(A) (B)

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

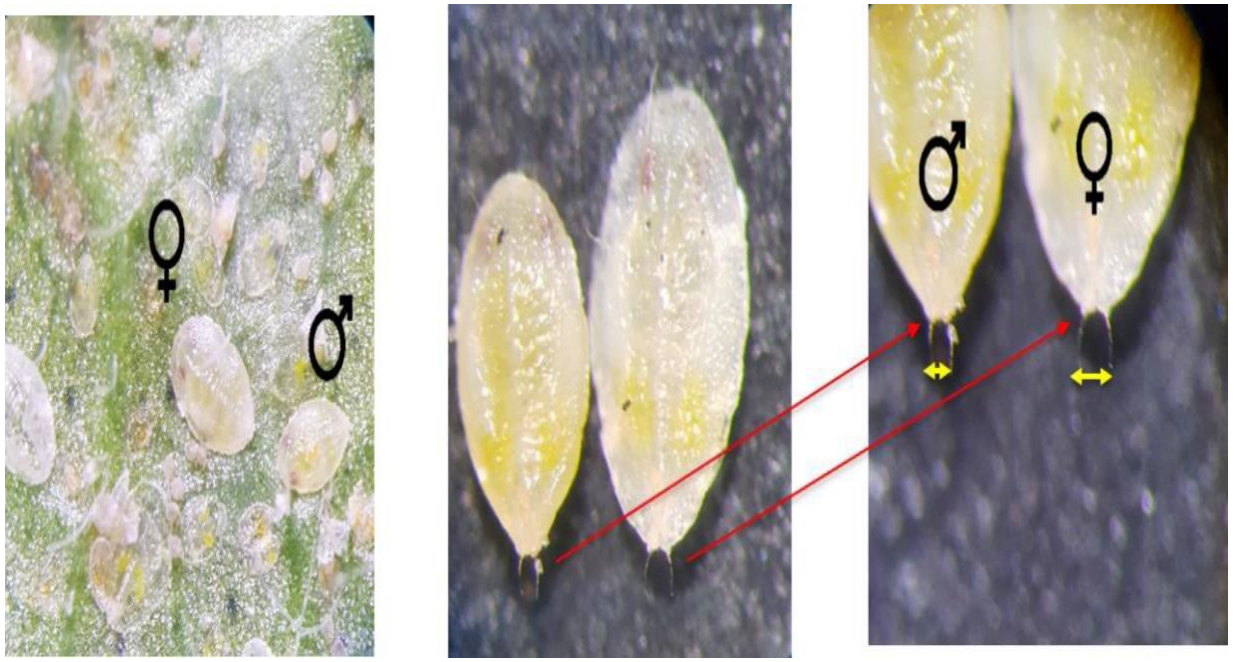
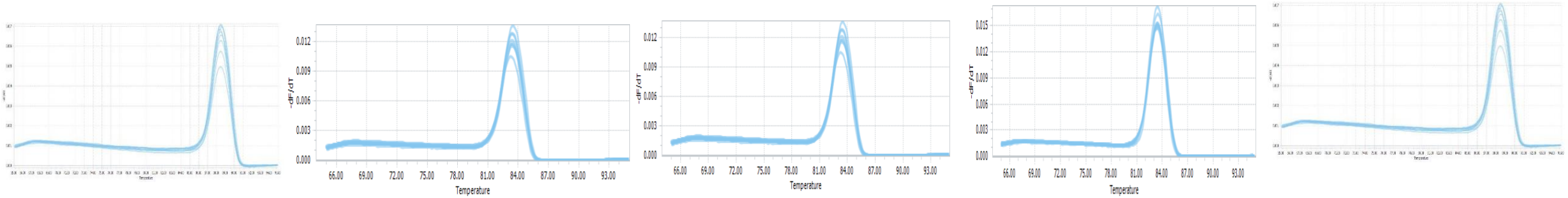


Figure S1b. Sexual dimorphism in puparia of *Bemisa tabaci* Asia II-1 (male and female). Size of double headed arrows (in yellow) depict the width of the caudal furrow - narrow in male pupa and wider in female pupa.



A. **B.** **C.** **D.** **E.**

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*).

