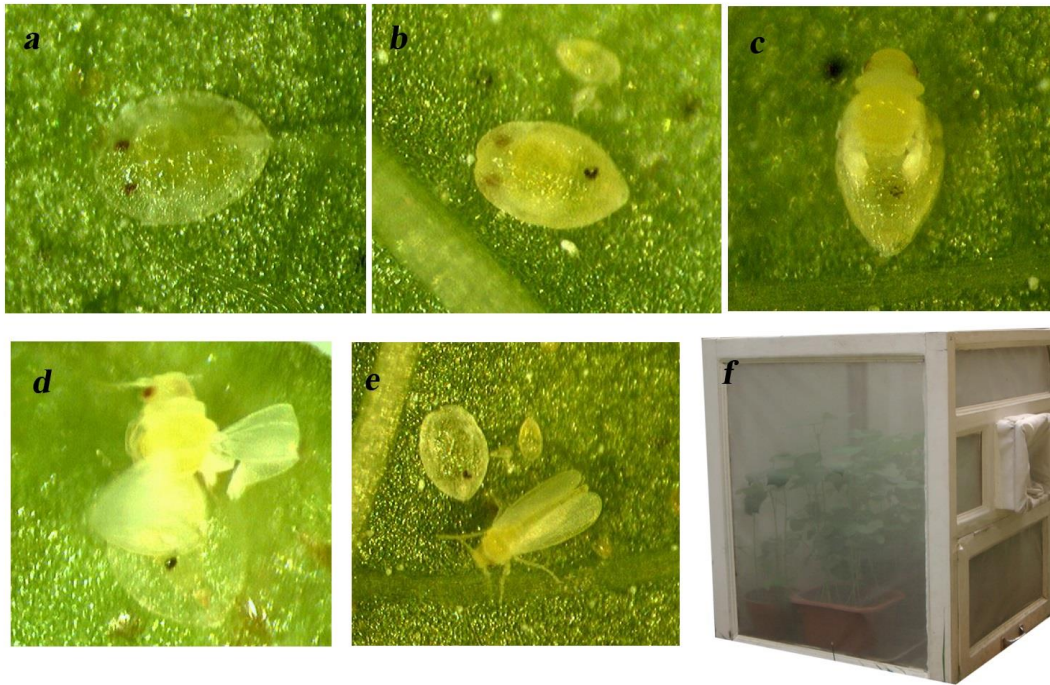


1 **Establishment of the cytoplasmic incompatibility-inducing *Wolbachia***
2 **strain wMel in an important agricultural pest insect**

