

## Supplemental material

Strain	Abbreviation	Country or area	Year	Reference
El Palmar-A	Ep-A	Spain	2005	This study
El Palmar-B	Ep-B	Spain	2005	This study
LaVar	Lv	France	2003	(Duron <i>et al.</i> 2006b)
Bifa-A	Bf-A	France	2002	(Duron <i>et al.</i> 2006b)
Bifa-B	Bf-B	France	2002	(Duron <i>et al.</i> 2006b)
Kol	Ko	Crete	2002	(Duron <i>et al.</i> 2006b)
Tunis	Tn	Tunisia	1992	(Ben Cheikh <i>et al.</i> 1998)
Keo-A	Ke-A	Cyprus	2003	(Duron <i>et al.</i> 2006b)
Keo-B	Ke-B	Cyprus	2003	(Duron <i>et al.</i> 2006b)
Istanbul	Is	Turkey	2003	(Duron <i>et al.</i> 2005)
Kara-C	Ka-C	China	2003	(Duron <i>et al.</i> 2006b)
Manille-A	Ma-A	Philippines	2003	(Duron <i>et al.</i> 2006b)
Aus	Au	Australia	2004	(Duron <i>et al.</i> 2005)
Slab	Sl	California	1950	(Georghiou <i>et al.</i> ,1966)
MaClo	Mc	California	1984	(Duron <i>et al.</i> 2006b)

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3 **Table S1.** Name, abbreviations, countries or areas of origin, year of collection and4 bibliographic references for *Culex pipiens* strains.

