SUPPLEMENTARY METHODS

Screening for and sequencing IS elements in SOPE and SZPE. We amplified IS903 in SZPE using a single primer, IS903IR, homologous to the 18 bp inverted repeats flanking the element (1055 bp amplicon), and we amplified IS256 in SOPE using primers IS256F1 and IS256R1 (1262 bp) (Table S1). The 10 μL PCRs contained 50-100 ng template DNA, 0.4 μM of each primer (0.8 μM of the single IS903IR primer), 1 mM dNTPs, 2.5 mM $Mg(OAc)_2$, and 0.5 U *Taq* DNA polymerase (Eppendorf, Westbury, NY) in 1× PCR buffer. We extracted DNA from ~25 dissected *S. oryzae* (for SOPE) and *S. zeamais* (for SZPE) larval bacteriomes using a DNeasy Tissue Kit (Qiagen, Valencia, CA). These weevils were from single lab-cultured populations of each species. The PCR profile was 94°C for 2 min; 35 cycles of 94°C for 30 sec, 54°C for 30 sec (60°C for IS256), 72°C for 1 min; 72°C for 5 min. We cloned these PCR products into a pCR 2.1 TOPO TA vector (Invitrogen, Carlsbad, CA), and fully sequenced at least three recombinant clones of each at the Genomic Analysis and Technology Core Facility at the University of Arizona.

Quantifying IS256- and IS903-like elements in SOPE and SZPE. We used quantitative PCR (qPCR) on a LightCycler (Roche Applied Science, Indianapolis, IN) to estimate the IS256 and IS903 genomic copy numbers in SOPE and SZPE. We used primers IS256F1 and IS256R2 (1223 bp) and IS903IR to quantify the number of full-length IS256 and IS903 elements, respectively, relative to the single copy chromosomal gene *murA*, using primers *murA*F4 and *murA*R3 (131 bp) (Table S1). Each 20 μL qPCR contained 10 ng template DNA and 0.5 μM of each primer (1 μM of the IS903IR primer) in 1× QuantiTect SYBR Green PCR Master mix (Qiagen). The PCR profile was 95°C for 15 min; 40 cycles of 94°C for 5 sec, 57°C for 15 sec (54°C and 60°C for the IS903 and *murA* reactions, respectively), 72°C for 90 sec (75

sec and 5 sec for the IS903 and *murA* reactions, respectively); followed immediately by a melting curve analysis of 95°C for 0 sec, 60°C for 15 sec, 95°C for 0 sec with a 0.2°C/sec transition rate. We generated standard curves for each amplicon (after 5), and ran each SOPE and SZPE reaction in triplicate (except SOPE IS256, which we ran duplicate reactions on two independent DNA extractions). For each IS element in each species, we calculated all nine genomic quantity estimates (three replicate IS estimates divided by three single copy chromosomal gene estimates; we calculated four genomic quantity estimates for SOPE IS256: two replicate estimates for each of two DNA extractions), and we present the median and range genomic quantity for each.

We confirmed the IS256 genomic estimates using a different qPCR primer pair, IS256F3 and IS256R3 (98 bp), and a different single copy chromosomal gene, *dnaN*, using primers *dnaN*F1 and *dnaN*R1 (153 bp) (Table S1). Each 20 μL qPCR contained 10 ng template DNA and 1.0 μM of each primer in 1× LightCycler FastStart DNA Master^{PLUS} SYBR Green I mix (Roche). The PCR profile was 95°C for 10 min; 14 cycles of 94°C for 5 sec, 68°C (–1°C per cycle) for 15 sec, 72°C for 5 sec; 26 cycles of 94°C for 5 sec, 55°C for 15 sec, 72°C for 5 sec, followed immediately by a melting curve analysis as above. We generated standard curves, ran replicate reactions, and calculated the median and range genomic quantities as above.

