



Figure S1a. Sexual dimorphism in *Bemisia tabaci* Asiall-1 (A) male adult and (B) female adult.

Figure S1b. Sexual dimorphism in puparia of *Bemisa tabaci* Asia II-1 (male and female). Size of double headed arrows (in yellow) depict the width of the caudal furrow - narrow in male pupa and wider in female pupa.



Figure S2a. Melt curve analysis of the different primer sets used for RT-qPCR studies, to ensure specific amplification (without any dimer formation) of the respective genes (A) double sex (*dsx*) (B) vitellogenin (*vg*) (C) vitellogenin receptor (*vgr*) (D) cytochrome p450 (*CYP450*) and (E) alkaline phosphatase (*ALP*).



Figure S2b. Gel electrophoresis after specific amplification of the double sex gene via RT-qPCR for different stages of *Bemisia tabaci* Asiall-1, depicting (A) egg (B) crawlers (C) 2nd instar (D) 3rd instar (E) red eyed pupa (F) male adult and (G) female adult.







Collection of leaves having red eyed pupa



Wrapping of tube with a luminum foil before temporary inactivation of white flies at -20 $\,$

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Separation of newly emerged whitefly



Collected newly emerged whitefly



Loading of sucrose diet having dsRNA



Setting up second layer over the sucrose diet



Feeding of whiteflies

Figure S4. Preparation of tubes for feeding dsRNA to *Bemisia tabaci* adults.



Figure S5. Gene structure of *Bemisia tabaci* double sex (*dsx*) gene based on alignment of cDNA with genomic DNA from Scaffold 130 of the MEAM1 whitefly genome using Splign (NCBI). The boxed numbers represent the specific locations of splice sites on the MEAM1 genomic DNA scaffold.

	MASDVTSGFQNKSRV	NSRAPRCCVI	CRNHGLKKL	VRGHKRYCPY	TLCPCRLCLA	TKERQVHMAK	TIKKRRYFLQD	LAMQAHPDPK	MPKHPEPIPQ	MPIIY Majority
	10	20	30	40	50	60	70	80	90	100
1 1 1 1	MASDVTSGFQNKSRV MASDVTSGFQNKSRV MASDVTSGFQNKSRV MASDVTSGFQNKSRV MASDVTSGFQNKSRV	NSRAPRCCVI NSRAPRCCVI NSRAPRCCVI NSRAPRCCVI NSRAPRCCVI	RCRNHGLKKLV RCRNHGLKKLV RCRNHGLKKLV RCRNHGLKKLV RCRNHGLKKLV	VRGHKRYCPY VRGHKRYCPY VRGHKRYCPY VRGHKRYCPY VRGHKRYCPY	FLCPCRLCLA FLCPCRLCLA FLCPCRLCLA FLCPCRLCLA FLCPCRLCLA	TKERQVHMAK' TKERQVHMAK' TKERQVHMAK' TKERQVHMAK' TKERQVHMAK'	FIKKRRYFLQE FIKKRRYFLQE FIKKRRYFLQE FIKKRRYFLQE FIKKRRYFLQE	DLAMQAHPDPK DLAMQAHPDPK DLAMQAHPDPK DLAMQAHPDPK DLAMQAHPDPK	MPKHPEPIPQ MPKHPEPIPQ MPKHPEPIPQ MPKHPEPIPQ MPKHPEPIPQ	MPIIY BtDsxM2.pro MPIIY BtDsxM1.pro MPIIY BtDsxM3.pro MPIIY BtDsxM4.pro MPIIY BtDsxF.pro
	KPPEVTTLPQGPPGP	GPVEPPRDTI	PTSPVGSLP	PPSHQKAASVI	KSEASLPTSS	PYRTPIDESL	WESSIGSKIRT	LIEMFNFQRI	VDIYSLLYVI	LRYTT Majority
	110	120	130	140	150	160	170	180	190	200
101 101 101 101 101	KPPEVTTLPQGPPGP KPPEVTTLPQGPPGP KPPEVTTLPQGPPGP KPPEVTTLPQGPPGP KPPEVTTLPQGPPGP	GPVEPPRDTI GPVEPPRDTI GPVEPPRDTI GPVEPPRDTI GPVEPPRDTI	PPTSPVGSLP1 PPTSPVGSLP1 PPTSPVGSLP1 PPTSPVGSLP1 PPTSPVGSLP1	PPSHQKAASVI PPSHQKAASVI PPSHQKAASVI PPSHQKAASVI PPSHQKAASVI	KSEASLPTSS KSEASLPTSS KSEASLPTSS KSEASLPTSS KSEASLPTSS	PYRTPIDESLI PYRTPIDESLI PYRTPIDESLI PYRTPIDESLI PYRTPIDESLI	WESSIGSKIRT WESSIGSKIRT WESSIGSKIRT WESSIGSKIRT WESSIGSKIRT	LIEMFNFQRI LIEMFNFQRI LIEMFNFQRI LIEMFNFQRI LIEMFNFQRI	OVDIYSLLYVI OVDIYSLLYVI OVDIYSLLYVI OVDIYSLLYVI OVDIYSLLYVI	LRYTT BtDsxM2.pro LRYTT BtDsxM1.pro LRYTT BtDsxM3.pro LRYTT BtDsxM4.pro LRYTT BtDsxF.pro
	SDVMEVYNKIVEGSR	ERANFRSQC	AHHVSFTYPS	[R						Majority
	210	220	230	240	250	260	270			
201 201 201 201 201	SDVMEVYNKIVE <mark>ACD</mark> SDVMEVYNKIVEGSR SDVMEVYNKIVEGSR SDVMEVYNKIVEV SDVMEVYNKIVEDKH	LRGAGAGVV ERANFRSQC ERANFRSQC APWHALEAT	AAALRASPEI AHHVSFTYPS AHHVSFTYPS ITHGHV <mark>T</mark>	PVQQLHLGHRI TR TR	RLHALPHPPRI	HRRPPQEHRQ'	TVLAHSLSRV			BtDsxM2.pro BtDsxM1.pro BtDsxM3.pro BtDsxM4.pro BtDsxF.pro