ORF	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
<i>pk1</i>	CCACTACATTGCGCTATAGA	ACAGTAGAACTACACTCCTCCA	1328
<i>pk2</i>	ATTATGATAAAGCTTGGTAAGAA	TTAGCCCTTCATAAATAGCTT	450
<i>ank1</i>	TGGCACGAATTATTGAGTTTAGT	TATCAACTTGAGTGTGAGGCT	682
<i>ank2</i>	CTTCTTCTGTGAGTGTACGT	ATGCTAGTAACTCTCTGCTGT	654-852
<i>ank3a</i>	ACGACAAATGGAATATAGTGGA	ATTCCGTGCATGGTCATGCCT	937
<i>ank3b</i>	TGCTGAAGGCAAGAGATTA	AGGGTCATCACTGCCTGGT	965
<i>ank4</i>	TCTATGAATGTGGTGGTATTCA	ATTCCGTGCATGGTCATGCCT	1876
<i>ank5</i>	TGGGTCAAGATACAAAAACA	AGTAGCTTTATGATTTCCCT	478
<i>ank6</i>	ATGAAGAAGGAGAGACGATA	TTAGCTCCGGCATGTAGCT	510
<i>ank7</i>	CAGCGAGTTACAGAAAGGCA	AGTGTATTGCCGCCACATCT	1322
<i>ank8</i>	ACCTGCTTGCTGCAGAACA	TCTTTTGCATCGCAGGTACT	925
<i>ank9</i>	TCTTCGCTATTAACAGTGGCT	AGCTTTTTCCCTTAAGTGCTCA	1953
<i>ank10</i>	TGGCATATAGTGATGGTGATGT	ACTGCAAATAATGCACCAGCT	1217
<i>ank11</i>	ATTGTAGGTAGAAAGGAAGCT	TGGTATATAACACCAAGGAGA	1038
<i>ank12</i>	ACTGTGTGCGAAATATGAAA	GATTTATGCTGTTCCCTCTGCA	532
<i>ank13</i>	TGGGTCAAGATACAAAAACA	AGTAACTTTATTATTTGATC	478
<i>ank14</i>	GGTGTATTTATGAACCCTGA	TCAGCTTTCTGAGTAACTGA	1382
<i>ank15</i>	GTAGTGGAGATTATGACGCT	TCCAATCTTCCTGCAAGTGT	914
<i>ank16</i>	TGTGTGATACAAAATCGGGA	GCTTTTGATGATGCACGCCT	1092
<i>ank17</i>	GAGATTCTCTTTTAGGAGGT	ACTGATTAAGGGACTTTTGA	1192
<i>ank18</i>	TAGGAGGGTTATGGTTACAA	TGATGATATACACCTTAGATA	1179
<i>ank19</i>	TGGGTATGATGGCTCTTTGT	AGCGCAGTACCAATATCCCT	872
<i>ank20</i>	ATGAGATTATGACAAGTGATC	ACATGCAATTGCACGAGCA	1097
<i>ank21</i>	ATGGTATGGCGTGTGAGGA	TGTTAAGCTTAGAGCTAGGA	1250
<i>ank22</i>	ATAGTGGCTAAAATAGCTTT	TGCAATATTTTCGTATTGTCT	642
<i>ank23</i>	ATGATTAATGAATTATTTGCTA	AATGAAGATGGCTTACTTGT	1040
<i>ank24</i>	AGTTAGTTAATGAATTTATTGA	GTCTTAAGTCCTTGTTTGCA	1185
<i>ank25</i>	ACATGTCCACAATTTTCGTAT	AGTGGTATCCCAATCAAA	873
<i>ank26</i>	AGAGGGAAAGGTTATGGTA	ACAAGCATCATGTTGCACT	1306
<i>ank27</i>	ATGAATTATTCAATCAGAAATA	ATGTCCATTATCAGCAGCTT	1728
<i>ank28</i>	GAGGTAAAGATGAATTTTGA	AGAGCATTATGTATACGTAT	1160
<i>ank29</i>	GATTAGGGTAAAAATTATGACA	TAACCCTACTAGTGCTAGC	902
<i>ank30</i>	AGGTATTTTATGTATCAAGA	ACAAGTAACTAGTAAGTCT	546
<i>ank31</i>	TTAGAGACACTAAAGGCACT	TCAACATATGTGATTCTATGTG	1492
<i>ank32</i>	GAATTATGGAATATGAACGGT	TTCATTAGGATCAGCACCT	1136
<i>ank33</i>	TGTACATATTAAGTTGCATGA	CTTAATGAACTTTCAGGACA	1089
<i>ank34</i>	CGCAAGATGAAGGTCTCGA	AGGCATTTTACCCTCCTCA	806
<i>ank35</i>	AAGATTCTATGCAACCTGCT	TTGCTTCTTTCATCTCCT	1358
<i>ank36</i>	AGGTAAATATGTTTCAGCACT	CTGCTGAACAGATACGTCA	1064
<i>ank37</i>	TTGTCAAGATGGCAATTGGT	CTTCTTGACTTTCATGTCT	1241
<i>ank38</i>	TGCTTGCTGAAAGTGGTGA	TCAACACTTTCATCATGGGT	991
<i>ank39a</i>	AAAGTTGAACATTCATCGGA	ACTCATTCAAGCCTTGAGCT	700
<i>ank39b</i>	ACACTTGAAGTCTCAGGCA	TCTTTCCTTCTTCTCGCT	1202

<i>ank40</i>	ACTGAATATGAGTCAACTGA	GATTTATGCTGTTCCCTCTGCA	519
<i>ank41</i>	AACTGGCGGAGATCTTGGA	TGTTCTTTCTCTCGTGACCT	708
<i>ank42</i>	TCAAGCACAGATGACCAGGA	TGTCTCTTGCTCAGTTACCA	870
<i>ank43</i>	GTTAACGGCCTCTCTGAGA	TGCAGCCTCTCAGATTGCT	886
<i>ank44</i>	CTAGAAAATGCGGCTCTGA	AATGCAGAAGTACCATCCT	1216
<i>ank45</i>	TTTCGAGAAGTAATGTAGGT	GCTACAGGTTGCTCTGCACT	985
<i>ank46</i>	AGTAATAACGAGGAACATCA	TGACACCTTACCTGAACAGA	695
<i>ank47</i>	ATATGTGGTACTCAGGAGA	TTACTGGCCTGTCCTTTCT	862
<i>ank48</i>	TAGAATACTCAATCTATGCT	ACGGTCGCTGATGCTGCA	667
<i>ank49</i>	AAGGATAGAAATATCACGA	TAACCTTGACGCTTGACCA	748
<i>ank50</i>	AGGAGAGTTTGATATGTATT	TCTGACCAGGACTTGACT	680
<i>ank51</i>	AGCGAACAAGACAGTGGCT	TGTGCTTTCTGTGGAGTGCT	1103
<i>ank52</i>	TCGTCTGTAGGAAAGGGATA	AGCCACTCCAAGTCTATTC	653
<i>ank53</i>	CTGCTCGGAGTGAATCAGGT	GAGCCGAAGGCTGCTAAGCA	835
<i>ank54</i>	AGGACAGATTCACAAGCAGT	TCCGCCAACTTCCTTCTGCT	841
<i>ank55</i>	GTAACTGCGTTACATTATG	GGTTTTTCCATCATCGTTTATA	204
<i>ank56</i>	TAAGAGGCGTGAGGCTAGCT	ATGCTATTTTGTATTGAAGGA	252