We also performed Southern hybridizations to confirm the IS256 and IS903 genomic quantities in SOPE and SZPE (7). Total genomic DNA (0.34 μg per lane) was digested with *Eco*RV for 12 hrs (following the protocol in Sambrook and Russell (7), pg. 6.40 to ensure complete digestion), and electrophoresed on a 1% agarose gel before being transferred to a Duralon-UV nylon membrane (Stratagene, La Jolla, CA). We produced digoxigenin-11-dUTP labeled probes with a PCR DIG Probe Synthesis kit (Roche), using primers IS256F3 and

IS256R3 to make a 98 bp IS256 probe, and IS903F1 and IS903R1 to make an 85 bp IS903 probe (Table S1). Hybridization was performed in DIG Easy Hyb solution (Roche) at 38°C overnight (following the manufacturer's recommendations), followed by two 5 min washes in 2× SSC, 1% SDS at room temperature, and three 15 min washes in 0.1× SSC, 0.1% SDS at 65°C. Hybridization signals were detected by a chemiluminescent reaction using CDP-Star (Roche) as substrate. Images were obtained from 15 min and 10 min exposures for IS256 and IS903, respectively.

Screening for *Wolbachia* in rice and maize weevils. Some *S. oryzae* populations harbor *Wolbachia* endosymbionts in addition to SOPE (1). Because IS elements are prevalent in some *Wolbachia* (8), its presence could skew our IS quantity estimates in SOPE and SZPE. Therefore, we screened for *Wolbachia* in our *S. oryzae* and *S. zeamais* lab cultures using the general *Wolbachia* primers *wsp*81F and *wsp*691R (9). The 20 μL PCRs contained 75-150 ng template DNA, 0.25 μM of each primer, 0.2 mM dNTPs, and 1.5 U *Taq* DNA polymerase in 1× PCR buffer. The PCR profile was 94°C for 2 min; 11 cycles of 94°C for 45 sec, 65°C (–1°C per cycle) for 45 sec, 72°C for 1 min; 24 cycles of 94°C for 45 sec, 55°C for 45 sec, 72°C for 1 min; 72°C for 5 min. As a positive control, we used DNA extracted from insects known to contain *Wolbachia* (Glassy-Winged Sharpshooters, *Homalodisca coagulata*).

Screening for IS256 and IS903 in rice and maize weevil genomes. Because bacterial endosymbiont genes can be transferred to the host genome (2), any IS256 or IS903 in the host would distort our IS estimates in SOPE and SZPE. Therefore, we screened for these IS elements in pure weevil DNA extractions (i.e., extracted from the head) from two and six *S. oryzae* and *S. zeamais* individuals, respectively. We confirmed that these DNA extractions all had PCR-quality DNA by amplifying part of the weevil's internal transcribed spacer region of the nuclear rDNA

using species-specific primers (see ref. 4). We then screened for IS256 and IS903 in these DNA templates using primers IS256F1 and IS256R1, and IS903IR as above (see "Screening for and sequencing IS elements in SOPE and SZPE"), and we used SZPE bacteriome DNA as a positive control in these PCRs.

Screening for and quantifying extrachromosomal IS256 circles. To assess whether IS256 forms extrachromosomal circles during transposition in SOPE and SZPE, we performed PCRs with outward-facing primers, IS256F4 and IS256R3 (Table S1), using total genomic DNA from dissected bacteriomes as template. PCRs were done as above, with a 63°C annealing temperature. We electrophoresed the PCR products on a 1% agarose gel, and gel-extracted a band from the SOPE and SZPE reactions corresponding to the predicted size for a circularized molecule. We cloned each gel-extracted PCR product and sequenced 13 and eight recombinant SOPE and SZPE clones, respectively, as above.

We quantified the number of putative extrachromosomal IS256 circles per SOPE and SZPE chromosome with qPCR, using the outward-facing IS256F4 and IS256R3 primers and the single copy chromosomal primers *dnaN*F1 and *dnaN*R1 (Table S1). These qPCR reaction conditions were identical to the second IS256 qPCRs above (i.e., using the Roche SYBR mix). We generated standard curves as above, and we ran SOPE and SZPE reactions in triplicate and calculated the median and range extrachromosomal IS256 quantities as above.