Figure S6. Multiple sequence alignment of the male and female specific transcripts of Asiall-1 Dsx protein. The region highlighted in yellow represents the DNA binding domain (OD1), also known as the DM domain. The OD2 domain (oligomerization domain) was not detected in these BtDSX isoforms. Comparable to what has been found in in other insect species, the sex specific BtDSX proteins differed at their C-termini.





≻A. mylitta M	20 IVSVGAWRRRAPDDCEERSDPGASSSAVPRAF	CARCRNHRLKVELKG		80 RVMALQTALRRAQAQDEARAR	100 AKETGGHLPGVELDRPEPI	1: PIVKAPRSPV
≻A. assamensis≬	4º VSVGAWRRRAPDDCEERSDPGASSSAVPRAF	40 CARCRNHRLKVELKG	HKRYDAYRYCNCEKCRLTADRQ	RVMALQTALRRADAQDEARAR	AKETGGHLPGVELDRPEPI	11 PIVKAPRSPV
>B. Mori M	20 Ivsmgswkrrvpddcersepgasssgvpraf <mark>pn</mark>	40 CARCRNHRLKIELKG	HKRYDN QHCTCEKCRLTADRQ	RVMAKQTAIRRAQAQDEARAR	100 ALELGIQPPGLELDRPVPI	
>D. Melanogast∦	20 Ivseenwnsdtmisdsdmidskndvcggassssgs	SISPRTPPNCARCRN	HGLKITLKGHKRYDRFRYCTCE	CRLTADRQRVMALQTALRRA	100 DAQDEQRALHMHEVPPAN	1: PÁATTĽLSHI
>Benisia tabac:	20 IASÓVTSGFQNKSRVNSRAPRCC <mark>VRCRNHGLKKL</mark>	40 VRGHKRYC₽¥TLCPC	RLCLATKERQVHMAKTIKKRRY	SU FLQDLAMQAHPDPKMPKHPEP	100 IPQMPIIYKPPEVTTLPQ	12 GPPGPGPVEF
>Rhodnius prol:	20 ISSGVGAASQQPMQQQQNPAQPAART <mark>PPNCARCR</mark>		MYRTCTCKKCHLTVEROREMAK	TALRRELAQDEARARAGLQP	100 ASPPPSATSPPPXTGQPI	1: ASHLSTASSI
>Acyrthosiphon≬		40 I I I I NLSS-DSECKNNGECV	60 INKKNRTSCKACRERKCLLVGM		100 DQNAAAAAAAYGHHPPHHI	1: HHQHHNGSSF
•						+
≻A. mylitta	-20 VVPPPPPRSLGSASCDSVPGSPGVSPVAPPPP	160 SVPPLQAMAPLMPPQ	QPAVSLETLVENCHKLLEKFHYS	200 WEMMPLOSKLAAMANSCEEA	220 SRKIDEAHCVVHQWRLYE	240 RSLÝSLLÉLQARH
≻A. mylitta ≻A. assamensis	-7° 1°° YVPPPPPRSLGSASCDSVPGSPGVSPYAPPPP -7° YVPPPPPRSLGSASCDSVPGSPGVSPYAPPPP	160 SVPPLQAMAPLMPPQ 160 SVPPLQAMPPLMPPQ	араусі фіту араусі фітуральні тара араусі фітуральні тара араусі фітуральні тара	200 WEMMPLOSXLAADSEEA 200 WEMASSDLEEA	220 SRKIDEAHCVVHQWRLYE 220 SRKIDEAHWVVHQWRLYE	240 Rislýslielaar 240 Hslýslikaar
>A. mylitta >A. assamensis >B. Mori	20 140 VVPPPPPRSLGSASCDSVPGSPGVSPYAPPPP -20 140 VVPPPPPRSLGSASCDSVPGSPGVSPYAPPPP 20 140 MIPPSAPRSLGSASCDSVPGSPGVSPYAPPPPS	160 SVPPLQAMAPLMPPQ svpplqampplmppq 160 vpppptmpplippq		200 WEMMPLOS LALOUSCEEA WEMPS LALOUSCEEA WEMPS LALOUSCEEA IEGNIKAVPSETLVENCHRLLE	220 SRKIDEAHCVVHQWRLVE 220 SRKIDEAHWVVHQWRLVE SRKIDEAHWVVHQWRLVE	210 R _S LÝSLLĖLQARH 45LČSLLKLQAQH 44 MGPT DEASRKTN
>A. mylitta >A. assamensis >B. Mori >D. Melanogasta	-20 140 VVPPPPRSLGSÅSCDSVPGSPGVSPYAPPPP -20 140 VVPPPPPRSLGSÅSCDSVPGSPGVSPYAPPPP -20 140 MIPPSAPRSLGSÅSCDSVPGSPGVSPYAPPPPS -20 140 MIPPSAPRSLGSÅSCDSVPGSPGVSPYAPPPPS -20 140 MIPPSAPRSLGSÅSCDSVPGSPGVSPYAPPPS -40 140 HHHVÅAPAHVHAHHVHÅHHAHAGGHHSHHGHVLI	160 SVPPLQAMAPLMPPQ svppLQAMPPLMPPQ vppptmppLIPPpQ ihdqqaaaaaaaaaaa	араусі <mark>180</mark> араусі <mark>471 уемснкці екгнус</mark> араусі 471 <mark>уемснкці екгнус</mark> араусі 471 уемснкці екгнус 180 рну шираагру саласті 180 рну шираагру саласті 180 аласті саласті саласті 180 аласті саласті саласті саласті саласті 180 аласті саласті саласті саласті саласті 180 аласті саласті саласт		220 SRKIDEAHCVVHQWRLYE SRKIDEAHWVVHQWRLYE SRKIDEAHWVVHQWRLYE KFHYSWEMMPL 220 KFHYSWEMMPL 220 SHHHHHQNPHQQPA	240 <u>R</u> slýsllélaar Hslćsllklaad <u>240</u> <u>41000000000000000000000000000000000000</u>
