

SUPPLEMENTAL MATERIAL

Long-range RNA pairings contribute to mutually exclusive splicing

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Table S1

Figures S1 to S10

Table S1 Primers used for the RT-PCR and PCR analysis

Primer	Sequence	Primer	Sequence
SRP cloning and sequencing			
DmSRP-5-1	CCGGAATTCAACGATACCTGGTTCGATCCG TTAA	DmSRP-3-1	CCGCTCGAGCTGAATGGTGCCTTTTTCATGGT
DmSRPM5-1	CCCAAGCTTATTAATAAATTTCAACTCTTGAG ATAAATTC	DmSRPM3-1	CCCAAGCTTAAATAATAAGAGACGTAAGGCAAG GCTTAAC
DmSRPM5-2	ATGCCATGGTAATTTCTGTGTGCAACATCCT C	DmSRPM3-2	ATGCCATGGTGTGTTTTCGGTTTGTTTGATTGAT TGGTATTG
DmSRPM5-3	CCCAAGCTTAAATAATATTATTACGCAATGC CAACTAG	DmSRPM3-3	CCCAAGCTTATTAATAATTCCTCTCTTGAGCAT TGGAG
DmSRPM5-4	ATGCCATGGTGTGGTTTTCTGACTGCATCCC GAGCGCTTCTAG	DmSRPM3-4	ATGCCATGGTCGTTTTCTATGGCAACTCAGTG
DmSRP-5-5	TCCGCCTCGGCATCGTTGTCAG	DmSRP-3-5	AAAGTCCGGCTCGCTTTGAGGC
DmSRPM5-7	CTATAATAAATTCATTAATAATATTCAACT CTTGAGATAAA	DmSRPM3-7	GTTGAAATATTATTAATTGAATTTATTATAGAGAC G
DmSRPM5-8	CTCTATAATAAATTCATTTTATTATTATCAA CTCTTGAGATAAA	DmSRPM3-8	TGAAATAAATAAATTTGAATTTATTATAGAGACG
DmSRPM5-9	CTCTATAATAAATTCATATTATTATTATCAA CTCTTGAGATAAAT	DmSRPM3-9	GTTGAAATAAATAATATTGAATTTATTATAGAGAC G
DmSRPM5-10	CGTCTCTATAATAAATTGTTATAATTTTATTT CAACTCTTGAG	DmSRPM3-10	GTTGAAATAAATAATTATAACAATTTATTATAGAGAC GTAAGGC
DmSRPM5-11	CATAGAAAACGAAGTTCCACACGAAAAGC TACTGCATC	DmSRPM3-11	GCAGTAGCTTTTCGTGTGGAACCTCGTTTCTAT GGCAACTCAGTGG
DmSRPM5-13	CATAGAAAACGTACTTCCACACGAAAAGCT ACTGCATCC	DmSRPM3-13	GTAGCTTTTCGTGTGGAAGTACGTTTCTATGGC AACTCAGTG
DmSRPM5-14	CATAGAAAACGTTCAACCACACGAAAAGCT ACTGCATCC	DmSRPM3-14	AGTAGCTTTTCGTGTGGTTGAACGTTTCTATGG CAACTCAG
DmSRPM5-15	AGAAAACGTTGAAGCACACGAAAAGCTAC TGCATCC	DmSRPM3-15	AGTAGCTTTTCGTGTGCTTCAACGTTTCTATGG CAACTC
DmSRPM5-17	AAACGTTGTAGGTGACGAAAAGCTACTGCA TCCCCG	DmSRPM3-17	CAGTAGCTTTTCGTACCTACAACGTTTCTATG GCAACTC
DmSRPM18-5	CGGGGTACCTATTCGATGCCGATTATTTAC	DmSRPM18-3	CGGGGTACCCAAAGATTGACATAGAAATATC
DmSRPM19-5	GCCGAGCTCAATGTCTAAACAAATTCAC	DmSRPM19-3	GCCGAGCTCTCTTCTAGGCTGTTTAATTAG
DmSRPM21-5	CGGGGTACCCCGCTGAATCAGGCGGGGATT TC	DmSRPM21-3	CGGGGTACCGAATTGAGCAATCAGAATGTTG
DmSRPM22-5	GCCGAGCTCGGTGCGCAATTAATTACCGATA	DmSRPM22-3	GCCGAGCTCGCGTCTCGAATTGGCGCGACTAAC