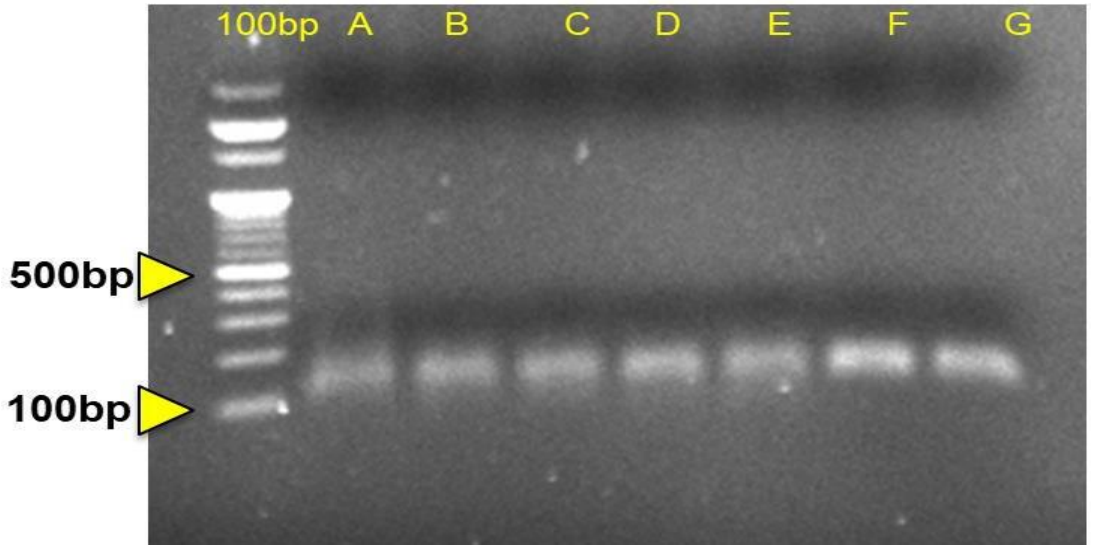


Figure S2b. Gel electrophoresis after specific amplification of the double sex gene via RT-qPCR for different stages of *Bemisia tabaci* AsiaII-1, depicting (A) egg (B) crawlers (C) 2nd instar (D) 3rd instar (E) red eyed pupa (F) male adult and (G) female adult.

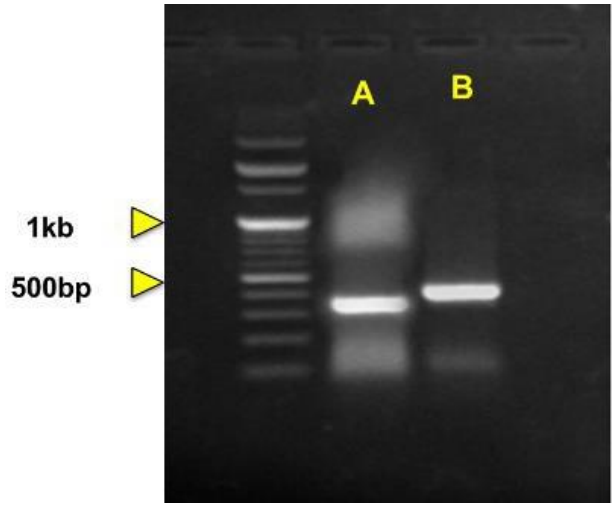


Figure S3. Gel electrophoresis of dsRNA after specific amplification of (A) ds*Btdsx* and (B) dsGFP.

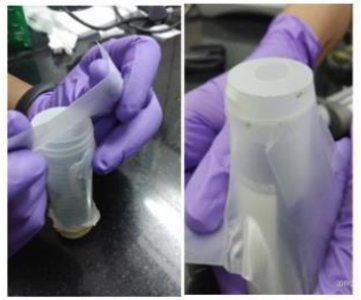
Collection of leaves having red eyed pupa

Wrapping of tube with aluminum foil before temporary inactivation of whiteflies at -20

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.

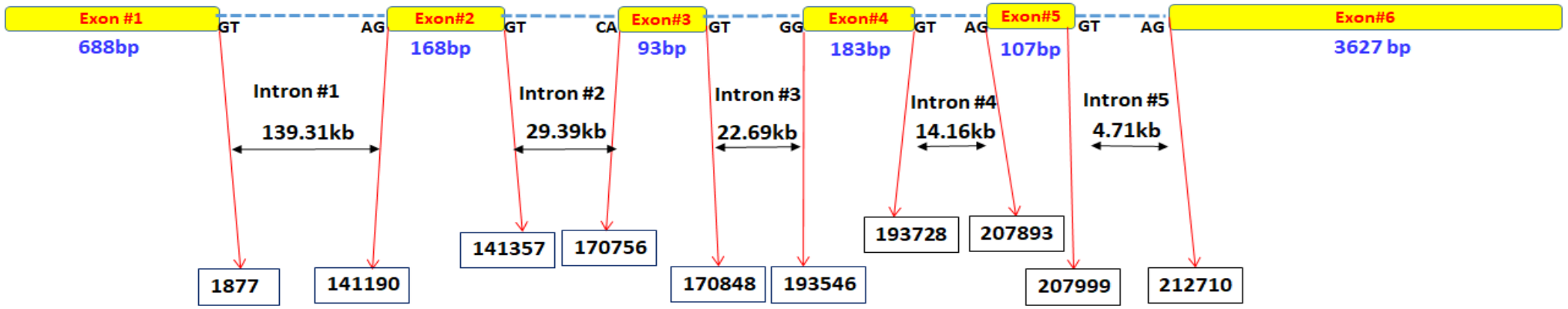


Figure S5. Gene structure of *Bemisia tabaci* double sex (*dsx*) gene based on alignment of cDNA with genomic DNA from Scaffold 130 of the MEAM1 whitefly genome using Splign (NCBI). The boxed numbers represent the specific locations of splice sites on the MEAM1 genomic DNA scaffold.