Real-time quantitative PCR

<i>pk1</i>	ATGCACGAAATCAGACACC	ACCAGAGGGTCTATATCTTC	322
<i>pk2</i>	TACATTATGCAGTAGACGCA	TTAGCCCTTCATAAATAGCTT	377
<i>ank2</i>	AGGCAATGATTGCTGGTGGA	GTAGCACAACCAACTACCAG	151
<i>G6PDH</i>	GCGGCGGGACTTTGAG	AATCCTGTTCCACCCCTTCA	160
<i>wsp</i>	AGAATTGACGGCATTGAATA	CGTCGTTTTTGTATTAGTTGTG	151

5

6 **Table S2.** List of the amplimers used to specifically amplify open reading frames (ORFs)  
7 containing ANK domains. Amplimers used in real-time quantitative PCR, including those of  
8 *G6PDH* and *wsp* genes, are also indicated. For each ORF are indicated forward and reverse  
9 primers and sizes of the resulting PCR products.

Female strain	Male strain														
	Ep-A	Ep-B	Lv	Bf-A	Bf-B	Ko	Tn	Is	Ke-A	Ke-B	Ka-C	Ma-A	Au	Sl	Mc
Ep-A	1 (21)	1 (3)	1 (11)	1 (16)	1 (9)	1 (8)	1 (10); 2 (2)	<b>3 (27)</b>	1 (10)	1 (24)	1 (19)	1 (9)	1 (9)	1 (9)	1 (13)
Ep-B	1 (15)	1 (17)	1 (7)	<b>3 (26)</b>	1 (3)	1 (5); 2(2)	1 (30)	<b>3 (22)</b>	1 (3)	1 (6)	1 (12)	1 (11)	1 (4)	1 (7)	1 (10)
Lv	<b>3 (11)</b>	1 (17)	1 (8)	1 (10)	<b>3 (17)</b>	1 (17)	<b>3 (21)</b>	<b>3 (26)</b>	1 (19)	1 (13)	1 (4)	1 (9)	1 (20)	1 (8)	1 (10)
Bf-A	1 (21)	1 (14)	1 (21)	1 (14)	1 (18)	1 (21)	1 (16)	<b>3 (15)</b>	1 (5)	1 (26)	1 (20)	1 (28)	1 (35)	1 (12)	1 (14)
Bf-B	1 (28)	1 (29)	1 (18)	<b>3 (31)</b>	1 (13)	2 (12)	1 (17)	<b>3 (27)</b>	1 (17)	<b>3 (28)</b>	1 (25)	1 (25)	1 (12)	1 (15)	1 (9)
Ko	1 (12)	1 (31)	1 (19)	1 (11)	1(11)	1 (18)	1 (15)	<b>3 (50)</b>	1 (13)	1 (13)	1 (7)	1 (22)	1 (12)	1 (30)	1 (24)
Tn	1 (20)	1 (23)	1 (35)	1 (27)	1 (20)	1 (33)	1 (55)	<b>3 (35)</b>	1 (15)	1 (17)	1 (24)	1 (20)	1 (13)	1 (66)	1 (54)
Is	<b>3 (18)</b>	<b>3 (21)</b>	<b>3 (40)</b>	<b>3 (5)</b>	<b>3 (11)</b>	<b>3 (26)</b>	<b>3 (30)</b>	1 (26)	<b>3 (12)</b>	1 (5)	<b>3 (7)</b>	<b>3 (5)</b>	1 (11)	<b>3 (34)</b>	1 (31)
Ke-A	1 (8)	1 (31); 2 (4)	1 (15)	1 (15)	1 (11)	1 (17)	1 (6)	<b>3 (15)</b>	1 (13)	1 (15)	1 (23)	1 (22)	1 (3)	1 (5)	1 (9)
Ke-B	1 (7)	1 (18)	1 (14)	1 (9)	1 (11)	1 (9)	1 (17)	<b>3 (10)</b>	1 (8)	1 (8)	1 (7)	1 (24)	1 (21)	1 (12)	1 (23)
Ka-C	1 (15)	1 (14)	1 (9)	1 (10)	1 (11)	1 (9)	1 (11)	<b>3 (7)</b>	1 (6)	<b>3 (23)</b>	1 (6)	1 (8)	1 (13)	1 (14)	1 (20)
Ma-A	1 (10)	1 (11)	1 (9)	1 (13)	1 (6)	1 (6)	1 (5)	<b>3 (11)</b>	1 (12)	<b>3 (17)</b>	1 (6)	1 (27)	1 (13)	1 (4)	1 (8)
Au	<b>3 (12)</b>	<b>3 (16)</b>	1 (23)	1 (5)	<b>3 (14)</b>	1 (11)	<b>3 (10)</b>	<b>3 (7)</b>	1 (11)	1 (18)	1 (7)	1 (18)	1 (6)	1 (12)	1 (9)
Sl	1 (9)	1 (33)	<b>3 (30)</b>	1 (18)	<b>3 (27)</b>	1 (39)	<b>3 (31)</b>	1 (33)	1 (14)	1 (19)	1 (23)	1 (26)	<b>3 (34)</b>	1 (14)	<b>3 (99)</b>
Mc	1 (16); 2 (4)	1 (15)	1 (36)	<b>3 (14)</b>	1 (11)	1 (9)	1 (19)	<b>3 (53)</b>	1 (17)	1 (9)	1 (4)	1 (16)	1 (9)	1 (43)	1 (44)