 >A. mylitta >A. assamensis >B. Mori >D. Melanogasti >Benisia tabac: 	20 140 VVPPPPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 VVPPPPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 VVPPPPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 MIPPSAPRSLGSÅSCDSVPGSPGVSPVAPPPPS 20 140 HHHVÅAPPAHVHAHHVÅHHAHGGHHSHHGHVLI 20 140 PRDTPPTSPVGSLPPPSHQKAASVKSEÅSLP	160 SVPPLQAMAPLMPPQ SVPPLQAMPPLMPPQ VPPPPTMPPLIPPPQ 160 ннqqааааааааааааарsai 160 тsspyrtpideslwe	180 QPAVSLET <u>IVENCHKLLEKFHYS</u> QPAVSLET <u>IVENCHKLLEKFHYS</u> PHYWWPGAFPVSPGHVSEQRLSC 180 PASHLGGSSTAASSIHGHAHAHH SSIGSKIRTLIEMFNFQRDVDIV	200 WEMMPLOSXLALOUSE WEMPSXLALOUSE EGNIKAVPSTLUMPSSDLEEA 200 200 200 200 200 200 200 20	220 SRKIDEAHCVVHQWRLYE SRKIDEAHWVVHQWRLYE SRKIDEAHWVVHQWRLYE 220 кFHYSWEMMPLQSX100 SHHHHHQNHHQHPHQQPA 220 SHHHHHQNHHQHPHQQPA 220 vEEVGNAQISGPNVPITC	240 RSLÝSLLÉLQARH HSLĆSLLKLQAQH 400 400 400 240 TQTÁLRSPPHSDH 240 RSPÍRPPÖDFPRJ
 >A. nylitta >A. assamensis >B. Mori >D. Melanogasta >Benisia tabac: >Rhodnius prol: 	20 140 VVPPPPPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 VVPPPPPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 MIPPSAPRSLGSÅSCDSVPGSPGVSPVAPPPP 20 140 HIPPSAPRSLGSÅSCDSVPGSPGVSPVAPPPPS 20 140 HHHVÅAPAHVHAHHVHÅHHAHGGHÅSHHGHVLI 20 140 PPRDTPPTSPVGSLPPPSHQKAASVKSEÅSLP 20 140 LQTTGGSNSGEVCWQSI I AL QEAL QLALDTLP	160 SVPPLQAMAPLMPPQ 160 SVPPLQAMPPLMPPQ 160 160 HHQQAAAAAAAAASSAI HHQQAAAAAAAASSAI 160 TSSPYRTPIDESLWE 160 LVYIILKFTKSYDEA	190 QPAVSLET <u>IVENCHKLLEKFHYS</u> QPAVSLET <u>IVENCHKLLEKFHYS</u> PHYWWPGAFPVSPGHVSEQRLSC 190 PASHLGGSSTAASSIHGHAHAHH 190 SSIGSKIRTLIEMFNFQRDVDIY 193 LKIIAENKPASLRVPKI	200 WEMMPL ÚSKLALÍVOST ÉEA 200 WEM RSKU STURKS SDLÉEA 200 16GŇIKAÝPSE <mark>TI VEŇCHRLLE 200 200 VHMAAAAAASVAQHQHQSHPH 200 SLLYVILRYTTSDVMEVYŇKI</mark>	220 SRKIDEAHCVVHQWRLYE SRKIDEAHWVVHQWRLYE SRKIDEAHWVVHQWRLYE 220 кFHYSWEMMPL QSX10 XFHYSWEMMPL QSX10 220 SHHHHHQNHHQHPHQQPA 220 SHHHHHQNHHQHPHQQPA 220 SHHHHHQNHHQHPHQQPA	240 240 HSLČSLLĽQARH 410 410 410 410 240 101 101 101 101 101 101 101 1

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Figure S7. Comparative schematic analysis of DSX protein sequences from Lepidoptera and Hemiptera, for identification of conserved Superfamily domains, using the Web CD Search Tool (NCBI; https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). The protein sequences from lepidopteran insects have a clearly identifiable DM1 (OD1) and oligomerization (OD2) domain (DSX-Dimer) while the latter identifier is missing in hemipteran insects. B. The equivalent domains (or lack thereof) in BtDSX proteins. C. Amino acid sequence alignments of the OD1 and OD2 domains in lepidopteran species and the OD1 domain in hemipteran insects.

