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Novel female-specific splice form of *dsx* in the silkworm, *Bombyx mori*

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Abstract The *Bombyx mori doublesex* (*Bmdsx*), a homologue of *doublesex* of *Drosophila,* is the bottom most gene of the sex determination cascade. *Bmdsx* plays a very crucial role in somatic sexual development. Its pre-mRNA sex-specifically splices to generate two splice variants; one encodes female-specific and the other encodes male-specific polypeptides which differ only at their C-termini. The open reading frame of *Bmdsx* consists of 5 exons, of which exons 3 and 4 are female-specific and are skipped in males. In the present study, we have identified a third splice form of the *Bmdsx* which is specific only to females and differs from the previously reported *Bmdsxf* isoform by the presence of 15 bp sequence. This new female splice form is generated as a result of alternative 5′ splice site selection in the third exon adding additional 15 bp sequence in exon 3 which results in alteration of the reading frame leading to incorporation of an early stop codon. Thus the protein encoded by this splice form is 20 aa shorter than the known BmDsxF. Initial results obtained from the study of *dsx* homologues in Saturniid silkmoths suggest that both the female-specific Dsx proteins are essential for female sexual differentiation. It remains to be seen whether female-specific multiple splice forms of *dsx* are characteristic feature of only silkmoths or widespread among lepidopterans. The findings that sex determination mechanism is unique in lepidopterans offer an opportunity to develop genetic sexing methods in beneficial as well as economically destructive lepidopteran pests.

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Introduction

Sex determination is a fundamental biological process of profound significance in the development, genome evolution and maintenance of proper sex ratio in a species (West et al. 2002). Organisms have evolved myriad mechanisms via which sex of the individual is determined (Bull 1983; Zarkower 2001). Within the insect species the sex determination pathway has been well investigated in *Drosophila melanogaster* (MacDougall et al. 1995; Cline and Meyer 1996; Schutt and Nothiger 2000; Sanchez 2008). The process of sex determination in *Drosophila* is thought to be governed by the signal initiated by X:A ratio (Bridges and Anderson 1925). *Sex-lethal* (*Sxl*), the top most gene of *Drosophila* sex determination cascade, responds to this signal (Penalva and Sanchez 2003; Cline 1983, 1978). However, in a recent study it has been shown that the X chromosome dose, not the X:A ratio, signals the sex determination cascade in *Drosophila* (Erickson and Quintero 2007). At blastoderm stage the early promoter (*Sxl-pe*) of *Sxl* gene gets activated in females by double dose of X signalling elements (XSE) whereas in males where XSEs are present in single dose it remains inactive (Erickson and Quintero 2007). This leads to early pulse of Sxl protein only in females. Once the activity of *Sxl* is established, the signal from XSEs is no longer needed (Sanchez and Nothiger 1983; Bachiller and Sanchez 1991). The *Sxl* late promoter (*Sxl-pm*) is active in both males and females but the transcript contains an in-frame stop codon. Because of the early pulse of Sxl protein only in females, the *Sxl* transcript from the late promoter is spliced to

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remove the stop codon thus producing functional Sxl protein (Bell et al. 1988; Bopp et al. 1991); the absence of this early Sxl pulse in males results in default splicing of *Sxl* transcript transcribed from late promoter, and production of truncated non-functional Sxl protein. An auto regulatory feedback loop ensures sustained production of functional Sxl protein throughout development only in females (Penalva and Sanchez 2003; Cline 1984; Bell et al. 1991; Keyes et al. 1992). The functional Sxl protein in females regulates the splicing of its immediate downstream target gene *transformer* (*tra*) by allowing the use of its distal 3′ splice site leading to the removal of the stretch encompassing the termination codon in the mature *tra* transcript, thus ensuring the production of full length Tra protein. In the absence of Sxl protein in males *tra* splices in a default mode, leading to the selection of proximal 3′ splice site including the stretch harbouring in frame stop codon resulting in truncated non-functional protein (Belote et al. 1989; Boggs et al. 1987; Valcarcel et al. 1993). Tra along with Tra-2 regulates sex-specific splicing of *doublesex* (*dsx*) pre-mRNA. The *dsx* is the most downstream gene of the *Drosophila* sex determination pathway. The *dsx* pre-mRNA sex-specifically splices to produce sex-specific transcripts which encode sex-specific proteins, DsxF in female and DsxM in male. These sex-specific Dsx proteins with common amino termini but sex-specific carboxyl termini execute somatic sexual differentiation (Burtis and Baker 1989; Baker et al. 1989; Slee and Bownes 1990; Steinmann-Zwicky et al. 1990; Hoshijima et al. 1991). Two more genes, *intersex* (*ix*) and *hermaphrodite* (*her*) are also required for the proper sex determination in *Drosophila*, but they are not the direct players in the sex determination pathway. The pre-mRNA of *ix* gene is not sex-specifically spliced and Ix protein is produced in both the sexes. Interaction of Ix with Dsx protein is essential for proper female sexual differentiation whereas male sexual differentiation does not require such an interaction (Chase and Baker 1995; Waterbury et al. 1999; Garrett-Engele et al. 2002).