	C		
DmSRPM24-5-1	GTCTCTATAATCGATTCAATTAATTTTATTTTC AAC	DmSRPM24-3-1	AAAATTAATTGAATCGATTATAGAGACGTAAGGC AAGG
DmSRPM24-5-2	AATTAATTGAATCGATTATTTATTACGCAATG CCAAC	DmSRPM24-3-2	GCGTAATAAATAATCGATTCAATTAATTTTTCAC TCTC
DmSRPM27-5	TAGAAAACCTTTGTTCACACAAAAGCTACT GCATCCCGAGCGC	DmSRPM27-3	AGCTTTTGTGTGGAACAAAGTTTTCTATGGCAAC TCAGTGG
DmSRPM28-5	TTGTTCCACACAAAAGCGTTTGCATCCCGA GCGCTTCTAGTC	DmSRPM28-3	GATGCAAACGCTTTTGTGTGGAACAACGTTTTCT ATGGC
DmSRPM29-5-1	ATTAATTTTTTCACTCTCTTGAGCATTTCA ACTCTTGAGATAAATTC	DmSRPM29-3-1	TGAAATGCTCCAAGAGAGTGAAAAAATTAATTG AATTTATTATAGAG
DmSRPM29-5-2	GAAAACGTGTTGCACACAGAAATTTGTTC CACACGAAAAGCTACTGC	DmSRPM29-3-2	GTGGAACAAAATTCTGTGTGCAACACGTTTTCT ATGGCAACTCAGTGGC
DmSRPM32-5	CAATTAATTTTTTCACAACTCTTGAGATAAA TTCAAAC	DmSRPM32-3	CTCAAGAGTTGTGAAAAAATTAATTGAATTTATT ATAG
DmSRPM33-5	CAATTAATTTTTTCACTCTTCTTGAGATAAA TTCAAACCAAC	DmSRPM33-3	TCTCAAGAAGAGTGAAAAAATTAATTGAATTTAT TATAG
DmSRPM35-5	TAATTTTTTCACTCTCTTGAGATAAATTC AACCAAC	DmSRPM35-3	TTTATCTCAAAGAGAGTGAAAAAATTAATTGAAT TTATTATAG
DmSRPM36-5	ACTTTGTTCACACAAAAGCGACTGCATCC CGAGCGCTTCTAG	DmSRPM36-3	CAGTCGCTTTTGTGTGGAACAAAGTTTTCTATGG CAACTCAGTGG
DmSRPM38-5	TTTTGTTCACACAAAAGCGTTGTATCCCG AGCGCTTCTAGTCTTAAC	DmSRPM38-3	TACAAACGCTTTTGTGTGGAACAAAATTTCTAT GGCAACTCAGTGGC
DmSRPM66-5	TTTTGTTCACACAAAAGCGTTGCATCCC GAGCGCTTCTAGTC	DmSRPM66-3	TGCAAACGCTTTTGTGTGGAACAAAATTTCTAT GGCAACTCAGTGGCC
DmSRPM67-5	TAAATCAATTAATTTAATTCAACTCTTGA GATAAATTC	DmSRPM67-3	GTTGAAATTAATTAATTGAATTTATTATAGAG
DmSRPM68-5	CATAGAAAACGATGTTCCACACGAAAAGCT ACTGCATC	DmSRPM68-3	GCAGTAGCTTTTCGTGTGGAACATCGTTTTCTAT GGCAACTCAGTGG
DmSRP-D1-5	CATGCCATGGTTATTACGCAATGCCAACTAG	DmSRP-D1-3	CATGCCATGGAGAGACGTAAGGCAAGGCTTAAC
DmSRP-D2-5	CATGCCATGGTACTGCATCCCGAGCGTTCT AG	DmSRP-D2-3	CATGCCATGGGTTTGTTTTGATTGATTGGTATTG
DmSRP-D7-5-1	GCCTTGCCCTTACGAACTCTTGAGATAAATTC AAAC	DmSRP-D7-3-1	GAATTTATCTCAAGAGTTCGTAAGGCAAGGCTTA ACCAC
DmSRP-D7-5-2	GCCACTGAGTTGCCATAGACATCCCGAGCG CTTCTAGTCTT	DmSRP-D7-3-2	GAAGCGCTCGGGATGTCTATGGCAACTCAGTGG CCAAG
DmSRP-D8-5-1	CCAATCAATCAAACTGTGTGCAACATCCT CTCTCAG	DmSRP-D8-3-1	GATGTTGCACACAGTTTGAATTGATTGGTATTGGT TAG
DmSRP-D8-5-2	GCTCCAAGAGAACGCAATGCCAACTAGTTT AATCG	DmSRP-D8-3-2	CTAGTTGGCATTGCGTTCTCTTGAGCATTGGAG CTTTTC