We also did PCRs to detect extrachromosomal IS256 circles that contain two IS256 elements facing in opposite directions (e.g., 6). To exclude the possibility of amplifying neighboring chromosomal elements, we first isolated extrachromosomal DNA from the dissected bacteriomes from each of two larval weevils using a PureLink Quick Plasmid Miniprep Kit (Invitrogen). We treated these minipreps with exonucleases III and VII (Amersham Biosciences,

Piscataway, NJ) to eliminate chromosomal DNA, as described by Loessner et al. (3), and we confirmed that they were free of chromosomal DNA by using these templates in PCRs with chromosomal *murA* primers. To detect double IS256 circles with opposite-facing IS256 elements, we performed PCRs with these extrachromosomal DNA templates using a single outward-facing primer, IS256F3 (Table S1), which could act as both the forward and reverse primer. The 10 μL PCRs contained 50-100 ng template DNA, 0.8 μM of the single primer, 1 mM dNTPs, 1.5 mM $Mg(OAc)_2$, and 0.5 U *Taq* DNA polymerase in 1× PCR buffer. The PCR profile was 94°C for 2 min; 3 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 3 min; 13 cycles of 94°C for 1 min, 67°C (–1°C per cycle) for 1 min, 72°C for 3 min; 20 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 3 min; 72°C for 10 min. We cloned this PCR product and sequenced the ends of three recombinant clones (using the M13F and M13R sequencing primers on the vector), as above. All clones had the same genomic region linking the two IS256 elements, so we sequenced the entire insert of only one of these clones, and used BLASTX to detect homology of open reading frames to sequences in GenBank.

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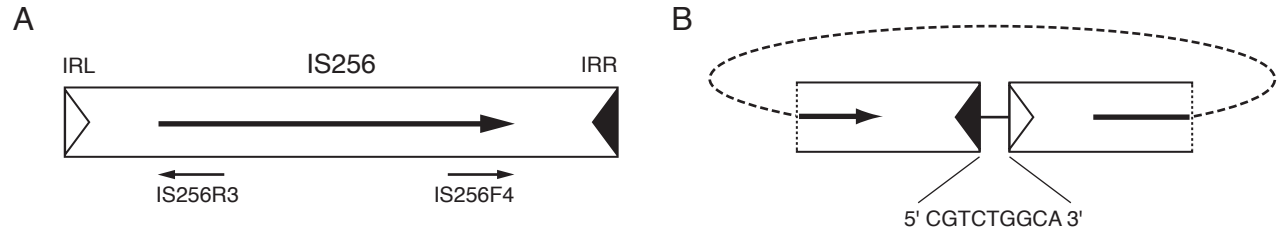
Supplementary Table 1. PCR primers used in this study.

Target gene	Primer name	Primer sequence (5'-3')
dnaN	*dnaN*F1	GCAAGATGGAAATCGGTTTC
	*dnaN*R1	TCGGCATAACGACATAGGC
IS256	IS256F1	GCCTGATTTTGATATGTTCAATCC
	IS256F3	TCGTGACGGTTCCTTTGAAC
	IS256F4	GAAAGGTTTCCCTGACGCTAT
	IS256R1	CCTTTTCTCAGAAGTGACCGTC
	IS256R2	CGATAATAAAGCGGCTCATTGC
	IS256R3	TCATCCCTTTGGCGTACAAC
IS903	IS903F1	AAAGTAGGTTATCACCGGCG
	IS903R1	CATGCAGACTCAGATGACCAC
	IS903IR	GGCTTTGTTGAATAAATC
murA	*murA*F4	TTACCGATTTTATTCGTCGCG
	*murA*R3	GAACCATTGCGTTCAACTCTG



C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SZPE 1  ··GCCGCTTTATTATCGAGTTCGGTGAACGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACACAGAATCGTGTACAGGGTCCGTCTGGCAGAGCCTGTTTAGAAA··
     2  ··GCCGCTTTATTATCGAGTTCGGTGACCGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACACAGAATC----------GAGCCTGTTTAGAAA··
     3  ··GCCGCTTTATTATCGAGTTCGGTGACCGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACA----------GAGCCTGTTTAGAAA··
     4  ··GCCGCTTTATTATCGAGTTCGGTGAACGCCTGGACGGTCACTTCTGAGAAA----------GAGCCTGTTTAGAAA··
     5  ··GCCGCTTTATTA----------GAGCCTGTTTAGAAA··
     6  ··GCCGCTTTATTATCGAGTTCGGTGACCGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACACAGAATCGTGTACAGGGTC---[83 additional bp >>]··