In the silkworm, *Bombyx mori* which has the female heterogamety (ZW), the W chromosome ensures the zygote to take the female development pathway. Screening of the *B. mori* genome with the *Drosophila* sex determination cascade genes revealed that except *dsx* no other genes could be identified as potential candidates of sex determination pathway in *Bombyx* except for the recent reports suggesting the involvement of W-borne zinc finger motifs as upstream regulators of sex determination pathway (Ajimura et al. 2006; Satish et al. 2006). *Bmdsx* plays a very crucial role in silkworm sexual development. The pre-mRNA of *Bmdsx* sex-specifically splices to produce male- and female-specific mRNAs that encode male-specific (BmDsxM) and female-specific (BmDsxF) polypeptides, respectively (Suzuki et al. 2001; Ohbayashi et al. 2001). The sex-specific splice forms of *Bmdsx* are expressed in almost all the tissues during larval, pupal and adult stages (Ohbayashi et al. 2001). *Bmdsx* pre-mRNA is made up of 6 exons: female-specific transcript (*Bmdsxf*) includes all the six exons whereas male-specific transcript (*Bmdsxm*) contains exons 1, 2, 5 and 6. Because of the skipping of exons 3 and 4 in *Bmdsxm*, the C-terminal region of BmDsxM is different from that of BmDsxF. The features of sex-specific splicing of *Bmdsx* and *dsx* seem to be similar but the mechanism of splicing in both the cases is quite different. Unlike in *Drosophila*, the default form of *Bmdsx* splicing is the female form as evident from the splicing pattern of the *Bmdsx* mini gene in the HeLa nuclear extract (Suzuki et al. 2001). Recently it has been shown that binding of BmPSI, a *Bombyx* homolog of P-element somatic inhibitor (PSI), a KH-domain RNA-binding protein, to the exonic splicing silencer (ESS) sequences in exon 4 is required for the skipping of exon 3 and exon 4, thus maintaining the proper male-specific splicing of *Bmdsx* pre-mRNA (Suzuki et al. 2008).

The molecular mechanism involved in sex determination pathway has gained importance because of its potential implication in SIT (Sterile Insect Technique) programs for the control and eradication of harmful insect agricultural pests. *dsx* gene is one of the potential candidate genes proposed to be used in SIT programs (Saccone et al. 2002). In light of this, it is important to investigate *dsx* gene in insects, particularly Lepidoptera which embraces a large number of pests where SIT potential is yet to be harnessed using emerging molecular genetic approaches (Marec et al. 2005). In the present study, we identified and analyzed the presence of a third splice form of *Bmdsx* pre-mRNA which is specific to females. In a recent study we have shown that, unlike *dsx* splicing pattern in other insect species, the pre-mRNA of *Antheraea assama dsx* gene (*Aadsx*) sex-specifically splices to produce six splice variants in females and one in male (Shukla and Nagaraju 2010). The *pre-mRNA* of *Antheraea mylitta dsx* gene (*Amydsx*) splices to generate two female-specific and one male-specific splice forms. The six female-specific splice variants of *Aadsx* could be grouped into two on the basis of their ORF; three splice forms coding for putative protein, AaDsxF1 and the other three code for putative protein, AaDsxF2. Both the female-specific AaDsx proteins (AaDsxF1 and AaDsxF2) are having all the features of a functional Dsx protein. They share common DNA binding (OD1 domain) and oligomerization domain (OD2 domain) but differ in their amino acid composition at their extreme C-terminal ends; the longer of the two female-specific proteins (AaDsxF1) has additional 21 aa whereas the shorter one (AaDsxF2) has an additional stretch of 3 aa. The difference in the ORFs of the two groups of female splice forms is because of the presence or absence of a 15 bp stretch (TACGGACTTTAATAG).