11

12 **Table S3.** Crossing relationships between *Culex pipiens* strains. Crosses were characterized by hatching rates (HR), indicative of CI levels. HR  
13 was classified into 3 categories: 1,  $HR \geq 70\%$  (compatible); 2,  $70\% > HR \geq 30\%$  (intermediate); 3,  $HR < 30\%$  (incompatible). Uni- and  
14 bidirectional incompatible crosses are shaded in gray and black, respectively. The number of egg-rafts collected is specified into brackets.

15 **Figure S1:** Alignment and schematic structure of alleles sequences of *Wolbachia* ANK proteins  
16 PK1 (A), PK2 (B), and Ank2 (C). Dashes correspond to identical amino-acid residues. Stars  
17 correspond to deletions. ANK domains are indicated by arrows above. The location of ANK motifs  
18 was determined using SMART v3.5 (<http://smart.embl-heidelberg.de/>; Schultz *et al.* 1998; Letunic  
19 *et al.* 2004). Transmembrane domains are represented by highlighted in alignment and black boxes  
20 in schematic structure. The distribution of alleles in strains is indicated.

# A

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<-----> <-----> <----->
PK1a PLHCAIEFDELSMVDLLLTCKKNINPFVEDNDGKTSLDYAKEGKKAAILKALINNKYGSEQDSLHLAAMVGEVN
PK1b -----E-----Q-----N-----I-----
PK1c -----E-----Q-----N-----I-----
PK1d -----
PK1e -----

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-----> <-----> <----->
PK1a AVRYLINRGIDVNSRNAIFHTPLHLAAGIGHVEVVKILVREGNAEIEVFDARNQTPMHYAVNKKLEIVKLLLE
PK1b -----GK-V--V--LH-----EN-----K-----D-----K
PK1c -----GK-V--V--LH-----EN-----K-----D-----K
PK1d -----
PK1e -----