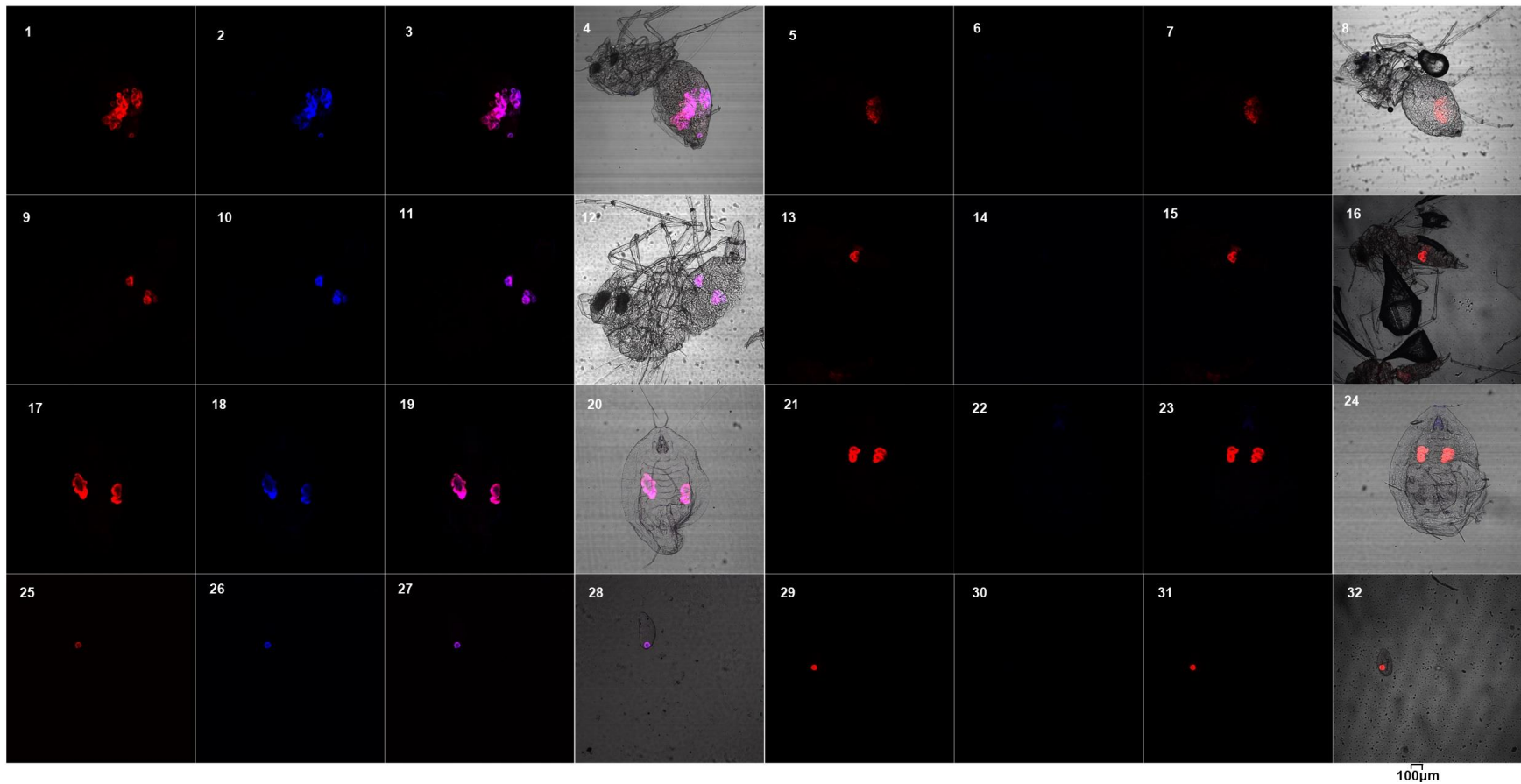
3 Xiao-Fei Zhou, Zheng-Xi Li*

4 Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road,
5 Beijing 100193, China

6 *Author for correspondence: Dr. Zheng-Xi Li, Department of Entomology, China
7 Agricultural University, 2 Yuanmingyuan West Road, Beijing 100193, China. Tel: +86 10
8 62732539; Fax: +86 10 62732539; E-mail: zxli@cau.edu.cn.