In *A. mylitta* also, the two female splice forms differ from each other by the presence or absence of the same stretch of 15 bp (Shukla and Nagaraju 2010). Conservation of this 15 bp sequence in the female-splice forms of *Aadsx* and *Amydsx* and its absence in the previously reported splice forms of *Bmdsx*, prompted us to reinvestigate the *Bmdsx* splice forms in the EST database of *B. mori*, which led to the identification and confirmation of existence of an another female splice form of *Bmdsx*. In spite of divergence of Bombycids and Saturniids from a common ancestor ~160.9 MYA, this 15 bp stretch was found to be conserved in the novel splice form of *Bmdsx*. We refer to this newly identified female splice form of *Bmdsx* as *Bmdsxf2* and the *Bmdsxf* reported earlier by Ohbayashi et al. (2001) as *Bmdsxf1* and the proteins encoded by these two forms as BmDsxF2 and BmDsxF1, respectively.

Bmdsxf2 differs from *Bmdsxf1* by the presence of 15 bp sequence (TACGGACTTTAATAG) towards the 3′ end of exon 3 which leads to early occurrence of stop codon. As a result, the putative protein encoded by *Bmdsxf2* (246 aa) is shorter by 18 aa compared to BmDsxF1 (264 aa).

Materials and methods

Identification of *Bmdsx* EST having additional 15 bp sequence (TACGGACTTTAATAG)

A stretch of 15 bp sequence (TACGGACTTTAATAG), which is the part of female-specific *doublesex* isoforms of *Antheraea assama* (*Aadsx*) and *Antheraea mylitta* (*Amydsx*) that generate a second novel type of female-specific Dsx protein in these silkmoths (Shukla and Nagaraju 2010), was searched in the EST database (http://www.ncbi.nlm.nih.gov/dbEST) of *B. mori*, using the BLAST 2.1 program (http://www.ncbi.nlm.nih.gov/blast/). Virtual translation and alignment of the obtained EST with the previously reported *Bmdsx* transcripts was done to confirm that the newly identified EST (BP121180) is a part of the *Bmdsx* transcript. Alignment of BP121180 with *Bmdsx* genomic DNA sequence was done to find the exact location of the 15 bp sequence, with respect to its position in the *Bmdsx* gene.

Designing of primers to confirm the presence of two female-specific transcripts of *Bmdsx* gene

Forward and reverse primers were designed for the regions flanking the 15 bp sequence which can amplify both the female-specific transcripts, one with the 15 bp and the other without this sequence, in the RT-PCR. To amplify either of the female-specific transcripts, keeping the forward primer constant, reverse primers were designed in such a way that they bind to either of the female-specific transcripts. *Bombyx mori* cytoplasmic A3 actin primers were used as endogenous control. The primer sequences used are listed in Table 1.

Insect species, RNA isolation and RT-PCR

A silkworm stock Pure Mysore (sex-limited), where male and female larvae could be distinguished by virtue of sex-limited larval markings (Nagaraja et al. 2005), was used as the source of RNA. The female larvae possess markings in the cephalic and thoracic segments while the male larvae lack the same and thus sex separation is made possible from early IV instar stage (Fig. 1). Tissues were dissected out from 5th instar larvae, washed in PBS (135 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO4, 1.4 mM KH_2PO_4), frozen immediately in liquid nitrogen and stored at −80°C, till RNA isolation. Total RNA was isolated from tissue samples using TRIZOL reagent (Invitrogen, USA), according to manufacturer's protocol. DNAse treated total RNA was denatured at 75°C for 10 min and immediately chilled on ice. First strand cDNA was synthesized for each RNA sample using SuperScript III reverse transcriptase (Invitrogen, USA), according to manufacturer's instructions, using 17-mers poly-T as primer. PCR was performed for each cDNA sample using Taq DNA polymerase (Fermentas, USA), with the gene-specific primers listed in Table 1. The PCR conditions were: 94°C for 2 min, 40