Supplementary Figure S8 Multiple sequence alignment of the DSX protein sequences (region common to both the sexes) belonging to various orders using CLUSTAL W.

	70 9	980 9	90 1,000	1,010	1,020	1,030	1,040	1,050	1,060	1,070	1,080	1,090	1,100	1,110	1,120	1,130	1,14	40 1,150
Consensus	SMVMSDSDMR	DSXDDVCGGX	SSSSSSS <mark>I</mark> SPRTPI	PNCARCRNHRL	<-IELKGHKRY(CKYRYCTC <mark>E</mark> K(RLTADRQR <mark>V</mark>		QAQ <mark>DE</mark> ARARX	MHE <mark>VPPXV</mark>	ELPTPLXPH	<mark> </mark> X	<mark>S</mark>	<mark>PXHSHGSN</mark> S	XHSVPASPG	SXAAA-PP	PSVPPPPT	MPPLXPPQQPAG
Identity			╼╼┚╼╹╴															
1. ADL40855.1 A. mylitta	MV SVGAWR R	RAP DD CEERS	DPGA <mark>SSS</mark> AV <mark>PR</mark> A <mark>P</mark> F	PNCARCRNHRL	K-V <mark>ELKGH</mark> KRY(CKYRYCNCEK(QAQ <mark>DE</mark> ARARA	KE⊤GGHL <mark>P</mark> G <mark>V</mark>	ELDRPEPPI	VK	APR <mark>S</mark> PVVPF	PPPRSL <mark>GS</mark> AS	CD <mark>SVP</mark> G SPG \	/Spyap- <mark>pp</mark>	PSVPPLQA	MAPLMPPQQPAV
2. ADL40846.1A. assamensis	MV SVGAWR R	rap dd ceers	DPGA <mark>SSS</mark> AV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-∨ <mark>ELKGH</mark> KRY(CKYRYCNCEK(QAQDEARARA	KE⊤GGHL <mark>P</mark> G <mark>V</mark>	ELDRPEPPI	VK	APR <mark>S</mark> PVVPF	PPPRSLGSAS	CDSVPGSPG\	/Spyap- <mark>pp</mark>	PSVPP LQA	MPPLMPPQQPAV
3. AAA17840.1 D.melanogaster	DT <mark>MSDSDM</mark> I	DSKN <mark>DVCGG</mark> A	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNH CL	K-ITLKGHKRY(CK FRYCTCEK(QAQDE QRALH	MHEVPPAN	IPAATTLLS	HHVAAPAH)	/НАННУНАННАР	IGGH hsh hghv	/L <mark>H</mark> Hqq A AAA	AA A PSA <mark>P</mark> A	SHLGGSS <mark>T</mark>	aassihghah a h
4. AAB99947.1 B. tryoni	DTIA <mark>DSDMR</mark>	DSKA <mark>DVCGG</mark> A	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNH CL	K-IT <mark>LKGH</mark> KRY(CK FR F <mark>CTCE</mark> K(QAQDE QRVLQ	IHEVPP√V	HG PTAL LN	H	L	- — — H <mark>H</mark> H H H L <mark>N</mark> Q	N <mark>H</mark> HAS A AAA	аа ааа ннн	I <mark>S</mark> TAIRSP	P HAEHGGGNVS S
5. AAQ82648.1 A. melifera						PCYCTCEK(K I TA N RQ Q <mark>M</mark>	/RQNMK l K R HI	LAQDKVKVRV	AE <mark>E</mark> VDPL <mark>P</mark> FG	iven t i ssvf	Q	PPRSLE	GSYD <mark>S</mark> SSGD <mark>S</mark>	PVSSHSSNG	HTGFGGS I	ITI <mark>PP</mark> TRK	L <mark>P PL</mark> H P HTAMVT
6. AFH41442.1 A. fraterculus	MS D S DML	DSKA <mark>DVCGG</mark> A	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNH CL	K−IT <mark>LKGH</mark> KRY(QAQDE QR I LQ	MHEVPP	NAPTALLN	H H	L	- — — H <mark>H</mark> H H H L <mark>N</mark> Q	N <mark>H</mark> HAT A AAA	AAAAH	HHISTAIR	S <mark>P</mark> AQTEHGSGG <mark>G</mark>
7. AFH41495.1A. sororcula	MSDSDML	DSKA <mark>DVCGG</mark> A	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNHGL	K-IT <mark>LKGH</mark> KRY(CK FR F <mark>CTCE</mark> K(QAQDE QRVLQ	MHEVPPVV	HAPTALLN	H H	L	-	NR HAT A AAA	AAAAH	HHMSTAIR	S <mark>PP</mark> QTEHGSGG <mark>G</mark>
