

## Doublesex target genes in the red flour beetle, *Tribolium castaneum*

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FIGURE 1S ClustalW alignment of TcDsx amino acid sequence coded by *Tcdsx* splice forms showing OD1/DM (grey shaded region) and OD2 (Yellow and blue shaded regions) domains. All the putative TcDsx proteins differ from each other at their C-terminus. The common region of OD2 (a) domain has been shown in yellow shaded region whereas female-specific regions (of OD2a) have been shown in orange. TcDsxM contains an additional OD2 (OD2b) domain (blue shaded region).

FIGURE 2S Schematic representation and sequence of female-specific exon (exon 3- in red color) of *Tcdsx* and its adjoining introns (black color) showing the presence of 3 putative Tra/Tra2 binding site (yellow line/shade), multiple Tra2-ISS binding site (grey line/shade) and a purine rich stretch (green line/ shade). Black lines within exon 3 in the schematic diagram represent the region of alternative splicing resulting in the production of three female specific isoforms.

FIGURE 3S Graphs showing the knockdown efficiencies of individual *Tcdsx* transcripts in all the three *Tcdsx* knockdown experiments; dsRNA injections targeting common region of *Tcdsx* (*Tcdsxf1*+*Tcdsxf2*+*Tcdsxf3*= C), *Tcdsxf1* and *Tcdsxf2* (*f1*+*f2*) and *Tcdsxf2* alone. Significant reduction ( $P < .05$ ) in the mRNA levels of target genes was observed in all the experiments.

Fig. 1S

**DM/OD1**

TcDsxF1 **MSSDSQDFDSKMDVNASSTSASPRTPPNCARCRNHRLKIALKGHKRYCKYRTCKCEKRL** 60  
TcDsxF3 **MSSDSQDFDSKMDVNASSTSASPRTPPNCARCRNHRLKIALKGHKRYCKYRTCKCEKRL** 60  
TcDsxF2 **MSSDSQDFDSKMDVNASSTSASPRTPPNCARCRNHRLKIALKGHKRYCKYRTCKCEKRL** 60  
Tcdsxm **MSSDSQDFDSKMDVNASSTSASPRTPPNCARCRNHRLKIALKGHKRYCKYRTCKCEKRL** 60  
\*\*\*\*\*

TcDsxF1 **TTERQVMMAMQTALRRAQAQDEAMLRSGSAVDPAIMQVPLKSPSPIHAIERSLDCDSSAS** 120  
TcDsxF3 **TTERQVMMAMQTALRRAQAQDEAMLRSGSAVDPAIMQVPLKSPSPIHAIERSLDCDSSAS** 120  
TcDsxF2 **TTERQVMMAMQTALRRAQAQDEAMLRSGSAVDPAIMQVPLKSPSPIHAIERSLDCDSSAS** 120  
Tcdsxm **TTERQVMMAMQTALRRAQAQDEAMLRSGSAVDPAIMQVPLKSPSPIHAIERSLDCDSSAS** 120  
\*\*\*\*\*

**OD2a**

TcDsxF1 **SQCSNPPPAIRKMTVPVAVPSSTSVNIGTIAQSTDILLEDCQKLLERFKYPWEMMPLMYAI** 180  
TcDsxF3 **SQCSNPPPAIRKMTVPVAVPSSTSVNIGTIAQSTDILLEDCQKLLERFKYPWEMMPLMYAI** 180  
TcDsxF2 **SQCSNPPPAIRKMTVPVAVPSSTSVNIGTIAQSTDILLEDCQKLLERFKYPWEMMPLMYAI** 180  
TcdsxM **SQCSNPPPAIRKMTVPVAVPSSTSVNIGTIAQSTDILLEDCQKLLERFKYPWEMMPLMYAI** 180  
\*\*\*\*\*

