



## Supporting Information

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Persistent Parental RNAi in the Beetle *Tribolium castaneum* Involves Maternal Transmission of Long Double-Stranded RNA

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# **Persistent parental RNAi in the beetle *Tribolium castaneum* involves maternal transmission of long double-stranded RNA**

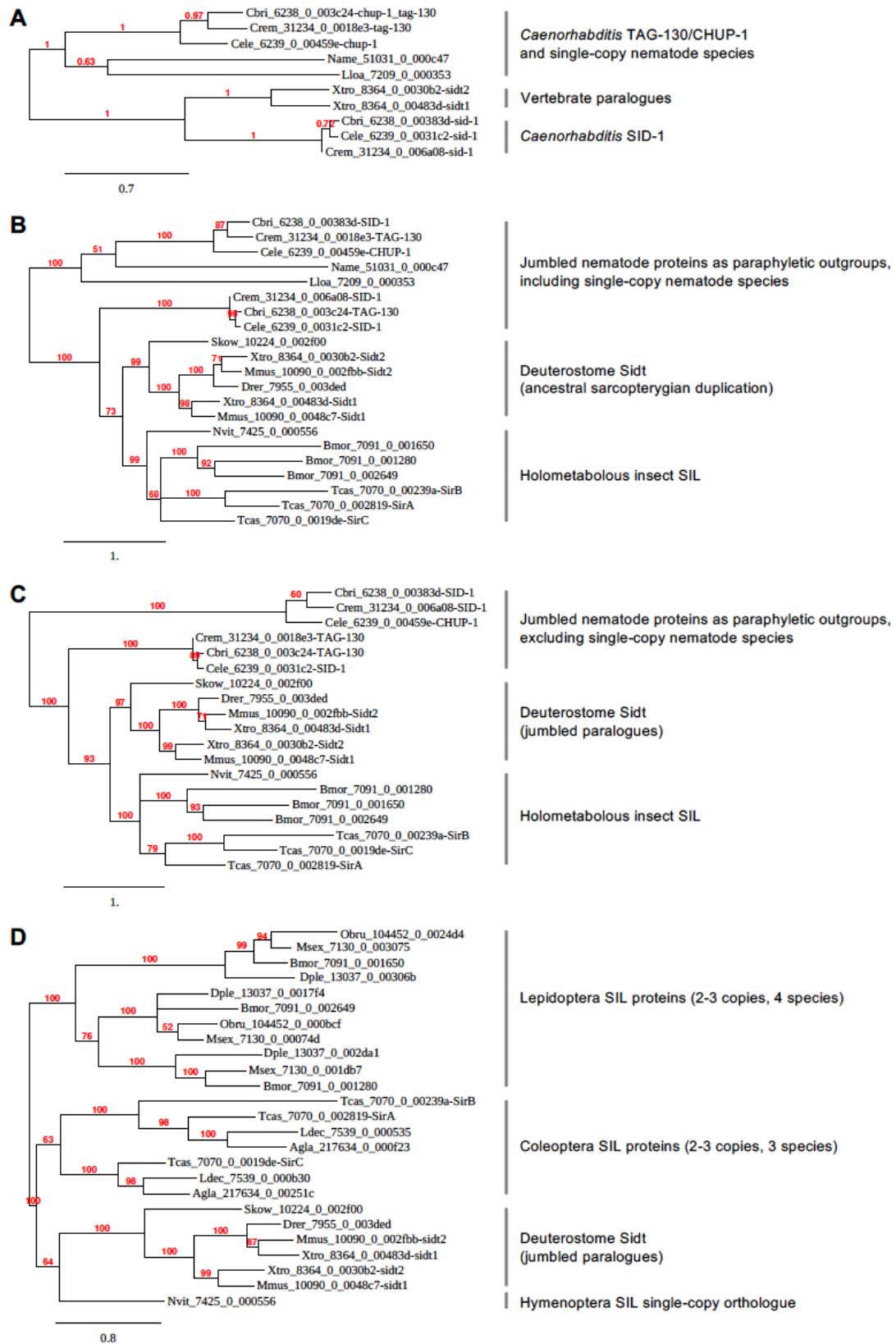
## **Supporting Information**

Figure S1. Additional phylogenies with species subsampling for SID-1/SIL proteins.