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-----> <-----> <----->
PK1a LGADVNSARVQNSMKLSPIHIAVSNTNYDERDLCLDILKCLIREPNAQVNLQDYENKTPHLHYAERLKTIEVLL
PK1b -----I-----V-----K-----
PK1c -----M-----V-----K-----R-----
PK1d -----K-----R-----
PK1e -----

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-----> <-----> <----->
PK1a TREDIDPLVKDDSGKTPFDYAKPEIKKALISNKYGSEKNSLLHLAAQRGETELVESILKEEIDIDISNNKSLSP
PK1b -----DA-----N-----
PK1c -----M-----I-DA-----V--G--
PK1d -----M-----I-DA-----V--G--
PK1e -----

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-----><----->
PK1a IYLAAEKGHLHVVKLLKKGANYTPVLHLAIKSNLELLKVLFNKNGALLCRDVTVVNFPTLHNKYIAQREIAD
PK1b -----T--K--SIK-**P-E-GRSVP IYYRCIEH-***-
PK1c -----
PK1d -----
PK1e -----T--K--SIK-**P-E-GRSVP IYYRCIEH-***-

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PK1a KRMKKHNNIICICITVSAVAMAAYIGLTAATISSAII FATITGIFALVVAIMVSEMKNRYIEKEFQKKMFMELE
PK1b AKVR-Y-SV--VFST-----T--N-----A-----S-----**
PK1c -----
PK1d -----
PK1e AKVR-Y-SA--VFSTV---I-V-----T--S-----T-----III-L-I--SR---NK-----**

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```

PK1a ECSSTV
PK1b -----
PK1c -----
PK1d -----
PK1e -----

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PK1a (Bf-A, Ko, Ep-A, Ep-B, Tn), PK1d (Is), PK1e (Ka-C, Ma-A)



PK1b (Bf-B, Mc, Sl), PK1c (Au, Ke-A, Ke-B, Lv)

# B

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                <-----> <----->
PK2a  MIKLGKIEKSLLESSFKKKETKRELHYAVDAKTVRLLEKGANVNAKDVEGYTALHLAVTEKRLEIVRELIKSG
PK2b  -----
PK2c  -----V-----V-----A-G-----
Pk2d  -----

-----> <----->
PK2a  GNVNAEEYGNKCTPLHLACMIGEKEIVKELVKAGGEIEQADKFGMTAMDYAKNSKEITEILKKEIDRIEKLFMK
PK2b  -----A-----G-----V-----T-----
PK2c  A-----I-----E-----A-----G-----V-----T-----
Pk2d  A-----I-----E-----A-----G-----V-----T-----

```



PK2a (Au, Bf-A, Is, Ka-C, Ke-A, Ke-B, Ko, Lv, Ma-C, Tn)  
 PK2b (Mc, Sl)  
 PK2c (Bf-B)  
 PK2D (Ep-A, Ep-B)

# C

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      <-----> <-----> /-----
Ank2b FFCECTLLTIAAENGHASVVEVLLKAEANVNAVDSNKWFTPLHVAAENGHASVVEVLLKAKANVNAVSGGHAS
Ank2c -----
Ank2d -----
Ank2e -----D-----
Ank2a -----E-----

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      -----> <-----> <----->
Ank2b VVEVLLKAKANVNAVSGSEGWTPLHVAAENGHASVVEVLLKAEANVNAVGI EGCTPLHFAAGNGHVDIVNLLLE
Ank2c -----E-----I--C-----
Ank2d *****
Ank2e *****NKWF-----
Ank2a *****

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      -----> <----->
Ank2b KGANVNAVDRYGKTPLDYAEGYAKNQDVVKALLDARGGSFVKARNKAMIAGGVNTLLGTAIAVALFTTGTITA
Ank2c -----
Ank2d -----
Ank2e -----
Ank2a -----

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Ank2b QLIPIVIAVVAVTAAALVVGATYELLKPSTKVDGAEKPIVNSINEQQRVTS
Ank2c -----
Ank2d -----
Ank2e -----
Ank2a -----

```



ANK2b (Bf-B, Mc, Sl)  
ANK2c (Is)



ANK2d (Ka-C, Ma-A)  
ANK2e (Au, Ke-A, Ke-B, Lv)



ANK2a (Bf-A, Ep-A, Ep-B, Ko, Tn)