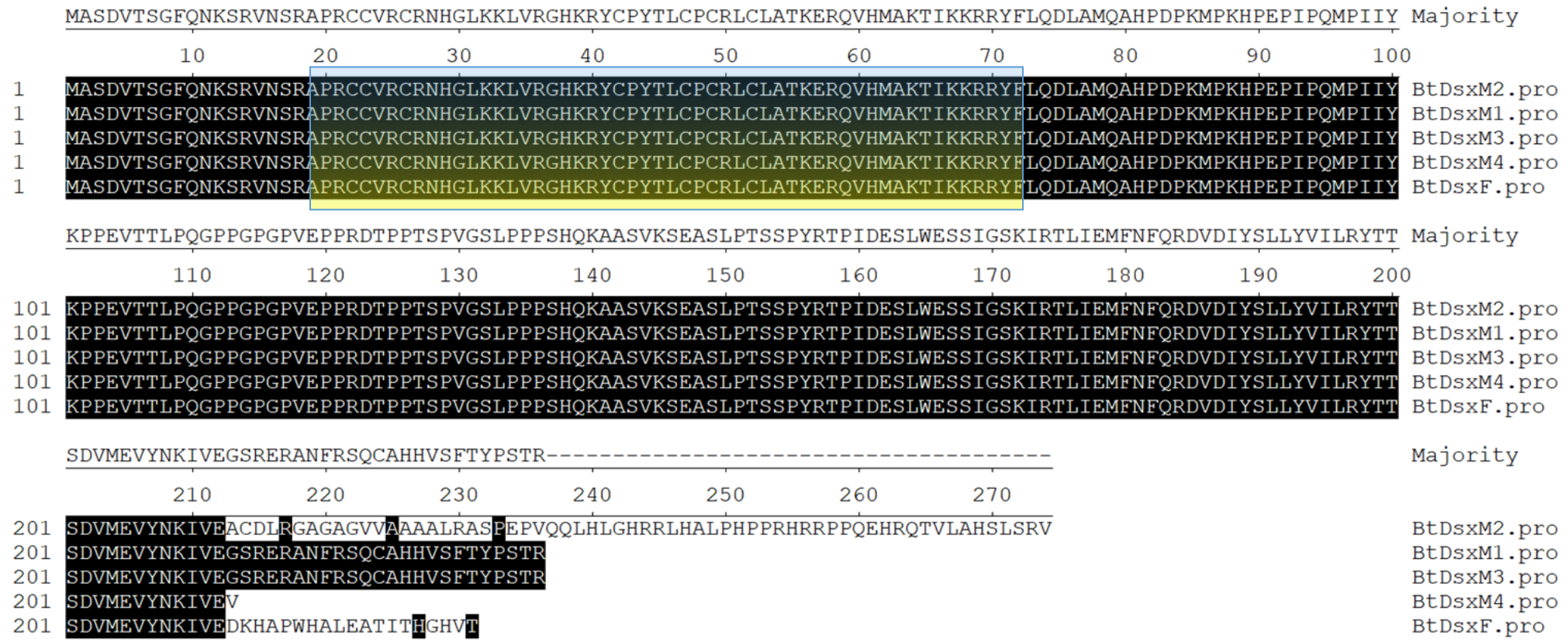
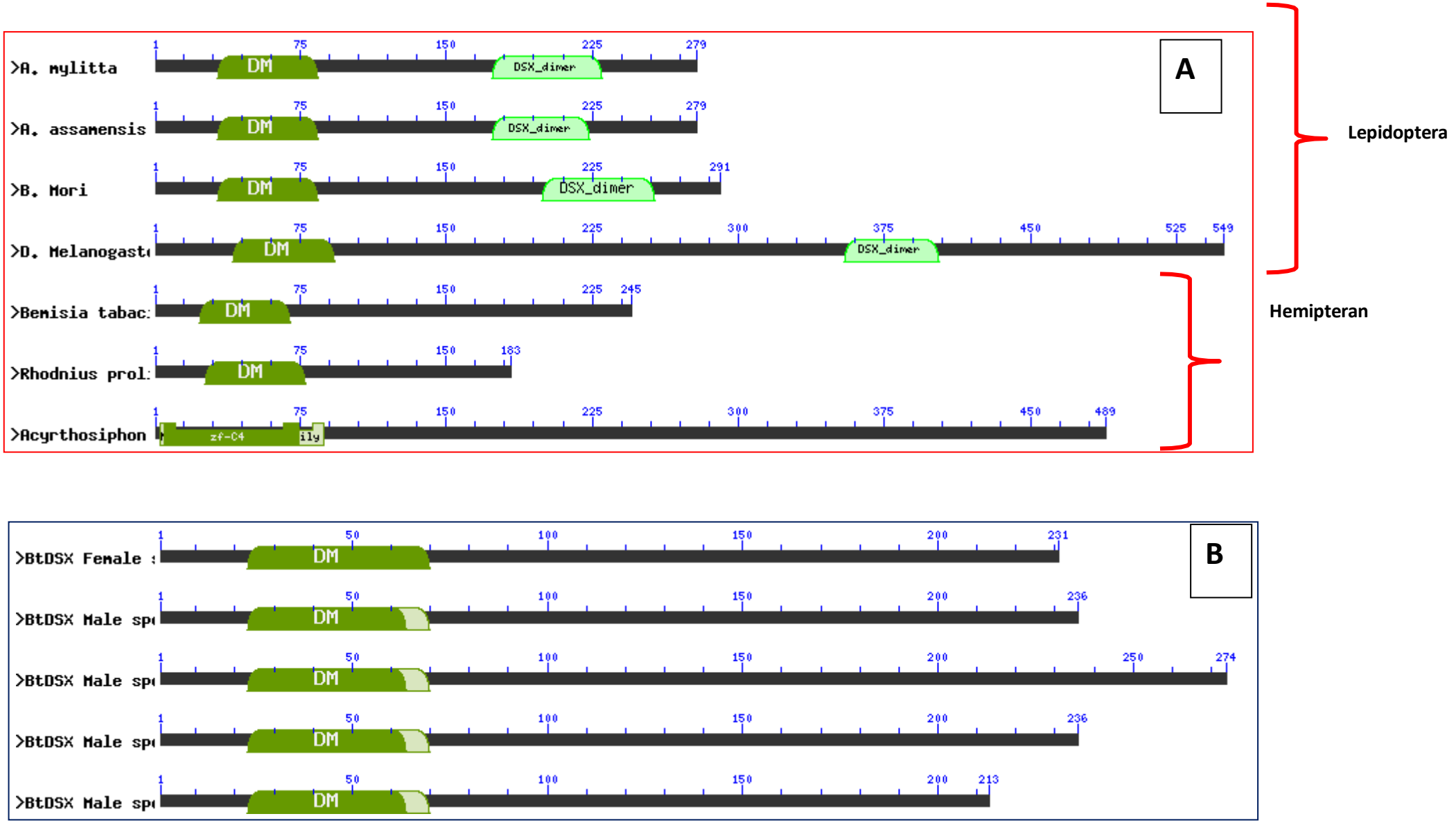


