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Am-tra2285 C
Am-tra2253 C
Am-tra2234 C

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Figure S1 Multiple Nucleotide Sequence Alignment of the Am-tra2 cDNAs Am-tra2²⁸⁵, Am-tra2²⁵³, Am-tra2²³⁴. Black (dsRNA-2) and grey (dsRNA-1) boxes mark the region that we used to produce our dsRNAs.

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A.m.  MSDIER-SSSRASAPRRPRTADGGLRDSRSHSRSRKSRERKESHRPVKEYSRSRSRSVSRGRKSYRSSKYASAG-----HRG
D.m.  M~DREPLSSGRLHCSARYKHKRSASS-SSAGTTSSGHK-----DRRSD-----YDYCGS-----
B.m.  MSDREr-SRSRTRNGSREPVPKPAVM-SRGHSRSR-SR----TPPPPKATSR-----KYRSPMLTSGLTVDGRTHS

                                           RNP-2
A.m.  SRSRSRSRSRSTHRFARYSRSRRSYFRSRYRECDRTIYRSHSRSPMSSRRRHVGNR----ENPSPSRCLGVFGLSIFFT
D.m.  --RRHQRSSRRRSR----SRSSSESPP----PEPRHR-----SGRSSDRERMHKSR----EHPQASRCIGVFGLNTNTS
B.m.  RSRSRSGS-ARRGYR-SRHSRTRRSYS----PRGSYR--RSHSHSPMSSRRRHLDGRVRLLENPTPSRCLGVFGLSLYTT

                                           RNP-1
A.m.  EQQVHHIFSKYGPVERIQVVIDAKTGHSGKGYCFVYFESLEDAKVAKEQCAGMEIDGRMRVDYSITQRAHTPTPGIYLGKPT
D.m.  QHKVRELFNKYGPIERIQMVIDAQTQRSRGFCFIYFEKLSDARAADSCSGIEVDGRRIRVDFSITQRAHTPTPGVYLGRQP
B.m.  EQQINHHIFSKYGPVVKVQVVIDAKTGRSRGFCFVYFEDMEDAKIAKNECTGMEIDGRIRVDYSITQRAHTPTPGIYMGKPT

A.m.  H-----LHDRG---WDGPRR----RDSSYRGSYRRSPSP-YNRRRGYDRSRSR--SYSPRRY
D.m.  RG-KAPRSFSPPRRGRRVYHDRSASPYDNYRDYDRNDYDRNLRRSPSRNRYTRNR-SYSRSRSPQLRRTSSRY
B.m.  ISSRGDNGYDRRRDRDDCYRGGGGGGGYRE----RDYYHRGYRHRSPSP-HYRRTR-RYERERSY----SPRRY

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Figure S2 Multiple Sequence Alignment of the Tra2 proteins of *Apis mellifera* (*A.m.*), *Drosophila melanogaster* (*D.m.*) and *Bombyx mori* (*B.m.*). The RNA binding domain (RBD) is highlighted in black, and the linker region is highlighted in dark grey. The arginine-serine rich motifs (RS1 and RS2) are shown in light grey. The RNP-1 and RNP-2 sequence elements are marked by black bars.

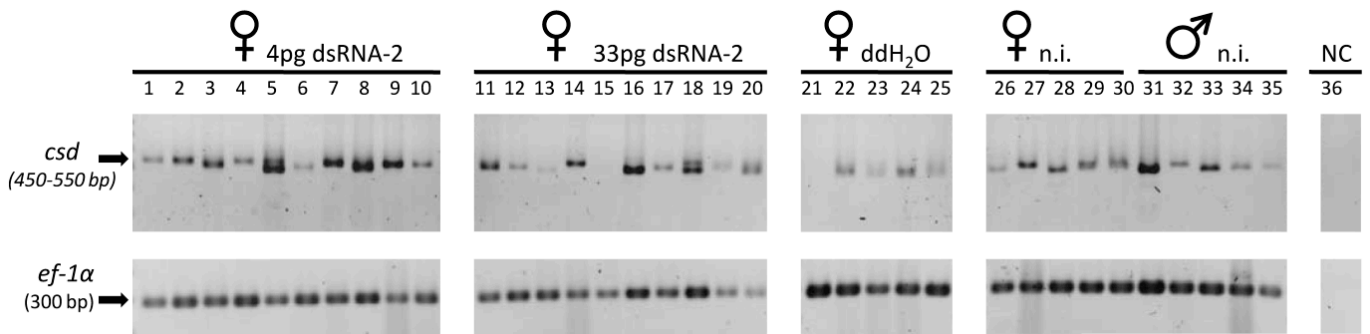


Figure S3 Constitutive splicing of *csd* transcripts in *Am-tra2* dsRNA-2 treated embryos. The *csd* mRNAs of individuals 77-80 hours after egg-laying were studied using semiquantitative RT-PCR. Early embryos were injected with 4 pg of *Am-tra2* dsRNA-2 (lanes 1-10), 33 pg of *Am-tra2* dsRNA-2 (lanes 11-20) or ddH₂O (lanes 21-25). The untreated female and male controls (labeled as n.i.) are shown in lanes 26-30 and 31-35, respectively. NC denotes our control PCR in which no cDNA was added (lane 36). Fragments corresponding to the *csd* transcripts including the hypervariable region (size of ~450-550 bp) were resolved by agarose gel electrophoresis and stained with ethidium bromide. The size of *csd* fragments varies due to length differences of the hypervariable that substantially varies between *csd* alleles. Because *csd* alleles can vary substantially in the nucleotide sequence (10-15%) we cannot amplify to the same extend all the *csd* alleles. We amplified cDNAs of the gene *elongation factor 1α* (*ef-1α*) as a relative control to semiquantify *csd* transcripts across embryonic samples.