8. AFH41496.1	MSDSDML	DSKA <mark>DVCGG</mark> A	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNHG L k	K−∎⊤ <mark>∎KGH</mark> KRY(CK FR F <mark>CTCE</mark> K(QAQDE QRVLQ	MHE <mark>V</mark> PP//V	HAPTALLN	H H 	L	HYH <mark>H</mark> PL <mark>N</mark> Q	N <mark>H</mark> HAT A AAA	AAAAH	HHISTAIR	S <mark>PP</mark> QTEHGSGG <mark>G</mark>
9. AFH41497.1	MS D S DML	DSKA <mark>DVCGG</mark> A	SSSSGSSISPRTPR	PNCARCRNHGLk	K-IT <mark>LKGH</mark> KRY(CK FR F <mark>CTCE</mark> KO			QAQDE QRVLQ	MHEVPPVV	HAPTALLN	 H H − − − − − − −	L	- – – H H H <mark>H</mark> P L <mark>N</mark> Q	N <mark>H</mark> HAT A AAA	AAAAH	HHMSTAIR	S <mark>PP</mark> QTEHGSGG <mark>C</mark>
10. AWC26116.1 B. tabaci																		
11. XP_037876116.1B. mori	MV SMG <mark>S</mark> WK R	rvp dd ceers	EPGA <mark>SSS</mark> GV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-IELKGHKRY(CKYQH <mark>CTCEK(</mark>			QAQ <mark>DE</mark> ARARA	lelgiq <mark>pp</mark> gl	EL DR PVPP	/VK	APR <mark>S</mark> PMIPF	SAPR <mark>S</mark> L <mark>GS</mark> AS	CDSVPGSPG\	/Spyapp	PSVPPPPT	MPPLIPPPQP
12. BAM93339.1 T. dicohotomus	MS	ds qeyeakmd	LNASSTSTN <mark>prtp</mark>	PNCARCRNHRL	K-IA <mark>LKGH</mark> KRY(CKYRHCKCEK(RLTSERQRM		QAQDEAMLRQ	gl – – – I <mark>PP</mark> QK	SPAP I HV <mark>P</mark> D)RN	FDCESF	GSSSTPSFPD	VTRKEHVEP\	/NNRETVGN	IND SNVKWG	GYCTSLDNKLSS
13. BAN82532.1L. dispar	MV SVGAWR R	rtp dd ceers	EPGAT <mark>SS</mark> AV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-∨ <mark>ELKGH</mark> KRY(CKYRNCHCEK(QAQDE ARQRA	GMQ -Q <mark>P</mark> GL	EL EHPEPPF	RVK	APR <mark>S</mark> PVIPF	PA <mark>P</mark> PR <mark>SMGS</mark> AS	CDSVPGSPG	ISPY AA-PP	⊤ <mark>sv</mark> psq <mark>pt</mark>	MLPLLPPQQPVIV
14. BAX24553.1P. memnon	MV SVGAWR R	R <mark>S</mark> P D ECDDRT	EPGA <mark>SSS</mark> GV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-V <mark>elkgh</mark> kry(CKYRYCTCEK(QAQ <mark>DE ARAR</mark> A	AEHGHP <mark>PP</mark> GI	EL ERGEP <mark>P</mark>	VK	-APRSPAVPAF	PL <mark>P</mark> PR <mark>S</mark> L <mark>GS</mark> S <mark>S</mark>	CDSVPGSPG\	/SpfApl <mark>Pp</mark>	SVPPPPT	MPPLL <mark>PPQQPA</mark> V
15. BCX65399.1C.punctulatus	ENSGEMGQE	GTRL <mark>DV</mark> L <mark>G</mark> TS	AAT <mark>SSSS</mark> LN <mark>PRTP</mark>	PNCARCRNHRL	K-IG <mark>LKGH</mark> KRY(<mark>ckyryc</mark> n <mark>c</mark> dk(QAQDE ARMAT	EVGMT I GGVN	IS I TASGE SF	АА	AAPGTTT	TAV I T <mark>S</mark> RSMEG	GSCDSSS <mark>S</mark> SPC	STGGRA <mark>P</mark> S	nvsr <mark>p</mark> rvn	P <mark>P</mark> QHNSATPVE F
16. BCX65401.1R. Speratus																		
17. QAB02856.1Bemisia tabaci																		
18. CAG5108149.1 C.congregata	MNIENE	DS STSIEVKS	NGTVN I GLN <mark>prtpi</mark>	PNCARCRNHRL	K-TA <mark>LKGH</mark> KRY(CKYRNCNCEK(dod ts rvr r	PE <mark>E</mark> VEPR <mark>P</mark> LT	LDRER	\SQ	PTRSLE	GSCD <mark>S</mark> SAD <mark>S</mark>	P I SNHGSTG	I H I VSVPNL	QKLS <mark>P</mark> TLN	NHTSSTT <mark>Q</mark> I SEP