DmSRP-D9-5	TTATTTCAACTCAGAGAGTGAAAAAATTAA TTG	DmSRP-D9-3	TTAATTTTTCCTCTCTGAGTTGAAATAAAATTA ATTG
DmSRP-D10-5	GGAACAAAATTTCTGTGATAGAAAACGTTG TTCCACACG	DmSRP-D10-3	GAACAACGTTTTCTATCACAGAAATTTGTTCCA CACAAAAG
DmSRP-5-3	CGGCTTGTTACATGAAAATGAACG	DmSRP-3-3	TCATGTACAAGCCGCAGGCATTGC
DmSRP-5-5	TCCGCCTCGGCATCGTTGTCAG	DmSRP-3-5	AAAGTCCGGCTCGCTTTGAGGC
DmSRPWA5-1	ATCCGCATCCGCCAGGATCCGCACCACCGC CCCACCTTCACC	DmSRPWA3-1	GGTGGTGC GGATCCTGGCGGATGCGGATTTCTAT AAG
DmSRPWA5-2	CCAGGATCCGCACCACCGCCCCACCTTCAC C	DmSRPWA3-2	CCAGGATCCAAGGGTTTCGGTTGGTTGGTAGG
DmSRPWA5-3	CGTAGTAAGGCTAGCACGAGTGGTGCCGCC AGCT	DmSRPWA3-3	AGCTGGCGGCACCACTCGTGCTAGCCTTACTACG
DmSRPWA5-4	CCAGGATCCCAGCAAACATTGCAAAACCA AAC	DmSRPWA3-4	TGCGGATCCTGGCGGATGCGGATTTCTATAAG
DmSRPWA5-5	CAATTGTGGTGC GATCAACCCCATTTATGG CGACGCG	DmSRPWA3-5	CGCCATAATGGGGTTGATATCGCACCACAATTGA CACATTC
DmeSRPM75-5-1	AAC TTATAATAAGATTATATTA AAAATTTCTG TGTGCAACATCCTC	DmeSRPM75-3-1	ATTTTAATATAAACTTTATATAAGTTTGTTTGAT TGATTGGTATTGG
DmeSRPM76-5-1	ACGTTAATATAAACTTTATATAACTGCAAT CCCGAGCGCTTCTAG	DmeSRPM76-3-1	GTATTATAATAAGATTATATTAACGTTTTCTATGG CAACTCAGTGGCC
DmSRP-SSE7-5	GTCAATCTTTGAAGGATTATTCGATGCCG	DmSRP-SSE7-3	CGGCATCGAATAATCCTTCAAAGATTGAC
DmSRP-SSE1-5	GTCAATCTTTGCAGCATGTATTCGATGCCG	DmSRP-SSE1-3	CGGCATCGAATACATGCTGCAAAGATTGAC
DmSRP-SSE4-5	CAGCCTAGAGCATCGGTAAATGTCTAAAC AAATTC	DmSRP-SSE4-3	GAATTTGTTT TAGACATTTACCGATGCTCTAGGCT G
DmSRP-SSE2-5	CAGCCTAGAGCATCGGTAAAGTACTAAAC AAATTC	DmSRP-SSE2-3	GAATTTGTTT TAGTCACTTACCGATGCTCTAGGCT G
DmSRP-SSW1-5	TCTGATTGCTCCATGGTCCAGTGCCCGCTG	DmSRP-SSW1-3	CAGCGGCCACTGGACCATGGAGCAATCAGA
DmSRP-SSE10-5	CCAACAACATTCTGATTTCTCTTTCCAGTG GCCGCTG	DmSRP-SSE10-3	CAGCGGCCACTGGAAAAGAGAAATCAGAATGTT GTTGG
DmSRP-SSW2-5	CGAGACGCTTGGTAGATATCGCCATTAATT ACCG	DmSRP-SSW2-3	CGGTAATTAATGGGCGATATCTACCAAGCGTCTC G
DmSRP-SSE6-5	CGAGACGCTTGGTAAAGTCGCCATTAATTA CCG	DmSRP-SSE6-3	CGGTAATTAATGGGCGACTTACCAAGCGTCTCG
SRP-JB-5-2	CTATTTRTGCAATGCTGCGGCYTG	SRP-JB-3-2	CCGCADGCATTRCASACRGGCTC
SRP-3-JB-5.2	CAGCAGCYGCRGCCGCVGYGGATGG	SRP-4.1-3.2	GTCGATTCATGCCGTTCATTTTATG
SRP-JB-5-1	CGATGCCGATTWKTTCACTGAG	SRP-JB-3-1	ATGGTRTCCTTYTTCATRGTC
SRP-3-JB-5.2	CAGCAGCYGCRGCCGCVGYGGATGG	MT-5	GGTACCTACTAGTCCAGTGTGG