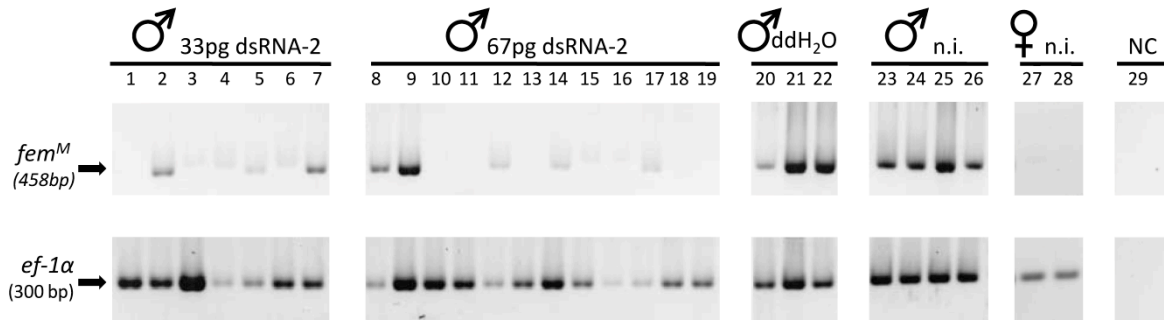


Figure S4 *fem* transcripts in male embryos treated with *Am-tra2* dsRNA-2. The male *fem* mRNAs of individuals 48 hours after egg-laying were studied using semiquantitative RT-PCR. Early embryos were injected with 33 pg of *Am-tra2* dsRNA-2 (lanes 1-7), 67 pg of *Am-tra2* dsRNA-2 (lanes 8-19) or ddH₂O (lanes 20-22). The untreated male and female controls (labeled as n.i.) are shown in lanes 23-26 and 27-28, respectively. NC denotes our control PCR in which no cDNA was added (lane 29). Fragments were resolved by agarose gel electrophoresis and stained with ethidium bromide. The corresponding fragment of the male *fem* transcripts has a size of 458 bp. We used amplification of the cDNAs of the gene *elongation factor 1α* (*ef-1α*) as a relative control to semiquantify *csd* transcripts across embryonic samples.

Table S1 Sequences of oligonucleotides that were used

to synthesize dsRNA	
#22M: tra-2_ds_FOR	TAATACGACTCACTATAGGGCGAAGTCGTAGTCGCAGCCGTAGTCGTT
#23M: tra-2_ds_REV	TAATACGACTCACTATAGGGCGACTGGTGTGGTGTATGAGCTCGTTG
#591	TAATACGACTCACTATAGGGAGTCGTAGTCAAGTCCTAGAAGACC
#592	TAATACGACTCACTATAGGGATTTCCCTGTTTCCAACATGAC
to analyze <i>Am-dsx</i> splicing	
#417	CTATTGGAGCACAGTAGCAAACCTG
#418	GGCTACGTATGTTTAGGAGGACC
#419	GAAACAATTTTGTTCAAAATAGAATTCC
to analyze <i>fem</i> splicing	
#412	CTGATTTTTCAATATTTACAGCTAAAACCTGTAC
#523	CAACATCTGATGAACTTAAACGG
#410	TGAAGTTAATAACATATTTTAAATTCATCAATGAAG
#566	TGTACCATCTGAAGATTCTAATTTTTTCG
to amplify <i>elongation factor-1α</i>	
#EM033	CGTTCGTACCGATCTCCGGATG
#EM034	GCTGCTGGAGCGAATGTTAC
in 5'RACE experiments	
5'RACE OLLI:	TGAACGGCTTCGTG
in 3'RACE experiments	
#33M (3'RACE J1 OUTER)	ACT CTC GCG AAT GTG ATA GGA CCA T
#34M (3'RACE J2 INNER)	TCA CAC TCC CGC AGT CCA ATG TCA T
3'RACE OLLI	AGAACAGTGTGCAG
to clone full ORF of <i>Am-tra2</i>	
#359	GATCGGATCCATGAGTGACATTGAGCGAAGTAGTAG
#421	TGACACGCGTTTAAATATCGACGTGGTGAATAAGAGC
to amplify <i>csd</i> transcripts	
#CS-1	ATGAAAAGAAAACCTTTTAGAAGAAAGAAC
#CS-2	TAAAATTTTATAGTTTTCATTGATGCGTAG