SOPE 1  ··GCCGCTTTATTATCGAGTTCGGTGACCGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACACAGAAT----------GAGCCTGTTTAGAAA··
     2  ··GCCGCTTTATTATCGAGTTCGGTGACCGCCTGGACGGTCACTTCTGAGAAAAGGCATTTACA----------GAGCCTGTTTAGAAA··
     3  ··GCCGCTTTATTA----------GAGCCTGTTTAGAAA··
     4  ··GC----------GAGCCTGTTTAGAAA··
     5  ··[<< 42 additional bp]----------GAGCCTGTTTAGAAA··
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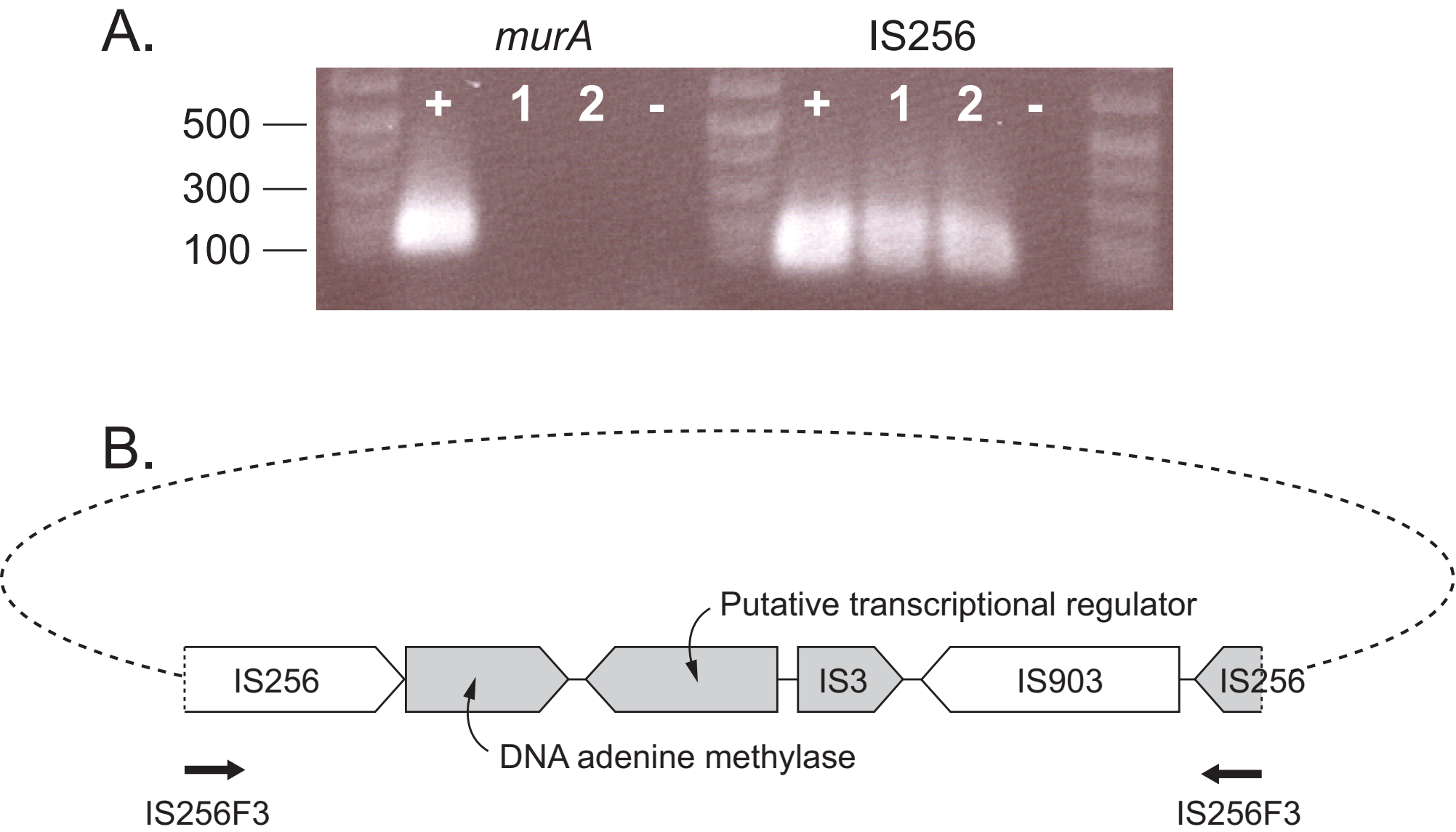
A.
murA
IS256
+ 1 2 -
+ 1 2 -
500
300
100
B.
Putative transcriptional regulator
IS256
IS3
IS903
IS256
DNA adenine methylase
IS256F3
IS256F3