Figure S6. Multiple sequence alignment of the male and female specific transcripts of AsiaII-1 Dsx protein. The region highlighted in yellow represents the DNA binding domain (OD1), also known as the DM domain. The OD2 domain (oligomerization domain) was not detected in these BtDSX isoforms. Comparable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

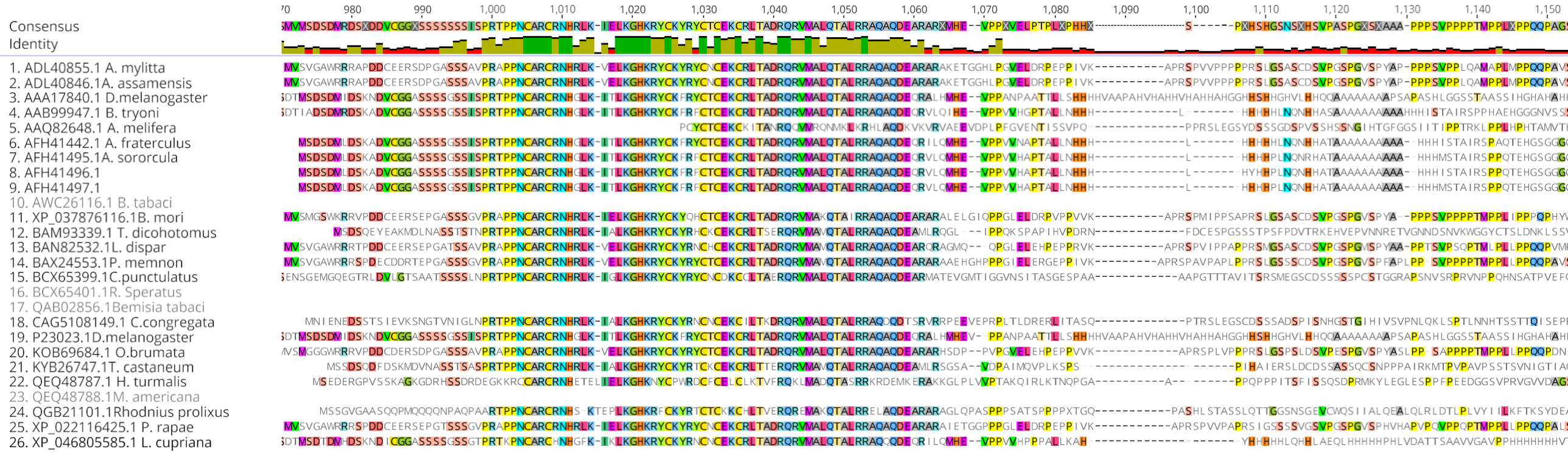

A
>A. mylitta
>A. assamensis
>B. Mori
>D. Melanogast
>Bemisia tabac
>Rhodnius prol
>Acyrthosiphon
DM
DSX_dimer
zf-C4
Lepidoptera
Hemipteran
B
>BtDSX Female
>BtDSX Male sp
>BtDSX Male sp
>BtDSX Male sp
>BtDSX Male sp

C


>A. mylitta
>A. assamensis
>B. Mori
>D. Melanogast
>Bemisia tabac
>Rhodnius prol
>Acyrthosiphon
DM
DSX_dimer

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.