***A. mellifera* Dscam cluster4**

Amcam4-5-1	CCCAAGCTTGCTCTTGCCGAACGAAATTTG GTC	Amcam 4-3-1	CGGGGTACCCGTAATGACGAGTCGTCC CTTG
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Amcam4-5-2	GCGTCATGAAGTGTCACATACCGAGCTTCG	Amcam4-3-2	GCGTCATGATCGCGCTGTTCCGC
Amcam4-5-X	TGCTCTAGACTTACCAAAGCAAAAGTTCAA AGTGG	Amcam4-3-X	TGCTCTAGAGATAGTTAITTAAGTATCCT TCTCACG
Amcam4-3-7	GTAATGACGAGTCGTCCCTTGG	Amcam4-M3-3A	TAACGAGACCGGTCCGTGATATTCTC
Amcam4-M1-5A	ATCACGGACCGGTCTCGTTATTAATG	Amcam4-M1-3B	CCGGTATGTACACTTCTCCGTCCTC
Amcam4-M2-5B	GAGAAGTGATACATACCGAGGATCC	Amcam4-M2-3M	CGCATGCAATTGACGACTCTACACG
Amcam4-M1-5-1	GCTTGGTGGTGGTGTAGCGTTTGTATCGTT ACTCGAAGCCCCTC	Amcam4-M1-3-1	ACGCTACACCACCACCAAGCTTACTCGT CGTGATGAAAGGTATGC
Amcam4-M2-5-1	ACGCTACACCACCACCAAGCACCATCCCAG GGTTAATGAACCATC	Amcam4-M2-3-1	GCTTGGTGGTGGTGTAGCGTACCGCCAG ACGTGGACCCGC
Amcam4-M3-5-1	TGTTTCGTATGACGGTGATCGAGGACTAGTC GAGGACGCGCTTCAATG	Amcam4-M3-3-1	CCTCGATCACCGTCATACGAACATTCCCT GACGTGTTCTTAGCGGTG
Amcam4-M4-5-1	CCTCGATCACCGTCATACGAACATGCTCGC GCTGTCCCAGACTTTC	Amcam4-M4-3-1	TGTTTCGTATGACGGTGATCGAGGATTCA ATAATTCGATAATTGTTTACTCG

B. mori Dscam cluster9

IE-F	CGATCGTGAACAACCAAACG	T7-promoter	TAATACGACTCACTATAGGG
Bmcam9-5-1	CGCGTCGACAATTCTCAGAGTCTCTCCCG	Bmcam9-3-2	CGCTCATGATAACGATAITATCATTTTTCT TAATT
Bmcam9-5-2	CGCTCATGACGATGCCGCTAGGC	Bmcam9-3-1	CGGGGTACCTTCAGCCCTTTTCCAAGTA
Bmcam9-3-7	CTTTAGCATCAGAGCCTTGG	Bmcam9-M1-3-N	GTGATCTCTTCATATGAATTAACAAACG
Bmcam9-M2-5-N	CGTTTTTAATTCATATGAAGAGATCAC	Bmcam9-M2-3-S	GGAACCGTACGTAAAGCCACATC
Bmcam9-M5-3-S	GTAAAGCCACATCTGGTGTAAGGAG	Bmcam9-M4-5-S	GTACGGTTCCATCCAAACAATCCAA
Bmcam9-M1-5-1	TTGGCGGGTATTAATTCGTTAGTCACACACG CCCCTG	Bmcam9-M1-3-1	AACGAATTAATACCGCCCAAAGTTACAC TGAAGATTTTCGTAAAGTAATCAATATG
Bmcam9-M2-5-1	CCTTTATTAATACCCGCCAAAGTTACAGTTA TAGAAATTACCCAAGCAAATTAG	Bmcam9-M2-3-1	TTGGCGGGTATTAATAAAGGATATAGGAG AAAATATAGAAG
Bmcam9-M3-5-1	CTTGAATCTATCGGCACCATTTTATAGATTATT GTAAGAATCGTTTCAA	Bmcam9-M3-3-1	AAATGGTGCCGATAGATTCAAGAGGTTT GGAAAAAATGTTTAA
Bmcam9-M4-5-1	ACCGGGTGCCGATAGAATCGAAAATCCCGT CTGTCTCTC	Bmcam9-M4-3-1	CGATTCTATCGGCACCCGGTTTAAATTTCAAGT ACGTTACTAG
Bmcam9-M5-5-1	CCATCTATCGGCACCTCGGAGCTGAACCAG TAAACGCAC	Bmcam9-M5-3-1	TCCGAGGTGCCGATAGATGGGGAGGGAC TGTGGGTGCA