19. P23023.1D.melanogaster	DT <mark>MSDSDM</mark> I	DSKNDVCGGA	SSSSGSS <mark>I</mark> SPRTPF	PNCARCRNHG <mark>L</mark> k	K-IT <mark>LKGH</mark> KRY(CK FRYCTCEK(QAQDE QRALH	MHEVPPAN	IPAATTLLS	HHVAAPAH)	/HAHHVHAHHAH	HGGH <mark>HSH</mark> HGHN	/L <mark>H</mark> HQQ A AAAA	AAA A PSA <mark>P</mark> A	SHLGGSS <mark>T</mark>	AASSIHGHAH A H
20. KOB69684.1 O.brumata	∕IVS <mark>M</mark> GGGWR <mark>R</mark>	RVP DD CDERS	DPGA <mark>SSS</mark> AV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-V <mark>ELKGH</mark> KRY(CKYRYCTCEK(QAQ <mark>DE</mark> ARARH	SDP−−PV <mark>P</mark> G <mark>V</mark>	EL EHPEPP\	/VK	APRSPL\	/P <mark>P</mark> PR <mark>S</mark> L <mark>GS</mark> PS	SLD <mark>SVP</mark> E SPG V	/Spyasl <mark>pp</mark>	SAPPPPT	MPPLL <mark>PPQQP</mark> DN
21. KYB26747.1T. castaneum	MSS	ds q d fdskmd	VNASSTSASP <mark>rtp</mark> i	PNCARCRNHRL	K-IA <mark>LKGH</mark> KRY(CKYRTCKCEK(RLTTERORM		DAQDEA MLRS	GSA −−<mark>V</mark>D<mark>P</mark>AI	MQVPLKSP	;		- – <mark>P</mark> I <mark>H</mark> A I ERSL	.DCDSS <mark>AS</mark> SQ0	SNPPPAIR	KMT <mark>P</mark> V <mark>P</mark> AV	PSSTSVNIGTIA
22. QEQ48787.1 H. turmalis	M <mark>S</mark> EDE	RGPVSSKA <mark>G</mark> K	GDR H <mark>SS</mark> DRDE GK KF	rc <mark>carcrnh</mark> ete	EL <mark>IELKGH</mark> KN <mark>y(</mark>	CPWRDCF <mark>CE</mark> L <mark>(</mark>	LK T VF RQ KL <mark>N</mark>	MADQTAS RRKI	RDEMK E <mark>RA</mark> KK	GL P L V <mark>VP</mark> ⊤AK		GA	A	<mark>P</mark> pqpppit <mark>s</mark>	F I <mark>S</mark> SQSD <mark>P</mark> RN	/KYLEGLES	P P F P E E D G	gsvprvgvvd <mark>ac</mark>
23. QEQ48788.1M. americana																		
24. QGB21101.1Rhodnius prolixus	MSSG	VGAA S Q Q P M Q	QQQNPAQPAA <mark>RTP</mark>	PNCARCRNHS-K	KTEP <mark>lkgh</mark> krf	CKYRTCTCKK(HLTVERQRE	MAKQTAL RR E	L aqdearar a	GLQPAS <mark>PP</mark> PS	ATSP <mark>P</mark> PPX1	-GQ	PA <mark>S</mark> HLSTA	ASSLQTT <mark>G</mark> GSN	ISGE <mark>V</mark> CWQSII	ALQE A LQL	rldtl <mark>p</mark> lv	Y I I <mark>L</mark> KFTKSYDE
25. XP 022116425.1 P. rapae	MV SVGAWR R	r <mark>s</mark> p dd ceers	EPGT <mark>SSS</mark> GV <mark>PR</mark> A <mark>P</mark>	PNCARCRNHRL	K-IELKGHKRY(QAQDE ARARA	I E T G G P <mark>P P</mark> G L	EL DR P E P P	VK	APRSPVVF	PA <mark>P</mark> RSIGS <mark>S</mark> S	VG <mark>SVP</mark> G SPG \	/ <mark>S</mark> phvha <mark>p</mark> v	₽Q <mark>V</mark> PPQ <mark>PT</mark>	MPPL LPPQQPAL
26. XP_046805585.1 L. cupriana	DT <mark>MSD</mark> T DM H	DSKND I <mark>CGG</mark> A	SSSSGSSGTPRTK	PNCARCHNHGF	K-IK <mark>LKGH</mark> KRY(QQ <mark>QDE</mark> Q R ILQ	MHE <mark>V</mark> PP/V <mark>V</mark>	hp <mark>p</mark> pa <mark>l</mark> lk <i>i</i>	(<mark>H</mark>		Y <mark>H</mark> H <mark>H</mark> HHLQ	0.000000000000000000000000000000000000	HHPHLVDA	TTSAAVVG	AV <mark>P</mark> PHHHHHHHV