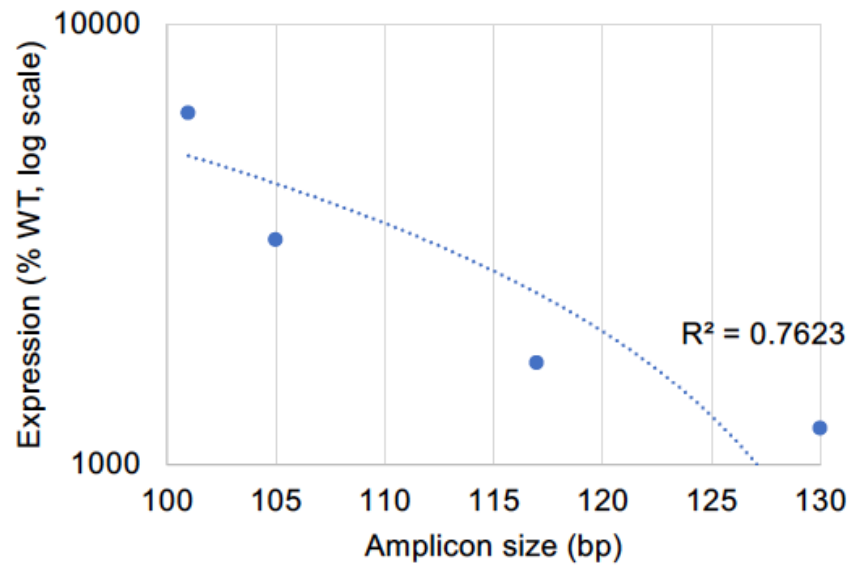
Figure S2. Negative correlation of nested RT-qPCR amplicon length and detection of dsRNA.

Table S1. Primers used in this study.

Table S2. Genome assembly versions queried by BLAST.



**Figure S1. Additional phylogenies with species subsampling for SID-1/SIL proteins.** (A-D) Maximum likelihood phylogenies of selected SID-1 homologues. The branch length unit represents substitutions per site. All nodes have  $\geq 50\%$  support. The designation “jumbled” highlights clades that conflate distinct genes (nematode SID-1 with TAG-130/CHUP-1, vertebrate Sidt1 with Sidt2), which did not occur across all trees.



**Figure S2. Negative correlation of nested RT-qPCR amplicon length and detection of dsRNA** (expression in excess of WT), assayed for *Tc-zen1* at 16-24 h, as in main text Figure 1C. Fragments 3 and 4 are shorter than Fragments 2 and 5. Logarithmic trendline for mean expression level (% WT) vs. amplicon length:  $R^2 = 0.76$ .

**Table S1. Primers used in the study.** Note that primers for RNAi (dsRNA synthesis) also included an adapter sequence, 5'-GGCCGCGG-3' (forward primers) or 5'-CCCGGGGC-3' (reverse primers), for subsequent amplification with T7 promoter universal primers (adapters not shown in table). The T7 universal primers are: 5'-universal primer 5'-GAGAATTCTAATACGACTCACTATAGGGCCGCGG-3', and 3'-universal primer 5'-AGGGATCCTAATACGACTCACTATAGGGCCCGGGGC-3'.