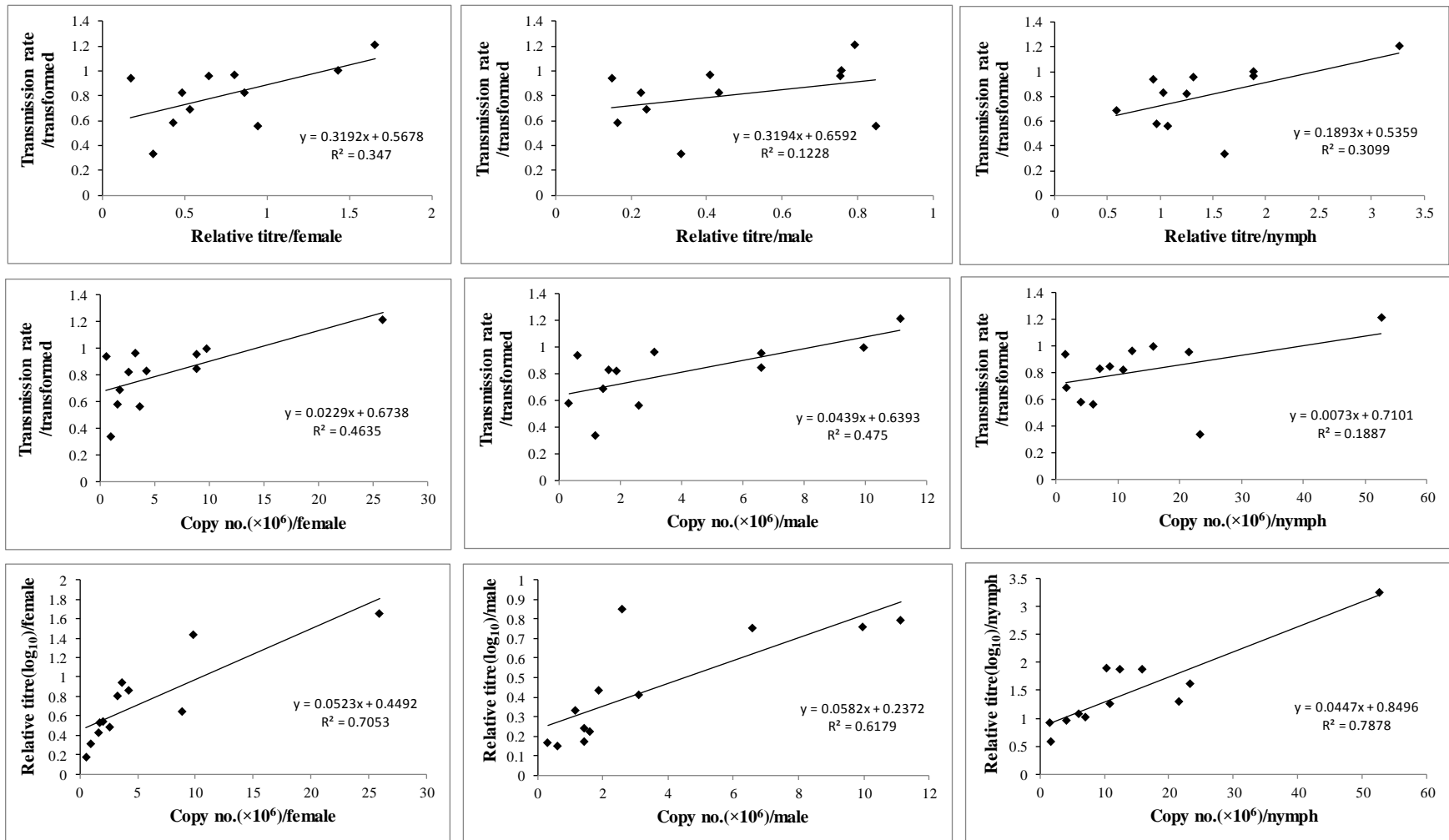


Supplementary Figure S1. Microinjection of *wMel* strain and establishment of isofemale lines. a, the 4th instar nymph (pseudopupa); b, 24h after injection; c, 48h after injection; d, 72h after injection (eclosion); e, G₀ adult; f, caged population of *wMel*-transfected *B. tabaci*.



Supplementary Figure S2. FISH analysis of *wMel*-transfected and wild-type *B. tabaci* at different developmental stages by using *Portiera*-specific probe (red) and *Wolbachia*-specific probe (blue). 1-4: Transfected female adult; 5-8: Wild-type female adult; 9-12: Transfected male adult; 13-16: Wild-type male adult; 17-20: Transfected nymph; 21-24: Wild-type nymph; 25-28: Transfected egg;

29-32: Wild-type egg. 1, 5, 9, 13, 17, 21, 25 and 29, *Portiera* channel only; 2, 6, 10, 14, 18, 22, 26 and 30, *Wolbachia* channel only; 3, 7, 11, 15, 19, 23, 27 and 31, merged images showing overlap of *Wolbachia* and *Portiera* channels in dark field; 4, 8, 12, 16, 20, 24, 28 and 32, merged images showing overlap of *Wolbachia* and *Portiera* channels in bright field.



Supplementary Figure S3. Linear regression analysis of the correlation between copy number of *wsp* gene, relative titre and transmission rate of *wMel*. Transmission rates (%) are transformed by arcsine square root before analysis. a-c, correlation between

relative titre of *wMel* in female adult (a), male adult (b) and nymph (c) and transmission rate; d-f, correlation between copy number of *wsp* gene of *wMel* in female adult (d), male adult (e) and nymph (f) and transmission rate; g-i, correlation between copy number of *wsp* gene and relative titre of *wMel* in female adult (g), male adult (h) and nymph (i).