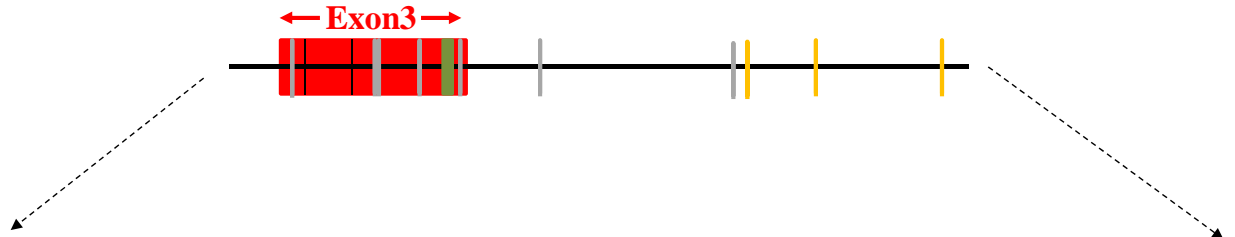
TcDsxF1 **LKDARADLEEASRRIDEGKRVVNEYSLHNLNMYDGVELRNST-----HNKNQDRRSF** 233  
TcDsxF3 **LKDARADLEEASRRIDE-----DNKNQDRRSF** 207  
TcDsxF2 **LKDARADLEEASRRIDEGKRVVNEYSLHNLNMYDGVELRNSTRQYG-----** 227  
TcdsxM **LKDARADLEEASRRIDEGRDTEILLDFCQRLKDKFQLSWKMISLVDVILKYAKDQDEAWR** 240  
\*\*\*\*\*

**OD2b**

TcDsxF1 **SSNPSFKSVNLQFKCNVLI-----** 252  
TcDsxF3 **SSNPSFKSVNLQFKCNVLI-----** 226  
TcDsxF2 -----  
TcdsxM **QIDEAFLEIRALAAVEAARYTYHHIPYSGLYPNAATAIYPPVYLPMSMYHPATLLGSVP** 300

TcDsxF1 -----  
TcDsxF3 -----  
TcDsxF2 -----  
TcdsxM **TSTSPSHSPPIVPRAIRPSSRA** 322

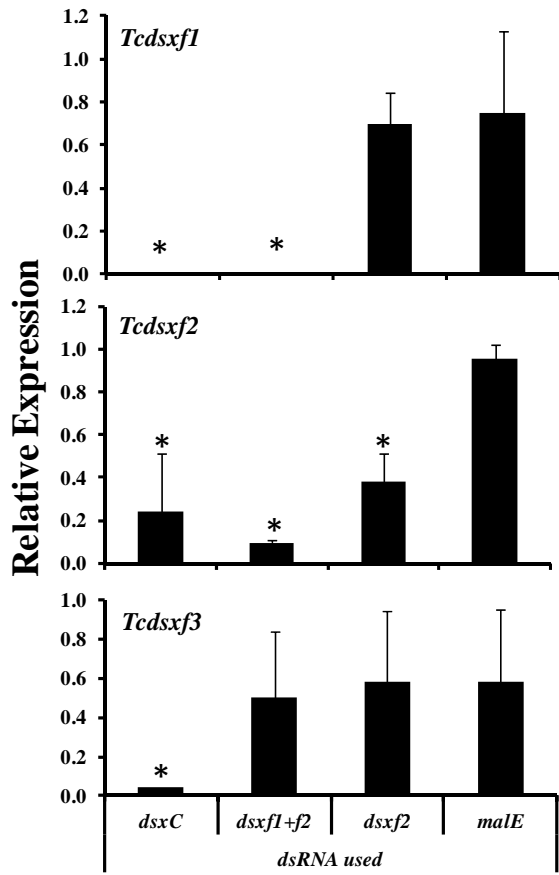
Fig.2S



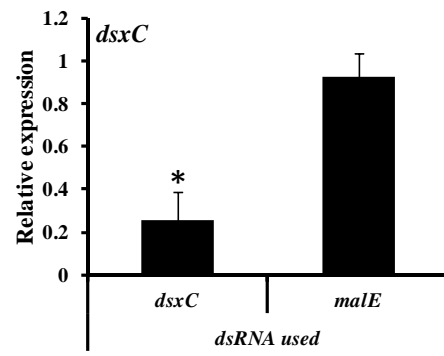
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TTCCCTAAAAATCTCAGCCTTAATCGGTGTTTTATTTTTGTGTTTTGTTGGCAG
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Fig. 3S