Table 1 Gene-specific primers used in the expression analysis of *Bmdsx*

Reaction	Primer name and sequence	Annealing temp (°C)	Amplicon size (bp)
1	**BdR2F (F)**: 5′-GCAGCGACTTGGATGAGG-3′	60	172, 187
	BmDsxR (R1): 5′ TTCATTGACGAAGACAGTACACC-3′		
2	**BdR2F (F)**: 5′-GCAGCGACTTGGATGAGG-3′	60	131
	BdR1R (R2): 5′-CAGCATTTTCTGGCGTGTC-3′		
3	**BdR2F (F)**: 5′-GCAGCGACTTGGATGAGG-3′	60	150
	BdR2R (R3): 5′-TTTCCAGCATTTTCTATTAAAGTCC-3′		
4	***B. mori* A3-actin (F)**: 5′-CACTGAGGCTCCCCTGAAC-3′	60	200
	***B. mori* A3-actin (R)**: 5′- GGAGTGCGTATCCCTCGTAG-3′		

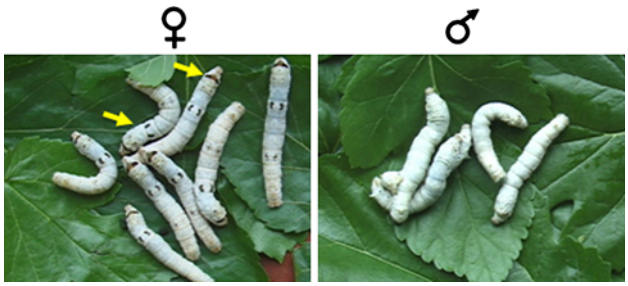


Fig. 1 Pure Mysore (sex limited) larvae. Female larvae possess crescent-shaped markings in the cephalic and thoracic segments (indicated by *yellow arrows*) while the male larvae lack the same

cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, followed by final extension at 72°C for 10 min. Amplicons were analyzed by agarose gel (2%) electrophoresis and confirmed by DNA sequencing.

Results

BLAST analysis and identification of EST- BP121180

When we were looking for the 15 bp conserved sequence of *A. assama* and *A. mylitta* female-specific *dsx* forms in the NCBI EST database (http://www.ncbi.nlm.nih.gov/dbEST/), a single EST (BP121180) from *B. Mori* EST database showed similarity to this sequence in a stretch, positioned after 795 bp relative to the earlier reported female-specific *Bmdsx* mRNA. Nucleotide alignment of the ORF of *Bmdsxf1* and EST- BP121180 is shown in Fig. 2. To avoid any changes, the N (a sequencing error) present in the EST-BP121180 was left as such. The only difference between *Bmdsxf1* and EST- BP121180 is the absence of 15 bp sequence (TACGGACTTTAATAG) in the former. After aligning this EST sequence with the *Bmdsx* mRNA and with the genomic DNA sequence of *Bmdsx* we found this sequence to be present immediately after exon 3 (without any intronic sequence in between) (Fig. 3), and due to its presence the reading frame of this newly found female-specific *dsx* transcript, *Bmdsxf2* is altered to encode 18 aa shorter protein as compared to BmDsxF1 encoded by *Bmdsxf1*. The new splice junction of exon 3 fulfils the GT-AG rule of splicing.

Expression analysis of female-specific *Bmdsx* mRNA variants

We performed reverse transcription polymerase chain reaction (RT-PCR) to determine the expression patterns of these two female-specific *Bmdsx* splice variants, using one specific forward primer (F) and three reverse primers (R1, R2, and R3) (Fig. 3). Primers F and R2 amplified *Bmdsxf1* whereas Primers F and R3 amplified *Bmdsxf2* in female-specific manner in all the organs tested, of V instar larvae. These primers also detected relatively weak expressions of these female variants in males, suggesting that both the female variants are expressed in male tissues at a low level (Fig. 4b and c). We confirmed that primers F and R1 amplified both the female-specific *Bmdsx* isoforms in different organs of 5th instar larvae (Fig. 4a).

Proteins coded by *Bmdsxf1* and *Bmdsxf2* splice forms

The BmDsxF2 contains both OD1(DM) and OD2 (Oligomerisation) domains and is exactly similar to the BmDsxF1 protein reported earlier except for the difference in their extreme C-terminal regions. The inclusion of 15 bp sequence at the end of 3rd exon in *Bmdsxf2* leads to the change in the reading frame after amino acid 243, relative to the BmDsxF1 and early occurrence of the stop codon compared to the *Bmdsxf1*. So, the putative protein BmDsxF2 is of 246 amino acids whereas the BmDsxF1 protein is of 264 amino acids. Both BmDsxF1 and BmDsxF2 differ at their C-termini by 21 amino acids (Fig. 5).