Supplementary Table S1. MLST and *wsp* sequences and PubMLST query results

Gene name	Fragment size (bp)	Allele sequence (5'-->3') ^a	Allelic type ^b
<i>hcpA</i>	515	<u>GAAATAGCAGTTGCTGCAAAGCAAGGGCTGCCCGATCCCGAACTCAACCCGCGCCTTCGCTCTGCTATATTTGC</u> TGCACGCAAGGAAAATCTACCAAAAAGATAAAAATAGAAACAGCAATAAAAAATGCAACTGGTAACGTTGCTGGAG AAAATTACGAGGAAATCCAATATGAAGGTCATGGGCCTTCTGGCACTGCACCTATTGTCCATGTTTTGACTAAT AATCGCAACCGAACTGCTTCTGAGGTACGTTATATATTTTTCTCGCAAGGGTGGAACTTGGGAGAAACAGGAAG TGTTAGTTACCTTTTCGATCATGTAGGCTTAATCGTCTATAAAGCAGAGGGTGTGAATTTTGATGATTTATTCA GTCATGGAATCGAATTAGAAGTATTGAATGTTGAGGAAAATGACAAAGAAGGATTACACGTTATAACTTGTGAA ATAAAAAGATTTTGGTAAAGTACGCGATGCCTTTTATGCAAAATTCGGAGAACCAGAACTTGCTCAACTTTC	hcpA:1
<i>fbpA</i>	509	<u>GCTGCTCCGCTTGGCATGATTGAAGCTGGTGTGCAACTTATGCTGGAAATGCTCCCACTTATTTTGAACCTTAA</u> TAGTTCCAACCTCTTTACATTCAAAGGATCTAACCTCTGATCAGGCAATAACCTCTTCTGTGAAAGATGCGCTGC GTTTGGGATGCTTAGCTGTGCGGATTTACTATATATCCTGGTTCTGCTAAGTGTTTCGATATGATGGAGGAAGCC CGTGGAATCATAGCTGAAGCCAAATCTTATGGACTTGCAGTAGTGCTATGGTCTTATCCACGCGGTGAAGGGAT TTCCAAAGAAGGTGAAACAGCAGTTGATGTTATTGCCTATGCTGCGCACATGGCAGCTTTGCTTGGCGCTAATA TAATAAAAGTAAAACCTTCCAATAAATATTTGGAAAGGGAGAAAATAGAAACAGAAAATATTGAATCATTATCT AAAAGAATTGAATATGTTAAAAGGTCTTGTTTTGCAGGGAAAAGAATAGTGATTTTCTCTGGCGG	fbpA:1
<i>gatB</i>	471	<u>GATTTAAACCGTGCAGGGGTTGCTTTAATGGAAATTGTTTTCAGAACCAGATCTCCGTTTCATCTGCGGAAGCTGC</u> AGAATGCATGAAAAAATTGAGGCAGATTTTGC GTTACATTGGTTCTGTGATGGTGATATGGAAAAGGGATCAC TTCGTTGTGATGCAAATGTTTTCTGTCCGCCTAAAAGGCAGTAGTACATTTGGCACTCGTTGTGAAATAAAAA ATCTGAACTCGATACGTTATATTGTGCAAGCTATAGACTATGAAATACAAAGACAAATTGAAATTTTAGAAAGT GGAGAAGAAATAAGTCAAGATACCTTATTGTTTGACGTTGCTTCCGGGAAAAACAAAAGTGATGAGAAGCAAAGA GAATGCAAGCGATTATAGATACTTCCCTGAGCCTGATTTATTACCTGTTGAGGTAAGCCAGGATAAAAATTGA TTTAATTCATCATCTTTGCCTGAGTTGCCA	gatB:1
<i>ftsZ</i>	524	<u>ATTATGGAGCATATAAAGGATAGTCATATGCTTTTCATCACAGCAGGAATGGGCGGTGGTACTGGAACCGGTGC</u> AGCACCGTAATTGCAAAAGCAGCCAGAGAAGCAAGAGCCGCAGTTAAGGATAGAGCGCCAAAAGAAAAAAGA TATTGACTGTTGGAGTTGTAACCTAAACCGTTCCGTTTTGAAGGTGTGCGCCGATGCGCATTGCAGAGCTTGGA CTTGAAGAAGTCAAAAATACGTGGATACACTTATTGTCAATCCAAATCAGAATTTATTTAGAATTGCAAATGA AAAAACTACATTTTCTGATGCATTTAACTTGCTGATAATGTTCTGCACATTGGCATCAGAGGAGTAACTGACT TGATGGTCATGCCAGGGCTTATTAATCTTGACTTCGCTGATATAGAAACAGTAATGAGCGAGATGGGCAAAGCG ATGATCGGCACCGGAGAGGCAGAAGGAGAAGATAGAGCAATTAGTGCTGCAGAGGCTGCAATATCCAATCCATT GCTCGA	ftsZ:1