Consensus
Identity
1. ADL40855.1 A. mylitta
2. ADL40846.1 A. assamensis
3. AAA17840.1 D.melanogaster
4. AAB99947.1 B. tryoni
5. AAQ82648.1 A. melifera
6. AFH41442.1 A. fraterculus
7. AFH41495.1 A. sororcula
8. AFH41496.1
9. AFH41497.1
10. AWC26116.1 B. tabaci
11. XP_037876116.1B. mori
12. BAM93339.1 T. dicohotomus
13. BAN82532.1 L. dispar
14. BAX24553.1P. memnon
15. BCX65399.1 C.punctulatus
16. BCX65401.1 R. Speratus
17. QAB02856.1 Bemisia tabaci
18. CAG5108149.1 C.congregata
19. P23023.1 D.melanogaster
20. KOB69684.1 O.brumata
21. KYB26747.1T. castaneum
22. QEQ48787.1 H. turmalis
23. QEQ48788.1 M. americana
24. QGB21101.1 Rhodnius prolixus
25. XP_022116425.1 P. rapae
26. XP_046805585.1 L. cupriana

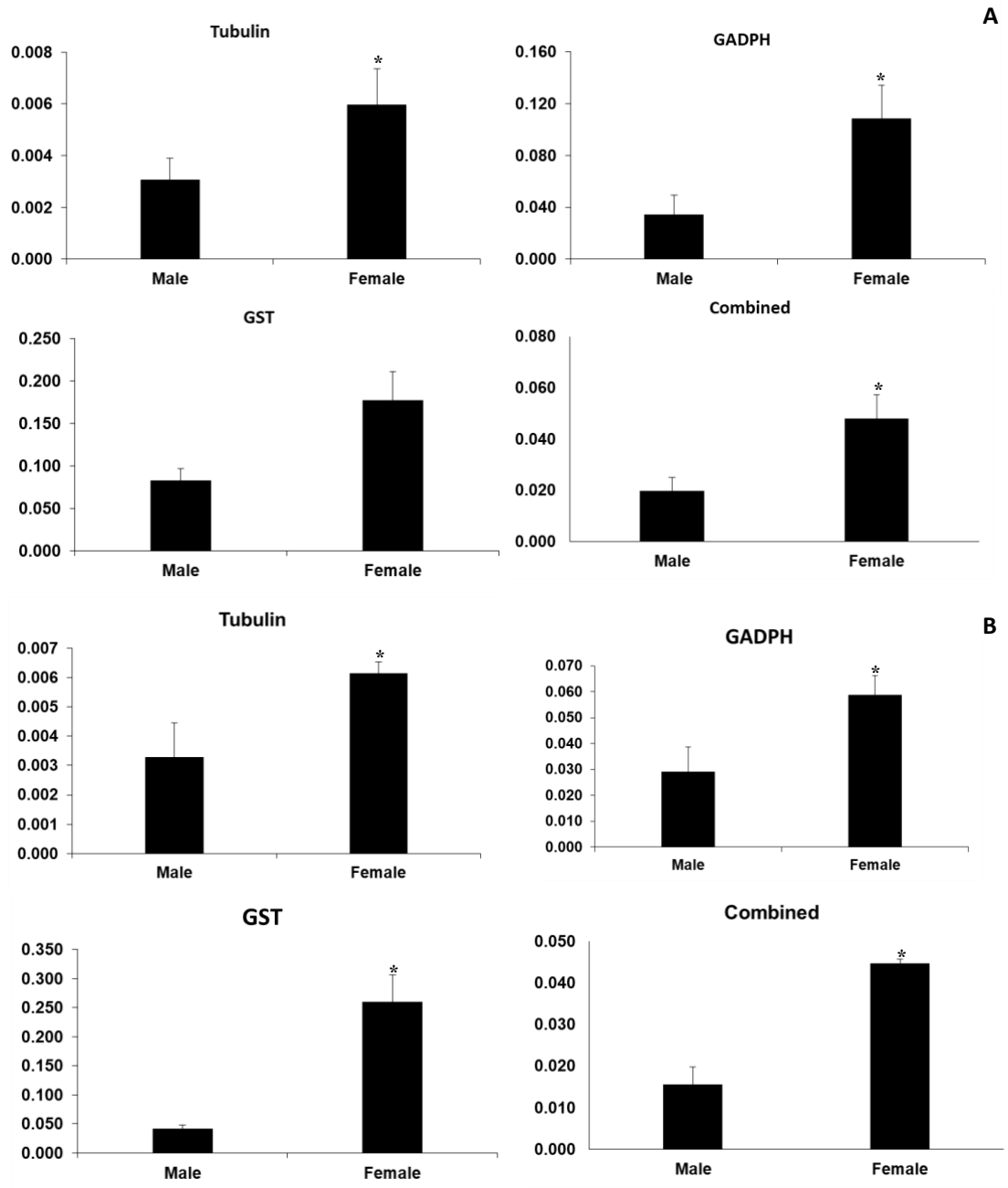
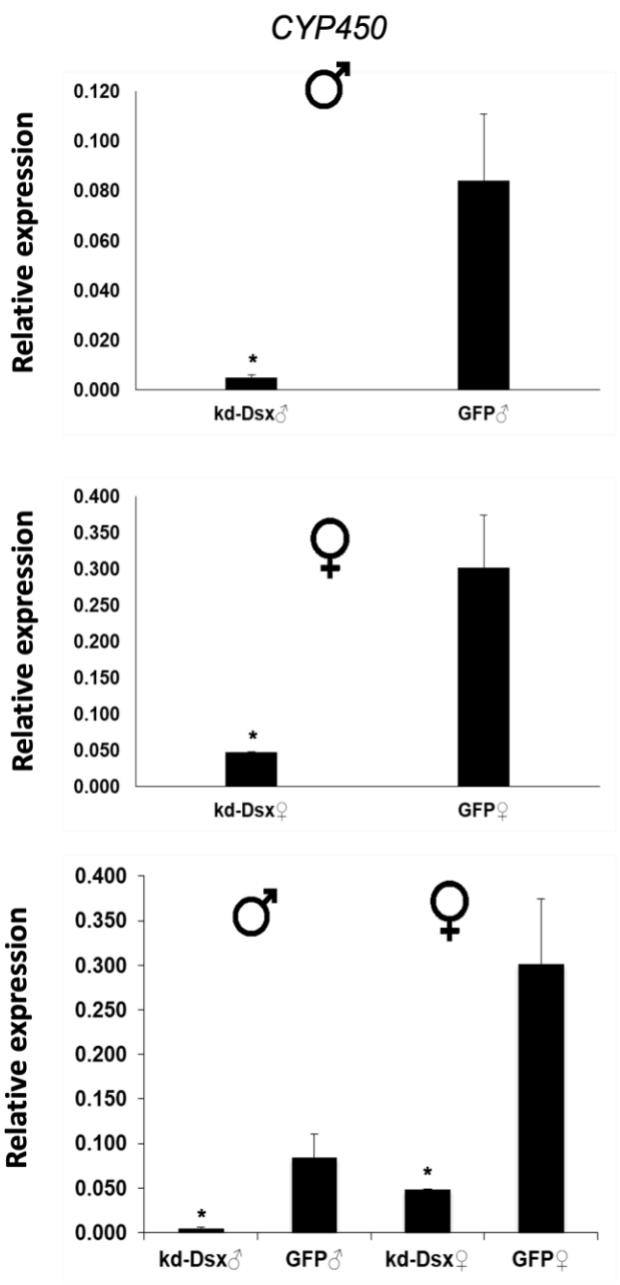