Application	Gene and fragment ID	Primer direction	Sequence (5' to 3')	Amplicon length (bp)
<b>RNAi</b>				
	<i>Tc-zen1</i> (TC000921)	forward	TCCCAATTTGAAAACCAAGC	688
		reverse	CGTTCCACCCTTCCTGATAA	
	<i>Tc-chs1</i> (TC014634)	forward (F1)	CACCAGGACTGTGCA	390
		reverse (R1)	GGCTTTTTGGACGAT	
	<i>DsRed2</i> (EU257621.1)	forward	AGTTCATGCGCTTCAAGGTG	600
		reverse	TGGTGTAGTCCTCGTTGTGG	
	<i>Tc-tup</i> (TC033536), <i>NOF 1</i>	forward (F1)	CGTGCGAGATGGTAAAACCT	306
		reverse (R1)	TTGCTCAAGCTGGTGTGTT	
	<i>Tc-tup</i> (TC033536), <i>NOF 2</i>	forward (F2)	CACGTTGAGGACGTGCTATG	347
		reverse (R2)	GCTGATGGGGTTGCTCTAAG	
	<i>Tc-gcl</i> (TC001571)	forward (F1)	CGTTGATCAGTGGTGTGCA	437
		reverse (R1)	TCGCTTCCTCCAGAAATGT	
<b>RT-qPCR</b>				
	<i>Tc-RpS3</i> (TC008261)	forward	ACCTCGATACACCATAGCAAGC	186
		reverse	ACCGTCGTATTCGTGAATTGAC	
	<i>Tc-zen1</i> (5'-3'):			
	Fragment 1 outside dsRNA	forward	TCCTGTTGTGAGTCAGTGCA	223
		reverse	CAGTTCCAATCAGAAGGTGGA	
	Fragment 2 inside dsRNA	forward	TGAAAACCAAGCCGTTCTGC	169
		reverse	CAGTTCCAATCAGAAGGTGGA	
	Fragment 3 inside dsRNA	forward	TCCACCTTCTGATTGGAAGT	161
		reverse	CGTTGGGGTTGAGTTTCTTG	
	Fragment 4 inside dsRNA	forward	CGGCCCAATTAGTGGAATTA	101
		reverse	ACGCTCACTCAGGTTCAAGT	
	Fragment 5 inside dsRNA	forward	CCATCGACAGTGCAAACCAA	130
		reverse	TCCTCTTGTTTGGGCAAAGC	
	Fragment 6 outside dsRNA	forward	CCATCGACAGTGCAAACCAA	190
		reverse	GTTAAAGCAGGCTGGGACAC	
	<i>Tc-chs1</i> (3')	forward	ATTCTGTAACCGGGACCTGG	
		reverse inside dsRNA	CCAGAAGGCGAAGATCAAGC	100
		reverse outside dsRNA	ATGAGGAAGTGGGAGAAGGC	186
	<i>DsRed</i> (5')	forward inside dsRNA	AGTTCATGCGCTTCAAGGTG	123
		forward outside dsRNA	GCTCCTCCAAGAACGTCATC	147
		reverse	CCTTGGTCACCTTCAGCTTC	

**Table S2. Genome assembly versions queried by BLAST.** These resources were interrogated with tBLASTn queries for selected SID-1 proteins (see main text Figure 5B). Accessed at the i5K@NAL site, most recent access date: 13 October 2021.

Taxonomic grouping	Species	Species abbreviation	Assembly version
Paraneoptera > Hemiptera > Sternorrhyncha	<i>Bemisia tabaci</i>	Btab	Genome Assembly - Bemisia tabaci genome assembly GCF_001854935.1 (ASM185493v1)
Paraneoptera > Hemiptera > Sternorrhyncha	<i>Diaphorina citri</i>	Dcit	Genome Assembly - NCBI-diaci1.1 (Current RefSeq assembly version)
Paraneoptera > Hemiptera > Heteroptera	<i>Gerris buenoi</i>	Gbue	Genome Assembly - Gbue.scaffolds.50_new_ids.fa
Paraneoptera > Hemiptera > Heteroptera	<i>Cimex lectularius</i>	Clec	Genome Assembly - Clec_Bbug02212013.genome_new_ids.fa
Paraneoptera > Hemiptera > Heteroptera	<i>Halyomorpha halys</i>	Hhal	Genome Assembly - Halyomorpha halys genome assembly GCA_000696795.3
Paraneoptera > Hemiptera > Heteroptera	<i>Oncopeltus fasciatus</i>	Ofas	Genome Assembly - Ofas.scaffolds_new_ids.fa
Paraneoptera > Thysanoptera	<i>Frankliniella occidentalis</i>	Focc	Genome Assembly - Frankliniella occidentalis genome assembly GCA_000697945.4
Holometabola > Hymenoptera	<i>Athalia rosae</i>	Aros	Genome Assembly - Aros01112013-genome_new_ids.fa
Holometabola > Coleoptera	<i>Anoplophora glabripennis</i>	Agla	Genome Assembly - Agla_Btl03082013.genome_new_ids.fa
Holometabola > Coleoptera	<i>Leptinotarsa decemlineata</i>	Ldec	Genome Assembly - Leptinotarsa decemlineata genome assembly GCF_000500325.1
Holometabola > Coleoptera	<i>Tribolium castaneum</i>	Tcas	Genome Assembly - Tribolium castaneum genome assembly Tcas5.2 (GCF_000002335.3), genomic scaffolds
Holometabola > Diptera	<i>Drosophila biarmipes</i>	Dbia	Genome Assembly - Drosophila biarmipes genome assembly GCF_000233415.1
Holometabola > Diptera	<i>Drosophila elegans</i>	Dele	Genome Assembly - Drosophila elegans genome assembly, ASM1815250v1
Holometabola > Diptera	<i>Drosophila kikkawai</i>	Dkik	Genome Assembly - Drosophila kikkawai genome assembly, ASM1815253v1