<i>coxA</i>	487	<p>TTGGGGCAATCAACTTTTATAGTTACTATATTTAACATGCGCACAAAAGGAATGTCATTAACATAAGATGCCACTG TTTGTTTGGTCTGTCTTGCTAACAGCATTATGTTGATTGTTGCCTTACCAGTGCTTGCCGGTGCTATAACTAT GCTTCTTACTGATCGCAATATTGGTACTTCCTTTTTTGGATCCTGCCGGTGGCGGCATCCTGTGTTATTTCAAC ATCTATTTTGGTTTTTTGGTCATCCAGAAGTTTACGTAATTATTTTTCCTGCATTTGGCATCATAAGTCAGGTT GTATCAACTTTTTCTCACAGACCTGTATTTGGTTACATAGGGATGGTTTATGCAATGATAGGTATAGCAGTATT TGGCTTTATGGTTTTGGGCTCACCATATGTTCACTGTTGGGCTTAGTGCTGACGCTGCTGCATTTTTTAGCACTA CCACAATTTTTATCGGTGTTATAACTGGCGTCAAAGTCTTTAG</p>	<i>coxA</i> :1
<i>wsp</i>	595	<p>TGGTCCAATAAGTGATGAAGAACTAGCTACTACGTTTCGTTTTGCAATACAACGGTGAATTTTTACCTCTTTTCA CAAAAAGTTGATGGTATTACCTATAAAGAAAGACAAGAGTGATTACAGTCCATTAAAACCATCTTTTATAGCTGGT GGTGGTGCATTTGGTTACAAAATGGACGACATCAGGGTTGATGTTGAAGGAGTTTATTCATACCTAAACAAAA TGATGTTAAAGATGTAACATTTGACCCAGCAAATACTATTGCAGACAGTGTAACAGCAATTTAGGATTAGTGA ACGTGTATTACGATATAGCAATTGAAGATATGCCTATCACTCCATACATTGGTGTGGTGTGGTGCAGCGTAT ATTAGCACTCCTTTGGAACCCGCTGTGAATGATCAAAAAAGTAAATTTGGTTTTGCTGGTCAAGTAAAAGCTGG TGTTAGTTATGATGTAACCTCCAGAAGTCAAACCTTTATGCTGGAGCTCGTTATTTCCGGTCTTATGGTGCTAATT TTGATGGAAAAAACAAGATCCTAAAAATCAACCGGACAGGCTGCTGATGCAGGCGCATACAAAGTTCCTTTAC AGCASTGTTGGTGCAGAAGCTGGAGTAGCGTTTAAATTTTT</p>	HVR1:1 HVR2:12 HVR3:21 HVR4:24

^a The underlined bases are sequences of sense and antisense primers.

^b The allelic profile for *gatB*:1, *coxA*:1, *hcpA*:1, *ftsZ*:1 and *fbpA*:1 is corresponding to ST-1; the HVR profile for HVR1:1, HVR2:12, HVR3:21 and HVR4:24 is corresponding to ST-31.