Figure S9. Relative expression of double sex (*dsx*) in male and female AsiaII-1 whitefly, using two different sets of primers targeted to two different regions of the Btdsx gene (A) Expression normalized individually (for F primer ACAAGTCGAGGGTCAATTCC; R primer GTGTACGGGCAGTAGCGTTT) using Tubulin, GADPH, 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.


CYP450
Relative expression
♂
0.120
0.100
0.080
0.060
0.040
0.020
0.000
*
kd-Dsx♂
GFP♂
Relative expression
♀
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
*
kd-Dsx♀
GFP♀
Relative expression
♂
♀
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
*
*
kd-Dsx♂
GFP♂
kd-Dsx♀
GFP♀
A

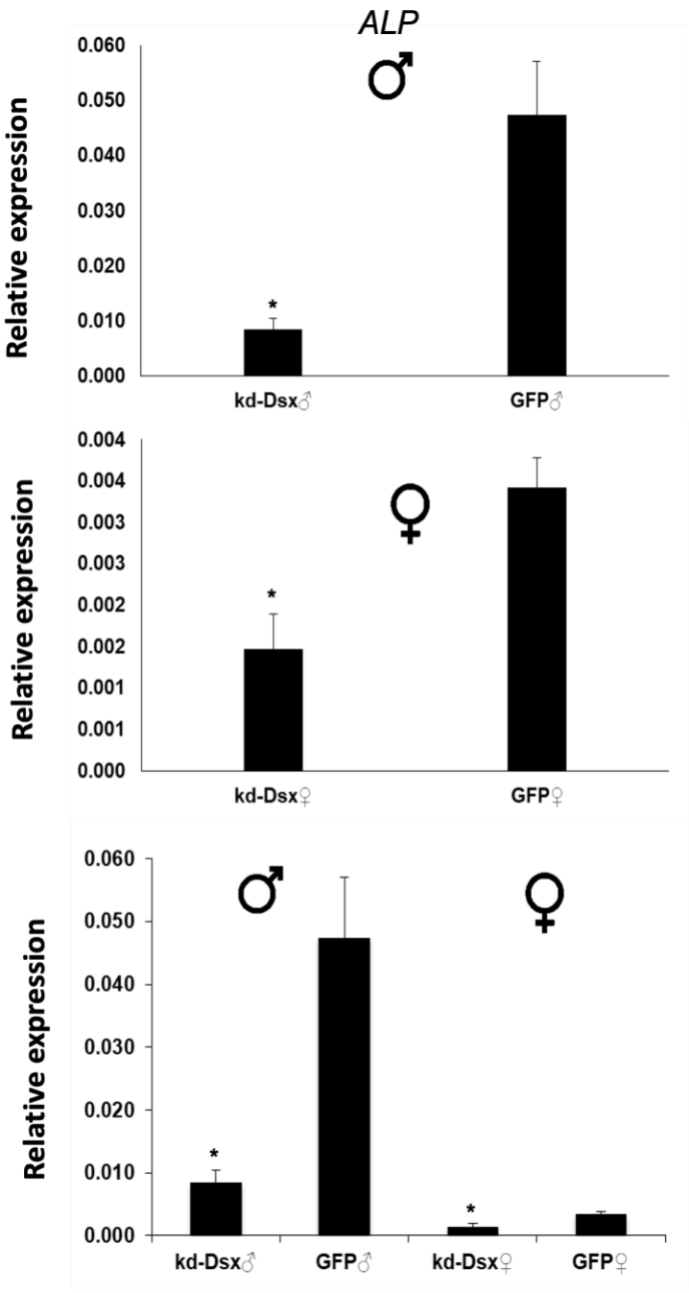

ALP
Relative expression
♂
0.060
0.050