Discussion

Sex-specific BmDsx proteins, BmDsxF1 in females and BmDsxM in males are encoded by the transcripts produced by the alternative splice forms of *Bmdsx* pre-mRNA as reported earlier by Ohbayashi et al. (2001). We found a third splice variant, *Bmdsxf2* which is female-specific and the putative protein, BmDsxF2 encoded by this splice variant is shorter than BmDsxF1 by 18 aa (Fig. 5).

BLAST search for the 15 bp sequence (TACGGA CTTTAATAG), which is conserved in the few female-specific transcripts of *Aadsx* and *Amydsx*, in the *B. mori* EST database picked up a single EST (BP121180) which completely matched with this 15 bp sequence, but many other hits (not related to *Bmdsx*) matching partially (12 out of 15 bases) to the query sequence were also picked up in the BLAST search. This raises a possibility that this sequence is present in the *Bombyx* genome to basically perform some regulatory functions related to alternative splicing and there may be no particular role of the shorter DsxF2 as a result of insertion of this 15 bp additional sequence. But knockdown of transcripts encoding shorter DsxF2 protein, in *A. Assama* that lead to complete abolition of expression of *Vitellogenin* and *Hexamerin* genes, the direct targets of the Dsx proteins and irregular differentiation of gonads, discounts such a possibility (Shukla and Nagaraju 2010). The knock-down effects seen at the molecular level is consistent with the reduced fecundity and complete failure of egg hatching in the treated batches. In order to analyze the function of BmDsx