	1,160	1,170 1,	180 1,190	o qoo	1,210	1,220	1,240	1,250	1,260	1,70	1.80 1,290	qoo	1,:i10	q20	1,330
Consensus		PHSSWXMXHXVL	VIENYAXNDLEE	ASR	QWRLHELNL	SLLELQAXXX	XPEXAP	HSLPXPLTTXRA	XPPAAX t	:lGSS(j(jli_(!	GRSSXXS	TSAXHXX TXVPTPX	QSLEGSCISS	SPSPSSTS <mark>GA</mark>	AILPXXS
dentity				_		_									
1. ADL40855.1 A. mylitta	ETLVINO{K EK	FHYSWEMMP LVL	VILNYAGSDLEE	ASR-KIDEAH		Y <mark>SLLELQA</mark> RKG T YA			SPPPAHL						
2. ADL40846.1 A. assamensis	ETLV ENCHKLL EK	FHYSWEMMPLVL	VILNYAGSDLEE	ASR-KIDEAHW	VHQWRLYEHSLO	CSLLKLQAQKGTYA	MCCSPRYVLAPEYAF	HLLP I PLTTORAS	SPPPAHL						
3. AAA17840.1 D.melanogaster	HMAAAAAASVAQH	QHQSHPHSHH	H		HQNH	HQHPHQQPATQ T AL	RSPPHSDHGGSVGPA	TSSSGGGAPSSS	NAAAATSS G	G-5SGG(lG(j(j(j(j	(SjSfG0GA.0(1GRSSGTIS	S TSAD HM: J:eA	O:S GSCDSSS	PSPSSTS":G88	&I]JE-'IS
4. AAB99947.1 B. tryoni	jlA.GGIGSAIT[S	:lilPGSVPPPE	HMTTVPTP Q	SLEGSSDTSSPSI	PSSTSGAVLPISW	GRKPS HPNGVNIE	LAQDVFLEHCQKLLE	KFRYPWEMMPLMYV	VILKD GAD	IEEASRRIEEGQH	WNEYSRQHNLNIYDGGE	ELRSTTRQCG			
5. AAQ82648.1 A. melifera	⁰QTLTSENVEILL	SSKLVELFLSL(GADATRA												
5. AFH41442.1 A. fraterculus	jMV:GGTVPTITIS	flvSAPPPE H													
7. AFH41495.1 A. sororcula	jgv:-QGTVBTIT\5	PVSAPPPE h h													
3. AFH41496.1	G GTV <mark>P</mark> TIT <mark>S</mark> ▼	₽∨ S APPPE <mark>H</mark> H													
9. AFH41497.1	j GGTVPTITIS	VSAPPPERH													
10. AWC26116.1 B. tabaci															
11. XP_037876116.1B. mori	∘GAFPVg P GHV S EÇ)RL S QEGNIKAVF	SETLVE CHRLL	EK- FHYSWErv	MPLVLVIMNYAR	RS DEASRKIYEGK	MIVDEYARKHNLNVF	DGLELRNS TR QKM	ALEINNISG\	/L S SSMKLFCE					
12. BAM93339.1 T. dicohotomus	<dllqdcf{k dki<="" td=""><td>FQYPWE MPLLY</td><td>KL P LEIA</td><td>SKRIDEGKGAE</td><td>QLVDFFYKIKDKI</td><td>F SWKMISLVQVI</td><td>LKSAKDDQEEAFRQI</td><td>DAAFLEILAIV 1</td><td>KYYYPQINT</td><td>ISSNILYPSLYPF</td><td>AFYPSMYLSAVYQNPI</td><td>LLNSIPPSPPLHSPIPP</td><td>PPRPRSRA</td><td></td><td></td></dllqdcf{k>	FQYPWE MPLLY	KL P LEIA	SKRIDEGKGAE	QLVDFFYKIKDKI	F SWKMISLVQVI	LKSAKDDQEEAFRQI	DAAFLEILAIV 1	KYYYPQINT	I SS NILYPSLYPF	AFYPSMYLSAVYQNPI	LLNSIPPSPPLHSPIPP	PPRPRSRA		
13. BAN82532.1 L. dispar	ETLVIS::O{K E	KFNY SW E MPL	YR DI	ASR-K 8EWA	'rllRS	S EK!A.RKAW	IH GCSTR Arp FAB	S PLP TTRRTP'	'PP,11/HL						
14. BAX24553.1P. memnon															
15. BCX65399.1 C.punctulatus	v1SA.!3SPSKSE	FP S L P FQDSSR	GFTLHEVTAQA	D S SDVSRISIQA	LLEMFRF EAL	P IYWLQVSQSDVT	MASNRI KGKTLHVF	ICYE I IYQTIY	YTIFI VYS						
16. BCX65401.1 R. Speratus															
17. QAB02856.1 Bemisia tabaci															
18. CAG5108149.1 C.congregata															
19. P23023.1 D.melanogaster	1MAAAAAASVAQH	H QSHPHS H H		Н	H HHQ	HPHC;KlPATQ T ALRS	PP SDHGGSVGPATSSSG	GGAPSSSNAA!A.AT	rssl'-J.GSSC	GGG:G_G_G_G_G_G	SS:G_GGAG HSSGTIS	TSAD HM.: PTeAO:	S GSCDSSSPS	PSSTS	Pl S
20. KOB69684.1 O.brumata															
21. KYB26747.1T. castaneum	ffillIDCC1K ER	FKYPWE MPLMY	KDM ADLEE	ASRRIDEGKR	NEYS rlhnln m	YDG RNSTRRDTE	ILLDFCQRLKDKFQL	SWKMIŚLVDVILKY	Y,11/KDQDE	AWRQIDEAFLEIR	ALAAVEAARYTYHHIP	YSGLYPNAATAIYPPVY	(LP S_ MSMY	ATLLGSSVI	KTLRDIVKH
22. QEQ48787.1 H. turmalis	EVSV\IIAITE NAI	FNFNVNTSCLLYI	」 K <mark>Y</mark> IS N NSVK	DARSIIQSA											
23. QEQ48788.1 M. americana															
24. QGB21101.1 Rhodnius prolixus	KIIAENKPASLR <mark>V</mark>	PKI													
25.XP_022116425.1 P. rapae	EPLVENCHKLLDI	FHYSWEMMP LVL	VILNYAGNDLEE	ASRKIDEGKMII	NEYARKHNUNIF	D RNSTRHDR	TKLEKFD								
26.XP_046805585.1 L. cupriana	AAAAAAISTIRSP	PHSDHSVNGGSS	AGGGGGNNGGGG	GGGSAGGGVVSS		NGMASSSS I ASSSTA	GPP HPSPDHHQQHN	NQHHH НН НРНРНЗ	SS VPPTAQS	VDSSCDSSSPSPS	STS[3AI SLPVNRK	VPEH QNGADMSIDLI	DYCQKL I EF	(FGYPWErvMP)	LMYVIL KDA1