0.040
0.030
0.020
0.010
0.000
*
kd-Dsx♂
GFP♂
Relative expression
♀
0.004
0.004
0.003
0.003
0.002
0.002
0.001
0.001
0.000
*
kd-Dsx♀
GFP♀
Relative expression
♂
♀
0.060
0.050
0.040
0.030
0.020
0.010
0.000
*
*
kd-Dsx♂
GFP♂
kd-Dsx♀
GFP♀
B

Figure S10a.

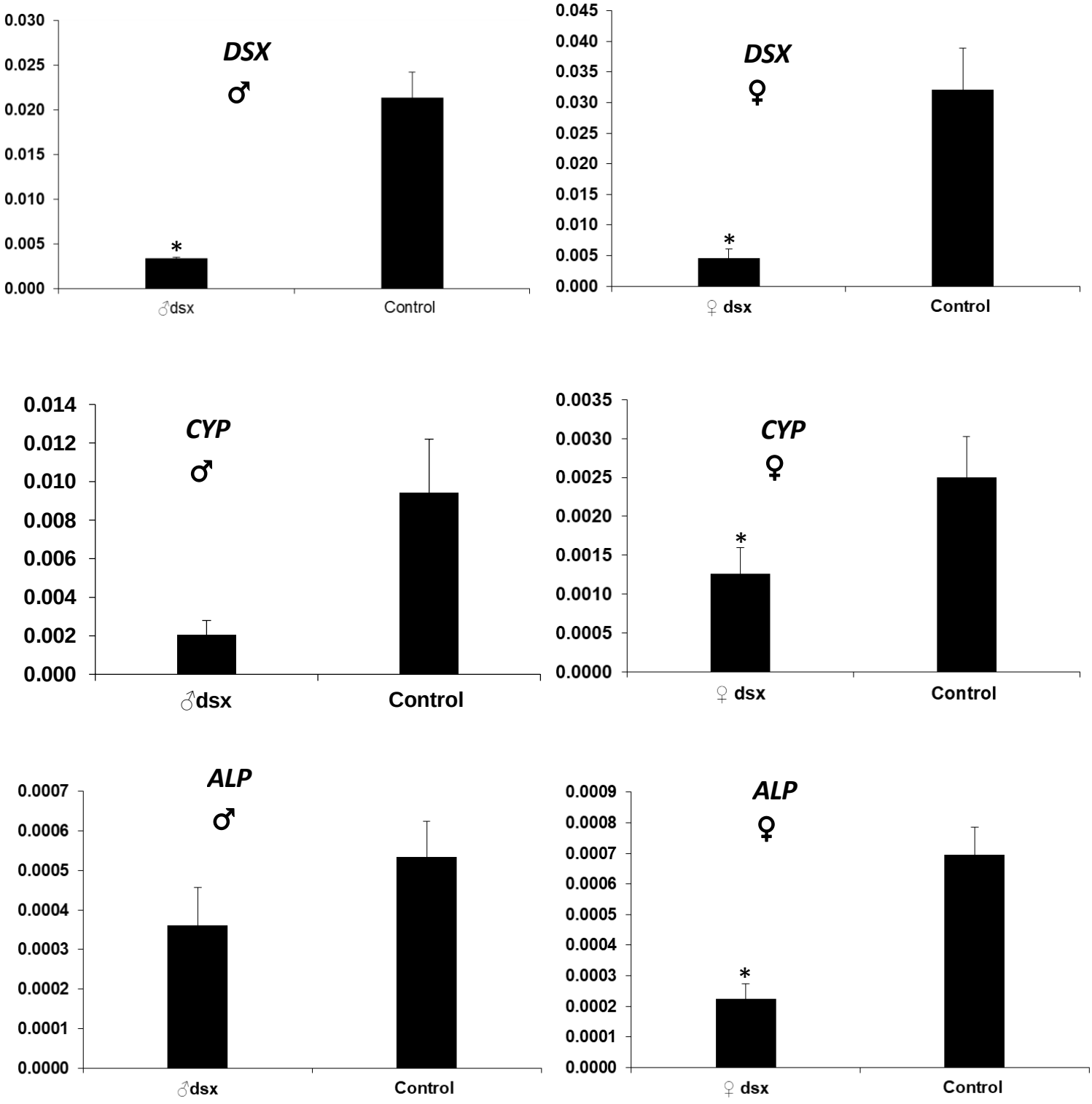

DSX
♂
0.030
0.025
0.020
0.015
0.010
0.005
0.000
*
♂dsx
Control
DSX
♀
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
*
♀ dsx
Control
CYP
♂
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
♂dsx
Control
CYP
♀
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
*
♀ dsx
Control
ALP
♂
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
♂dsx
Control
ALP
♀
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
*
♀ dsx
Control