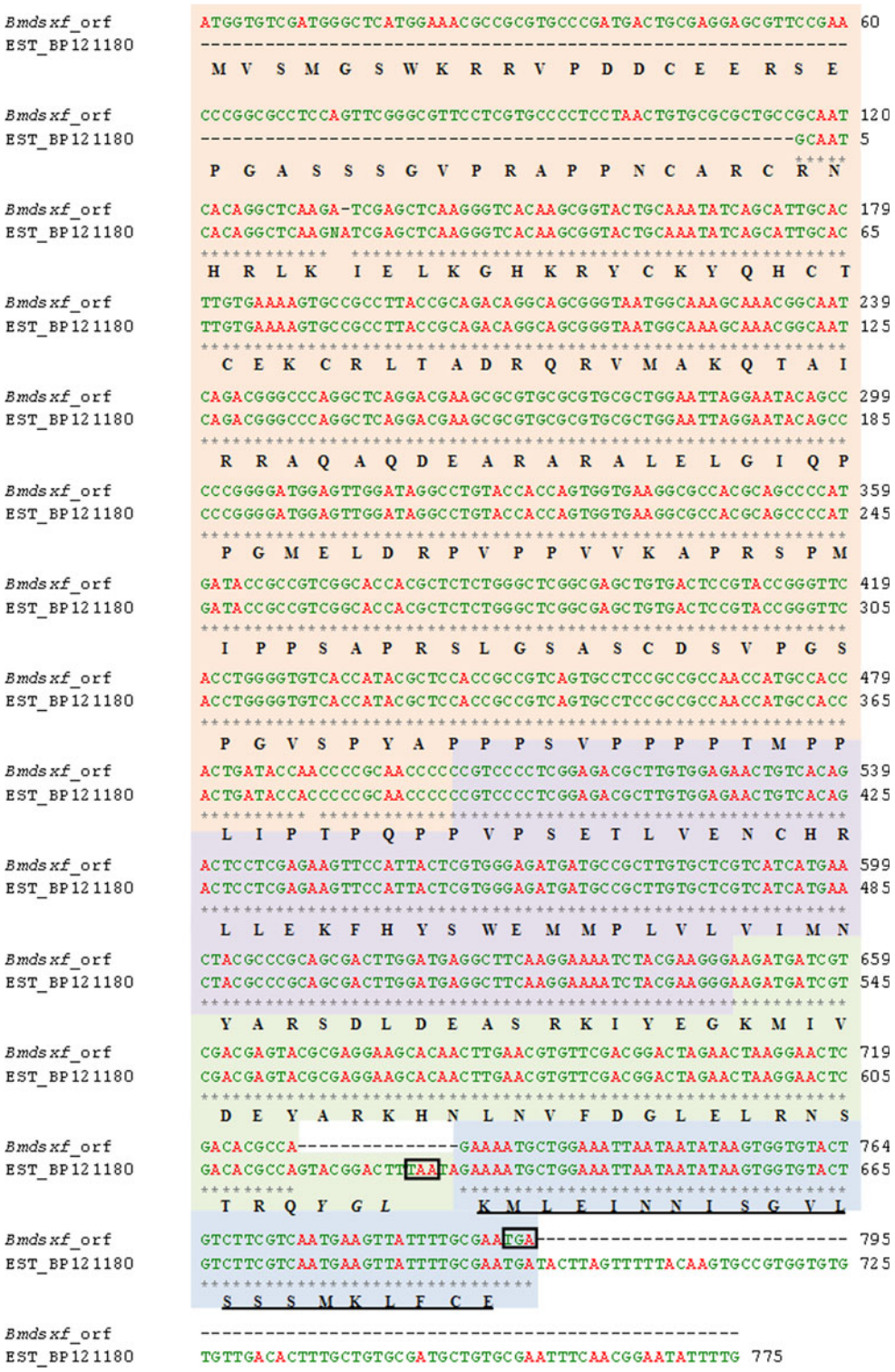


Fig. 2 ClustalW alignment of the *Bmdsxf*-ORF (named as *Bmdsxf 1*) and EST-BP121180 (named as *Bmdsxf 2*) showing the presence of additional 15 bp sequence after exon 3 in the latter. The amino acid coded is given below the alignment; the amino acid in italics (YGL) is coded by EST-BP121180 (*Bmdsxf2)* whereas underlined amino acids are specific to BmDsxF1. First four exons (shown in different *colours in background*) of *Bmdsx* gene are covered by ORF of *Bmdsxf1*. The unshaded region after exon 3 shows the difference of 15 bp sequence between *Bmdsxf1* and EST-BP121180. Because of the presence of this 15 bp sequence, the stop codon in the EST-BP121180 occurs 66 bp earlier compared to the stop codon of *Bmdsxf1* splice form, as a result of which the new splice form of *Bmdsx* (named as *Bmdsxf2*) produces BmDsxF2 protein shorter by 18 aa. The stop codons have been shown by the *black boxes*

proteins, Suzuki et al. constructed silkworm transgenic lines ectopically expressing $BmDsx^{F1}$ or $BmDsx^{M}$ (Suzuki et al. 2003, 2005). Though there was no change in the morphological characters of the male moths ectopically expressing $BmDsx^{F1}$, they showed increased expression of *Vitellogenin* (*Vg*) and *Sp1* mRNAs compared to that in normal males (Suzuki et al. 2003). However, expression level of these genes was 20 fold and 200 fold less than those of normal females, respectively. On the other hand, female moths expressing transgenic $BmDsx^{F1}$, showed enhanced expression of *Vg* and *Sp1* which is consistent with the expected increase in the $BmDsx^{F1}$ due to additional copy of the

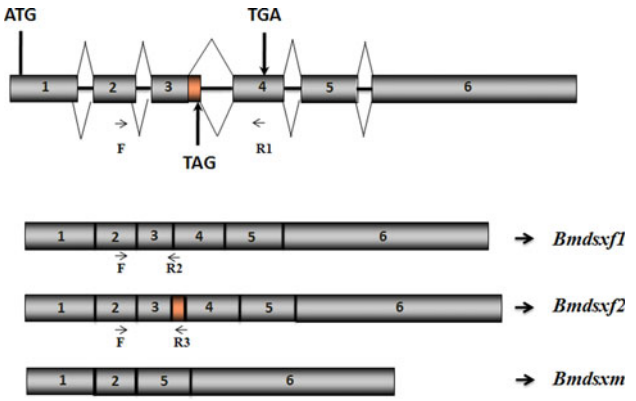


Fig. 3 pre-mRNA of *Bmdsx* splices to produce three products i.e., *Bmdsxf1*, *Bmdsxf2* and *Bmdsxm*. *Boxes* represents exon, whereas lines represent introns. Exons have been numbered. The *red coloured* region in *Bmdsxf2* is the additional portion generated due to the alternative 5′ splice-site selection. Stop codon of respective splice forms are shown by *vertical arrows* whereas primer positions are mentioned by *horizontal arrows*. Priming region for primers F and R1 are present in both the female forms whereas the priming region for primers R2 and R3 is present in *Bmdsxf1* and *Bmdsxf2* splice forms, respectively

