## SUPPLEMENTAL METHODS

***Sitophilus zeamais* collections.** We collected small quantities of residual corn kernels from empty storage bins in IN, NE, and PA (N = 1-2 neighboring bins from a single farm in each state). These samples were heavily infested with *S. zeamais*, so we were able to extract larvae directly from this corn. Conversely, we obtained the KS sample from a full bin of corn, so the infestation was relatively light. Because extracting larvae from this corn would have been too labor intensive, we collected larvae from the $F_1$ generation of a culture initiated using field-collected adults (N ≈ 30 adults; we were unable to determine the sex ratio of these live weevils because unambiguously sexing *S. zeamais* requires genitalia dissection). For each sample, we cracked kernels and dissected bacteriomes from ≥30 larvae per population, and obtained sufficient quantities of DNA from 25, 22, 25, and 27 individuals from IN, KS, NE, and PA, respectively.

***Wolbachia* screening.** Some *S. zeamais* populations harbor facultative *Wolbachia* endosymbionts as well as obligate SZPE endosymbionts (1). Because *Wolbachia* can harbor high IS loads (4), any bacteriome-associated *Wolbachia* could obscure our SZPE IS quantity estimates. Therefore, we used PCR to screen for the presence of *Wolbachia* in all our DNA templates, using the general *Wolbachia* primers *wsp*81F and *wsp*691R (5). The PCRs were similar to Zhou et al. (1998), but the profile was 94°C for 2 min; 35 cycles of 94°C for 30 sec, 68°C-55°C touchdown (-1°C/cycle for 14 cycles, followed by 21 cycles at 55°C) for 30 sec, 72°C for 90 sec; 72°C for 5 min. As a positive control, we used DNA extracted from *Camponotus* spp. ants that were known to contain *Wolbachia.*

**IS256 and IS903 quantification**. Each 20 μL qPCR contained 10-20 ng template DNA and 0.4 μM of each primer in 1× ABsolute SYBR Green qPCR Master mix (ABgene, Epsom, UK). The qPCR profile was 95°C for 15 min; 30 cycles of 95°C for 20 sec, 68°C-59°C touchdown (-0.3°C/cycle) for 20 sec, 72°C for 20 sec (90 sec for the IS256); followed immediately by a melting curve analysis of 60°C to 95°C with a 0.2°C/sec transition rate. We ran every reaction in triplicate, and we used equation (1) from Pfaffl (ref. 2) to calculate the relative quantity of each IS element in each SZPE isolate. This equation requires calculating the amplification efficiency of each amplicon, which we did by running qPCRs on three independently derived five-fold serial dilutions of SZPE DNA. For each of these standard curves, the relationship between log DNA concentration and calculated threshold cycle was linear ($R^2 > 0.98$), and each amplification efficiency fell within the expected range of 1.6-2.1 (3). Furthermore, we eliminated all experimental templates from the analysis that fell outside the calculated linear range of any of the standard curves. We calculated the IS quantities of all SZPE isolates relative to an internal control (a concentrated SZPE purification), which were then converted to fold-difference quantities relative to the SZPE isolate with fewest chromosomal IS copies.

**Statistical analysis**. We compared IS256 and IS903 quantities among weevil populations using a Kruskall-Wallis nonparametric analysis of variance, followed by a post-hoc Scheffe's test for multiple comparisons.


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**Table S1. PCR primers used in this study.**

| Organism | Target gene | Primer name | Primer sequence (5'-3') | Reference |
|---|---|---|---|---|
| SZPE | IS256 | IS256F1 | GCCTGATTTTGATATGTTCAATCC | Plague et al. (2008) |
| | | IS256R1 | CCTTTTCTCAGAAGTGACCGTC | |
| | IS903 | IS903F1 | AAAGTAGGTTATCACCGGCG | Plague et al. (2008) |
| | | IS903R1 | CATGCAGACTCAGATGACCAC | |
| | *murA* | *murA*F4 | TTACCGATTTTATTCGTCGCG | Plague et al. (2008) |
| | | *murA*R3 | GAACCATTGCGTTCAACTCTG | |
| weevil | ITS-2 | ITS3 (forward) | GCATCGATGAAGAACGCAGC | Peng et al. (2003) |
| | | So (reverse) | CCGTTTAAACGATTTCATCC | |
| | | Sz (reverse) | CGATTGTACGAGACGGGCA | |
| *Wolbachia* | *wsp* | *wsp*81F | TGGTCCAATAAGTGATGAAGAAAC | Zhou et al. (1998) |
| | | *wsp*691R | AAAAATTAAACGCTACTCCA | |