Figure S9. Relative expression of double sex (*dsx*) in male and female Asiall-1 whitefly, using two different sets of primers targeted to two different regions of the Btdsx gene (A) Expression normalized individually (for F primer ACAAGTCGAGGGTCAATTCC; R primer GTGTACGGGCAGTAGCGTTT) using Tubulin, GADPH, GST and a combination of all three of the housekeeping genes (denoted as Combined) (B) Expression normalized individually (for F primer AAGCTGAGACTCGCAGACC; R primer ACGCGGCGAATGGAATTTT) using *Tubulin, GADPH, GST* and combination of all three of the housekeeping genes (denoted as Combined). * indicates a significant difference compared to the control (p=0.05). Error bars represent the standard error of the mean.







Figure S10b





Figure S10 a-c. Impact of *Bemisia tabaci* Asiall-1 doublesex (*Btdsx*) gene knockdown (using dsRNA amplified from different regions of the *Btdsx* gene) on mRNA levels of double sex, cytochrome p450 (*CYP540*) and alkaline phosphatase (*ALP*) in male and female adults. The expression data was normalized with tubulin as an internal control. * indicates significant difference as compared to control (p=0.05) using Student T-test. Error bars represent the standard error of mean. *Btdsx* primer combinations employed in S10 a-c respectively were:

Fprimer:TAATACGACTCACTATAGGGACATGGCCAAGACGATCAAGAAG RPrimer:TAATACGACTCACTATAGGGTGACTCCCACAGTGATTCGT

Fprimer:TAATACGACTCACTATAGGGGAAGCTGAGACTCGCAGACC Rprimer:TAATACGACTCACTATAGGGGACGCGGCGAATGGAATTTT

Fprimer:TAATACGACTCACTATAGGGAAGAAACTCGTGCGTGGACA Rprimer:TAATACGACTCACTATAGGGTCTGATGTCGTATATCGGAGAATGAC

Supplementary Table 1. *Bemisia tabaci* Asiall-1 male and female adult combinations for feeding dsRNA against double sex (*dsx*) gene

S. no	Male and female combination	
1	dsdsx fed ♂ + dsdsx fed ♀	
2	dsGFPfed ♂ + dsdsxfed ♀	
3	dsdsx fed ♂ + dsGFPfed ♀	
4 (Control)	dsGFP fed ♂ + dsGFP fed ♀	

Supplementary Table 2. Attributes of the *Bemisia tabaci* Asiall-1double sex (*dsx*) gene based on alignment with genomic scaffold 130 of the MEAM1 whitefly genome

Exon	cDN	A	Genom	ic DNA	Length of	ength of Splicing site Siz	
details	Start	End	Start	End	exon (bp)		(bp)
Exon #1	1	688	1190	1877	688	<exon>GT</exon>	139313 (Exon#1- Exon#2)
Exon #2	689	856	141190	141357	168	AG <exon>GT</exon>	29399 (Exon #2- Exon#3)
Exon #3	857	949	170756	170848	93	CA <exon>GT</exon>	22697 (Exon #3- Exon#4)
Exon #4	950	1132	193546	193728	183	GG <exon>GT</exon>	14164 (Exon #4- Exon#5)
Exon #5	1133	1239	207893	207999	107	AG <exon>GT</exon>	4712 (Exon #5- Exon#6)
Exon #6	1240	4866	212710	216336	3627	AG <exon></exon>	

Supplementary Table 3. Eggs laid by females after feeding of dsRNA (400ng/ μ l) to *Bemisia tabaci* Asiall-1 male and female adults in different combinations. * indicates significant difference as compared to control (p=0.05) using Student T-test Error. N = sample size

Treatments	Mean ±S.E. _m Fecundity (N)
dsdsx fed male + dsdsx fed female	8.27±0.20 * (273)
dsGFP fed male + dsdsx fed female	12.3±0.22 * (406)
dsdsx fed male + dsGFP fed female	15.06±0.14 * (497)
dsGFP fed male + dsGFP fed female (Control)	21±0.51 (2079)

Supplementary Table 4. Mean percent hatching rate of eggs after feeding of dsRNA (400ng/ μ l) to *Bemisia tabaci* Asiall-1 male and female adults in different combinations. * indicates significant difference as compared to control (p=0.05) using Student T-test. N = sample size

Treatments	Mean ±S.E. _m percent hatching (N)
dsdsx fed male + dsdsx fed female	68.58±0.2* (183)
dsGFP fed male + dsdsx fed female	61.60±0.18* (244)
dsdsx fed male + dsGFP fed female	68.83±0.16* (348)
dsGFP fed male + dsGFP fed female	85.46±0.11 (1790)

Supplementary Table 5. Impact of double sex (*dsx*) gene knockdown on male:female ratio of offspring after feeding of dsRNA (400ng/µl) to *Bemisia tabaci* Asiall-1 male and female adults in different combinations.

Treatments	Mean ±S.E. _m percent female population
dsdsx fed male + dsdsx fed female	46.98±0.18
dsGFP fed male + dsdsx fed female	52.70±0.15
dsdsx fed male + dsGFP fed female	47.76±0.20
dsGFP fed male + dsGFP fed female (control)	48.43±0.11