**A**



**B**



**TABLE 1S: List of sex-specific *Tcdsx* transcripts and protein isoforms.**

<b><i>Tcdsx</i>Splice variant</b>	<b>Length (bp)</b>	<b>exons included</b>	<b>Predicted protein</b>	<b>protein length (aa)</b>
<i>Tcdsxm</i>	1788	1, 2, 4, 5, 6	TcDsxM	332
<i>Tcdsxf1</i>	2264	1, 2, 3, 4, 5, 6	TcDsxF1	252
<i>Tcdsxf2</i>	2428	1, 2, 3, 4, 5, 6	TcDsxF2	227
<i>Tcdsxf3</i>	2186	1, 2, 3, 4, 5, 6	TcDsxF3	226

**TABLE 2S: List of primers and their sequences used in the study.**

<b>Primer name</b>	<b>Primer sequence</b>
<b>dsRNA</b>	
dsxCommon	GTCCTCCGACAGCCAAGACT
	CTGAGGAGTCGCAGTCCAGA
dsxf1+f2	AAAGCGTGTGTGAATGAGTA
	TTGAATTTCGAAGCTCCACTC
dsxf2	CCATCACCCAAAATTTGTAGTTTCG
	AAAGGGGACATGCATTACTGTTTC
RT-PCR	
dsx(F1-R1)	ACAGTCCGCGACAAGGTTGC
	CATGCCGTAAATGACCTGCTAA
Dsx(F2-R2)	AGCACTTCCGTCAACATTGG
	TTTGGCAGAAGTCTAACAGAATTTCA
dsxqCommon	TTCAAGTATCCGTGGGAAATGA
	CGGGAAGCCTCTTCCAAGT
qRTf1R	GTCTTGGTTCTTGTATGCGTTG
qRTf2R	GAAACTACAAATTTTGGGTGATGG
qRTf3R	CGGTCTTGGTTCTTGTATCCTCA
G13602	TTGCAAATGCTGGGTGGTGAAGAC
	AGCGTGTGCGTTGATAACTTGCTG
G10839	AACGCACACGATTTTCGACCAAGTG
	ACGGCAGCATTAACTTGGTTGCTC
G14653	AACTCAACGTCGCCTCCCTTGTAT
	GTCCGCGTGGTTGAACTTCACTTT
G07775	ATACTAGCGTCTCCAATTGCGGGT
	TAGGCCCTAGCAAAGGCCCAATTA
G05384	AAACCGTCTCCTCGATGATGTGCT
	ACTTCATGCCATTAGGCTCTCGT
G7186	CCTTACGGCCAACACCACACAAAT
	TGGCTCCCTCCATATGACCAACAT
G03183	AAATCGCGCTCCGATCTCAGTCTT
	ATACGGCGTGTGTACGGAGTCTT
G08596	AGGTGACGTATTTGCTATCGCCCA
	AAGTGTCGCAATCCTCCTCTGGTT
G04042	ACTCGCGATGTATCAGTTCTGCGT
	TTGGTCCCTTGTACACGTGGAGTT
G15076	TGGCCATCAGGTCCATTCACTACA
	ATATCCGCAGCCTTGAAAGCCTA
G07776	ACCATTGCTTGGACCACTCTTTGC

	CCAAGTTTGTGTACCAAAGGCGCA
G06658	CGTCCATTCAATCGCCCACCATTT
	ACGTCATTGTCGCTCTCCAATCA