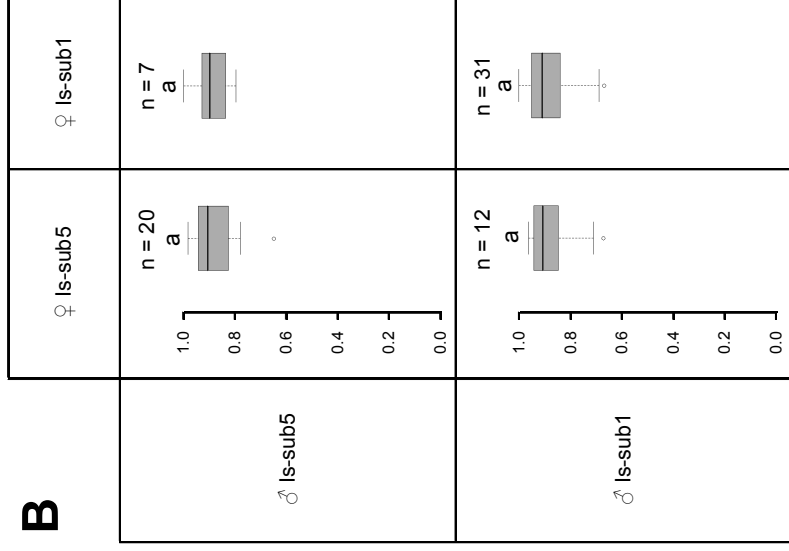
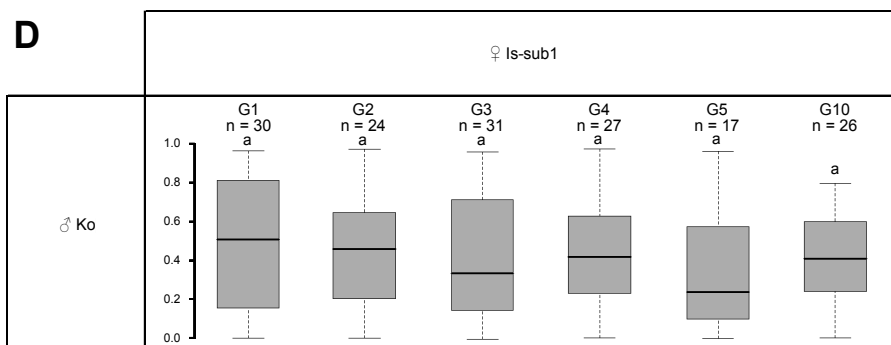
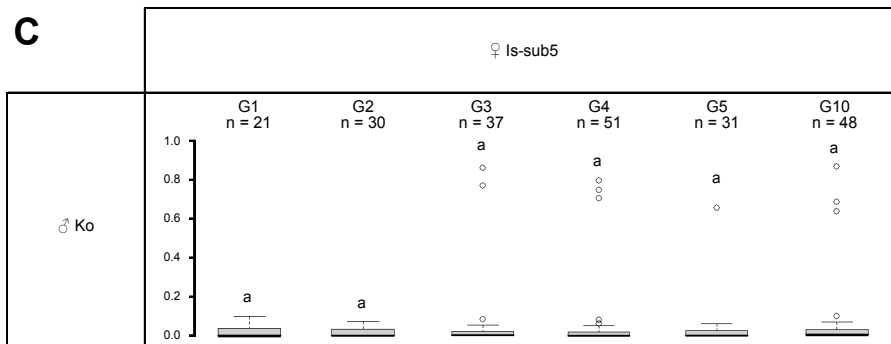
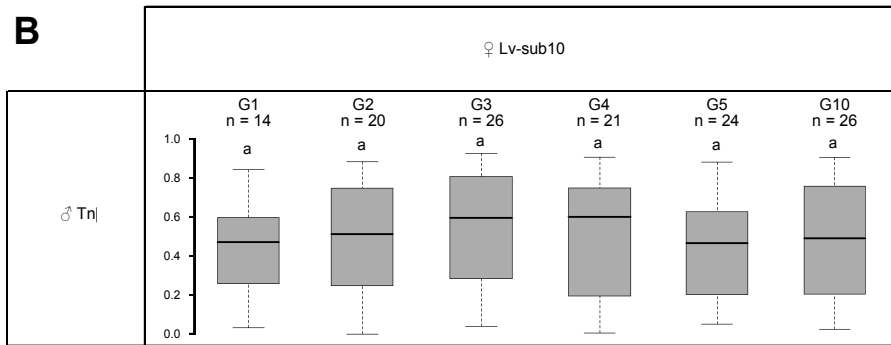
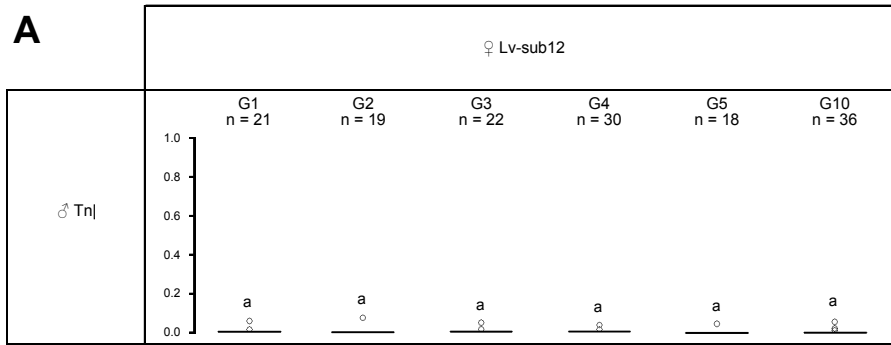


Figure S4



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Figure S5