Figure S10b

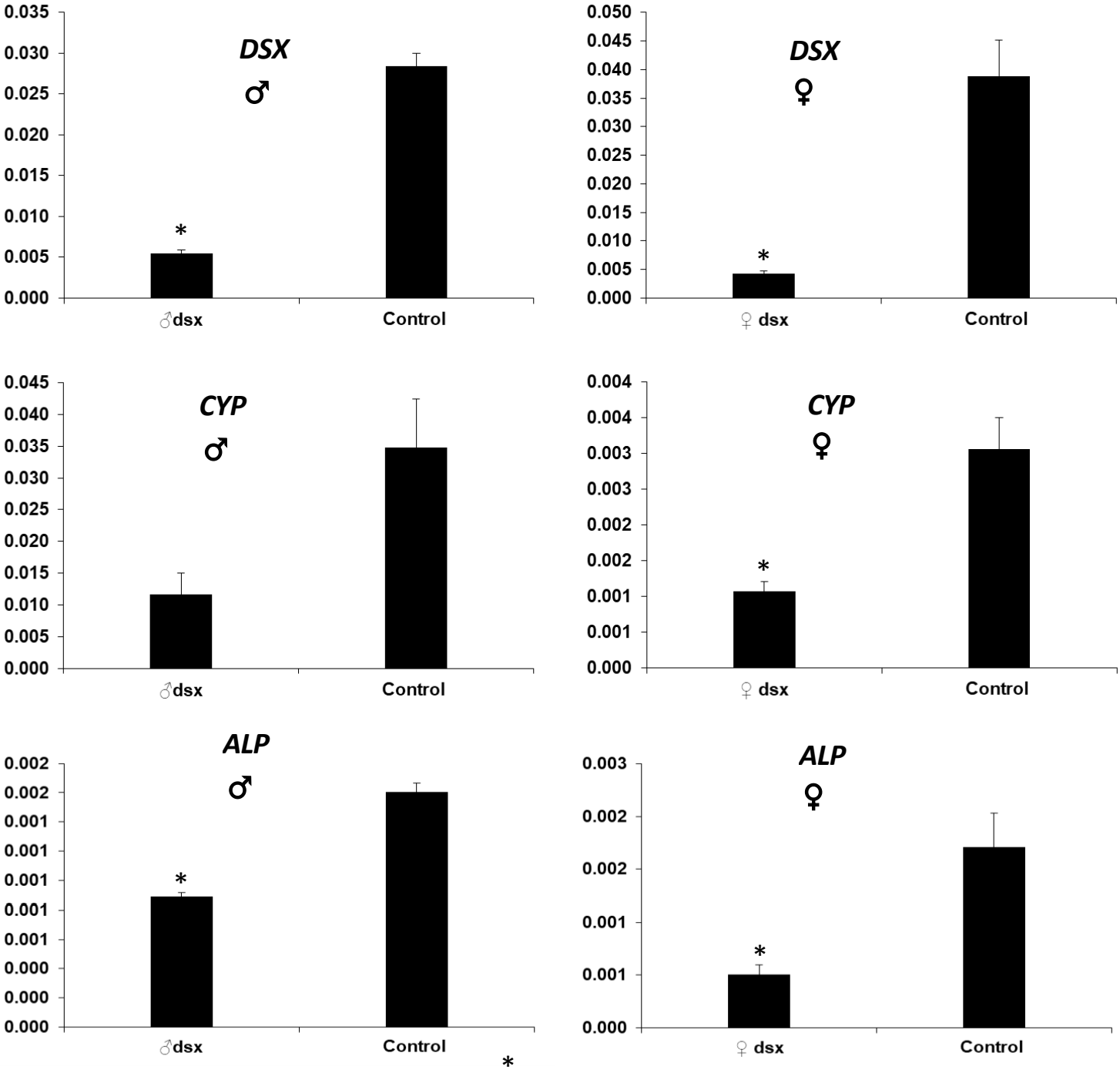


Figure S10c

Figure S10 a-c. Impact of *Bemisia tabaci* AsiaII-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* AsiaII-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* AsiaII-1double sex (*dsx*) gene based on alignment with genomic scaffold 130 of the MEAM1 whitefly genome

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

Supplementary Table 3. Eggs laid by females after feeding of dsRNA (400ng/µl) to *Bemisia tabaci* AsiaII-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/µl) to *Bemisia tabaci* AsiaII-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* AsiaII-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