Supporting Information

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1 CAGTTTCTTAGCCATTCCTCAAAAAAACAACCACCCCTTTAAGAAGGTTTTTCTAGTCTTTCTGGGGTTCACTGATATGCTTTTTCTGTTGGCGATAACTATG 101 M P P V R R O P R Y O O L T P F E R G R I 201 V G L R E G G M S V R E I A A R V N R G V A T V L R C I R A W E GTCGGGCTACGTGAAGGTGGCATGTCGGGTTCGGGAAATTGCAGCTCGTGTGAATCGTGGAGTGGCTACTGTCCTGCGATGTATTCGGGCATGGGAGGAAG 301 E G R E H R A R G S G R P R G T T A R Q D R Y L H F L A F R D R H V AAGGGAGAAAAATCGAGCGAGGGGTTCTGGACGGCCACGAGGTACTACCGCACGCCAGGATCGGTACCTCCACTTTTTAGCCTTTCGAGACCGGCATGT 401 S T R R I G D O W Y A A K G R P V T M A T V Y R R I R S F G L H S 501 Y R P H L V L P L T P O O R O H R L D W C R A R E N W D L E W N S 601 V F S D E S R F C L G M H D G R Q R V R R V R G E R * N V A F 701 ${\tt TGGTCTTTTCGGAT}{\tt GAATCGCGGTTTTGTTTGGGCATGCATGATGGTCGACAAAGGTTAGAAGGTACGTGGGGAACGG{\tt CGTGA}{\tt CACGTGGCCTTTTCTGT}$ E L P V A R T V G V M T W G A T A Y D S R S P L V F T E G S M T A 801 Q R Y V Q E V L E P V A V P Y V Q T I E N A S F Q Q D N A T P H CAGCGCTATGTACAGGAGGTTCTGGAACCTGTCGCAGTACCATATGTGCAAACTATTGAAAACGCGTCGTTTCAACAGGATAACGCCACGCCACACCCA 901 A R F T L R Y L E E V Q V Q V L P W P P R S P D L S P I E H I W D S 1001 I G R R V T N L P Q P P Q T L A D L R R E I L T A W E A L P Q D E 1101 AATAGGTCGGCGAGTGACGAATTTACCCCAGCCTCCACAAACGCTAGCGGACCTGCGACGCGGAAATTTTGACTGCTTGGGAGGCCCTGCCCCAAGACGA I N H L I R S M P R R V A E C I H A R G G P T H Y 1201 ATTAATCATTTAATTAGAAGTATGCCACGGAGGGTTGCAGAGTGTATACATGCACGTGGAGGGCCCAACCCATTATTAAGTTTTTTTGTTAAAATTTCTA 1301

1401 TTTTTTGATAAGTAGTA**TA**

Fig. S1. DNA sequence and conceptual translation of the Tc1-1 element in the M^1 insertion. The sequence begins and ends with the duplicated TA target motif in bold. The 35-nt inverted terminal repeats (ITRs) are underlined, with palindromic regions shaded. Authentic (TAA) and premature (TGA) stop codons are in bold. The otherwise fully intact ORF is interrupted by a single C \rightarrow T transition that converts R to the latter stop codon at nucleotide 781. The diagnostic residues of the catalytic DD34E motif are boxed.

	1 80
Tc1-1	MPPVRRQPRYQQLTPFERGRIVGLREGGMSVREIAARVNRGVATVLRCI <u>RAWEEEGREHRARGSGRPRGTTARQDRYLHF</u>
Tc1-2	
Tc1-3	
	81 <u>16</u> 0
Tc1-1	LAFRDRHVSTRRIGDQWYAAKGRPVTMATVYRRIRSFGLHSYRPHLVLPLTPQQRQHRLDWCRARENWDLEWNSVVFSDE
Tc1-2	
Tc1-3	EEE
	161 240
Tc1-1	SRFCLGMHDGRQRVRRVRGER*NVAFSVELPVARTVGVMIWGAIAYDSRSPLVFIEGSMTAQRYVQEVLEPVAVPYVQTI
Tc1-2	GLRR
Tc1-3	GLRRR
	241 320
Tc1-1	ENASFQQDNATPHSARFTLRYLEEVQVQVLPWPPRSPDLSPIEHIWDSIGRRVTNLPQPPQTLADLRREILTAWEALPQD
Tc1-2	<u>-</u>
Tc1-3	T
	321 346
Tc1-1	EINHLIRSMPRRVAECIHARGGPTHY*
Tc1-2	*
Tc1-3	*

Fig. S2. Alignment of Tc1 proteins. Tc1–1: translation of transposase gene in the *M*¹-associated *Tc1* insertion (LG3). Tc1–2 and Tc1–3 are closely related elements found in a non-*M*¹ (GA2) genome. Tc1–2: translation of transposase gene found within an intron of EST DT789639 (GLEAN_00717; LG2). Tc1–3: translation of transposase gene in the *Tc1* element corresponding to GLEAN_02110 (LG unknown). *, stop codon. Dashes indicate identity with Tc1–1. Note premature stop codon in Tc1–1 at residue 182. Boxed residues: Tc1-DD34E motif [Shao H, Tu Z (2001) Expanding the diversity of the IS630-Tc1-mariner superfamily: Discovery of a unique DD37E transposon and reclassification of the DD37D and DD39D transposons. *Genetics* 159:1103–1115]. Black underline: transposase_5 DNA-binding domain (pfam01498). Blue underline: COG3415 DNA binding domain.

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Fig. S3. Two gene fragments from the *M*¹-associated *Tc1* insertion and their functional counterparts. (*A*) An apparently functional copy of the *ATP synthase* subunit *C* (*ATPsyntC*) gene on LG10 of the non-*Medea* strain GA2 (*Upper*) and the truncated copy from the *M*¹ insertion (*Lower*). (*B*) An apparently functional copy of elongation initiation factor 3 (eIF3) on LG8 of the GA2 strain and the full-length but defective copy from the *M*¹ insertion. Thick bars represent exons, and thin bars represent introns. Diagonal cross-hatching indicates UTRs. Shaded portions of exons are deleted in the *M*¹ copy of *eIF3*. Dashed lines indicate deleted portions of the large intron in the *M*¹ copy of *ATPsyntC*.



Fig. S4. Phylogenetic analysis of DUF1703 proteins. Shown are unrooted neighbor-joining trees based on multiple alignment of complete amino acid sequences with bootstrap values for 1,000 replications indicated. Accession numbers are given for the representative 14 bacterial/archeael proteins, and GLEAN numbers are given for *Tribolium* proteins. Scale indicates distance, rate of change per amino acid sequence.



Fig. S5. M^1 -associated sequence variation flanking the *Tc1* insertion site. Homologous M^1 and non- M^1 (GA2) sequences were aligned by using AlignX. The *x* axis is the distance in nucleotides from the Tc1 insertion point. The *y* axis represents the number of nonidentical residues in each contiguous 100-nt segment. The large strain difference in the segment 200–300 nt centromeric (to the left) of the insertion point reflects the copy number difference in the tandem repeat (five copies in non- M^1 , approximately two copies in M^1).

DNA C