transgene *Bmdsxf1* (two copies of endogenous *Bmdsxf1* + single copy of transgene *Bmdsxf1*) (Suzuki et al. 2003). Transgenic males, having only single dose of $BmDsx^{F1}$ encoded by *Bmdsxf1* should show 50% expression of downstream target genes compared to normal females. But the increase was found only to be marginal. The reason attributed by authors for such a low level of expression of *Vg* and *Sp1* in transgenic males expressing $BmDsx^{F1}$ is the antagonistic effect of endogenous BmDsxM. But the effect of $BmDsx^{M}$ on sexual differentiation of the organs and on the expression of *Vg* and *Sp1* was more pronounced in the transgenic females ectopically expressing $BmDSX^{M}$; *Vg* expression was reduced by 25% as compared to that in normal females. In addition, female-specific structures were repressed; instead certain male genital structures appeared in these transgenic females (Suzuki et al. 2005).

The less pronounced effect of ectopically expressed $BmDsx^{F1}$ compared to the ectopically expressed $BmDsx^{M}$ in the opposite sexes in regulating the sexual differentiation process and change in the morphology and expression levels of *Vg*, *Sp1* and *PBP* genes, as suggested by the

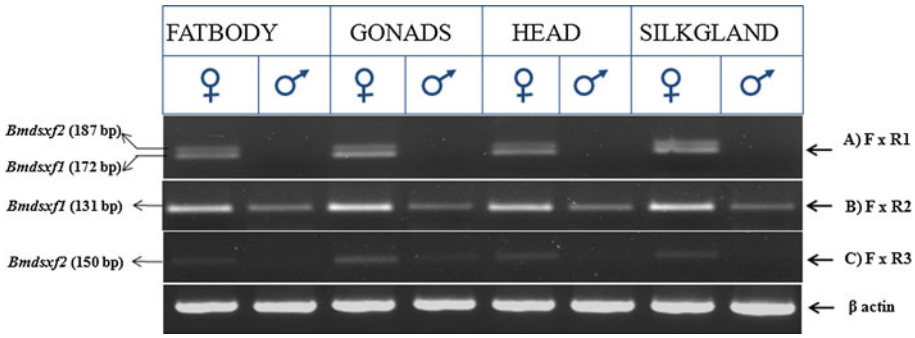
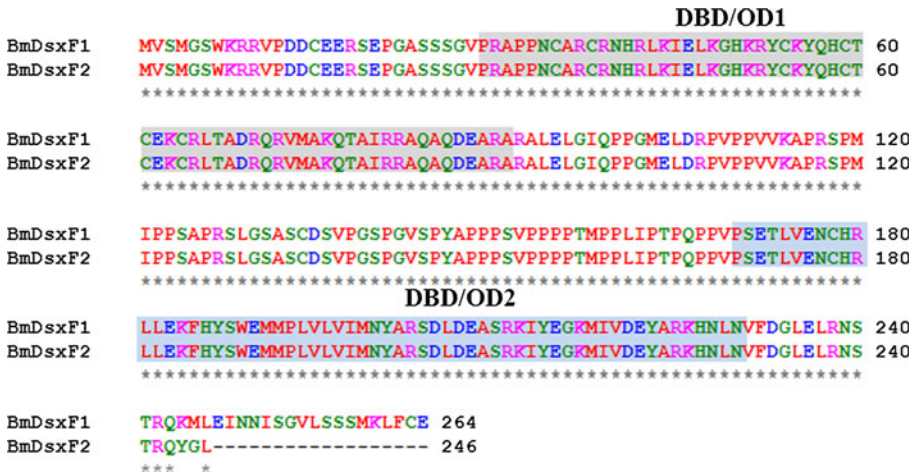


Fig. 4 Expression analysis of two female-specific splice forms of *Bmdsx* (*Bmdsxf1* and *Bmdsxf2*). **a** Amplification of both the female-specific *Bmdsx* forms. **b** Amplification of the *Bmdsxf1* splice form. **c** Amplification of the *Bmdsxf2* splice form. *Bmactin* (GenBank accession no. DC549679) is the loading control. Faint amplification in male panels could also be seen for *Bmdsxf* primers used, similar results have also been reported earlier (Ohbayashi et al. 2001)

Fig. 5 ClustalW alignment of BmDsxF1 and putative BmDsxF2 proteins. BmDsxF2 is coded from female splice form of *Bmdsxf2* having 15 bp insertion after exon3, relative to *Bmdsxf1* transcript. *Grey shaded* region represent the DNA binding domain (OD1 domain) whereas *blue shaded* region represent the oligomerisation domain (OD2 domain)



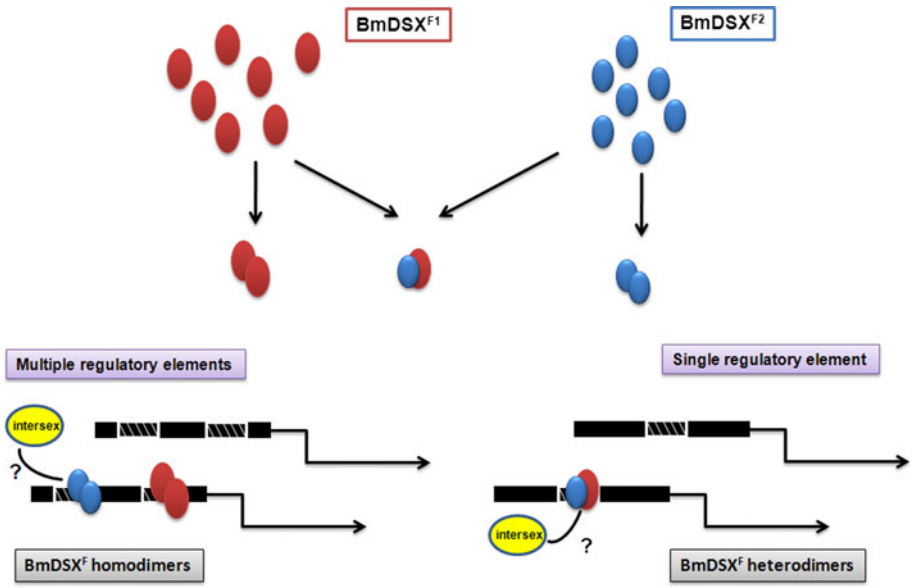


Fig. 6 Model explaining the mode of action of two BmDsx proteins in females. **a** Two different regulatory elements present in the downstream target genes (*Vitellogenin*, *Sp1* and *PBP*) and the BmDsx homodimers independently regulate those elements. **b** Single regulatory elements are regulated by the BmDsx heterodimers

authors, probably involves the recruitment of additional factor(s) by the BmDsx^{F1} to exert its effect fully on female sexual differentiation. In *Drosophila*, *intersex*, a gene implicated in female sexual development is expressed in both the sexes and functions together with *doublesex* (*dsx*) to regulate terminal sex differentiation in females. Recent studies on Ix proteins isolated from *Maruca vitrata* (Cavaliere et al. 2009) and *B. mori* (Arunkumar and Nagaraju manuscript communicated) and their partial rescue of *Drosophila ix* mutant (Cavaliere et al. 2009; Siegal and Baker 2005) suggest that it may be functionally conserved to exhibit its role in sex differentiation in co-ordination with Dsx. In light of this, finding of a novel female-specific splice form of *dsx* in lepidopterans, which codes for another female-specific putative protein, opens up a new avenue to unravel the sexual differentiation process governed by BmDsx proteins in the lepidopterans. We propose a model (Fig. 6) to explain the pathway of female sexual differentiation governed by the two female-specific Dsx proteins. BmDsxF2 might be acting as an additional factor in co-ordination with BmIx, in addition to BmDsxF1, to execute female sexual differentiation. However, further investigations are required to know whether the production of two female-specific Dsx proteins is confined only to silkmoths or is a common feature of all lepidopterans. Also, we need to decipher the mode of action of the female BmDsx proteins on the downstream target genes in female sexual differentiation.

Conclusions

Many recent developments that include the success of transgenesis in lepidoptera (Peloquin et al. 2000; Tamura et al. 2000), characterisation of chromosomal region that harbours female sex determining genes on the W chromosome in the female heterogametic system like silkworm (Abe et al. 2008; Ajimura et al. 2006; Satish et al. 2006), RNAi applications in lepidoptera (Gandhe et al. 2007; Uhlirova et al. 2003; Kanginakudru et al. 2007), hold promise to develop genetic sexing strategies in lepidoptera. With the role of many upstream players that determine the sex-specific splicing pattern of *dsx* is becoming clearer, the *dsx* is emerging as a potential candidate gene proposed to be used in SIT programs (Saccone et al. 2002). In light of this, our finding that the *dsx* encodes two proteins in the female silkmoths not only provides additional information to the molecular technologies involved in SIT in lepidopteran pests but also adds new dimension to the unique sex determination mechanism of moths which differs from flies, fruitflies and bees.

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