Dscam cloning and sequencing

PnuDs9-5-1	TAAATCTAATGAATACCAAGCGCA	PnuDs9-3-1	TATTCGCCGCTGTGGCTAGCCAT
PnuDs9-5-2	ATCAAGGAATTACAACAACGAAGA	PnuDs9-3-2	TAAAGCCATTGTCTTCACGCCCAT
PnuDs9-5-3	CGAAAACGTTGAGCGCATGAGCG	PnuDs9-3-3	CAAACCTCAAACGGTAATATCTGT
PnuDs9-5-4	ACAGATATTACCGTTTGAGTTTG	PnuDs9-3-4	CGGCTATGCAAGTGTAATTGCCA
PnuDs9-5-5	TGGCAATTACACTTGCATAGCCG	PnuDs9-3-5	TGCGCTTGGTATTCAATAGATTTA

PnuDs9-5-6	GGCGAATCTGTACATCTTGACTGC	PnuDs9-3-6	GTTGATCGCCGTGTGTTATAATG
PnuDs9-5-7	GCGTCCTCACAATAGAGTCGGTG	PnuDs9-3-7	CAAGCTTGCAATGCCTGCAGGTCG
HarDs9-5-1	CGTAGCCAAGAACTCTCAGG	HarDs9-3-1	ATAACTGTTAATACGATTCCCAT
HarDs9-5-2	TTAGTGGTATACACAGGGGCACG	HarDs9-3-2	CAAAGGTAAGTCCCCCTTCCCGAC
HarDs9-5-3	ACTCTGGGAACGGTATACTCATA	HarDs9-3-3	GCATGAATATTGCCGCTGTGGC
HarDs9-5-4	TTTGCCGGAGAGTCTGTACAGTTG	HarDs9-3-4	ATGTTTAGAGGCAAATCGCCCTTG
HarDs9-5-5	ACTAACAGAGCTGGTTCCACCAATC	HarDs9-3-5	CTCAGGGCCAGCAATGAAAGGTGC
HarDs9-5-6	TCCGTCGACGCAACACACGCAGGG	HarDs9-3-6	CGTCGTAATATCTCGCTGAAGGGTC
HarDs9-5-7	GCAAATCATCAAGGGAACACACG	HarDs9-3-7	CCTTGTCTAGTGGGCTCAATAATCC
CsuDs9-5-1	TGACTCGAGTCAACACCGTGACA	CsuDs9-3-1	GCATCTGAGCCTTGAGCAAAAG
CsuDs9-5-2	CCAACGGTACCCTTGTGATCGAGA	CsuDs9-3-2	ACTGCCAATAGCGATGCCTATCG
CsuDs9-5-3	CGATAGGCATCGCTATTGGCAG	CsuDs9-3-3	TGGCAGATCTCCCTTAGTGACCG
CsuDs9-5-4	GTACGGTCACTAAGGGAGATC	CsuDs9-3-4	CAACTGGACGGATTCTCCAGC
CsuDs9-5-5	TTGCTGACTATATCTAGTGCCACG	CsuDs9-3-5	GTACGTGGCAGTCGAGGTGTACCGA
CsuDs9-5-6	AATAGTGGGAACATACCTGCACG	CsuDs9-3-6	AGGCAGGTCTCCCTTTGTTGCGAC
CsuDs9-5-7	TTTATGCCCGCCAAAGCTACATCG		

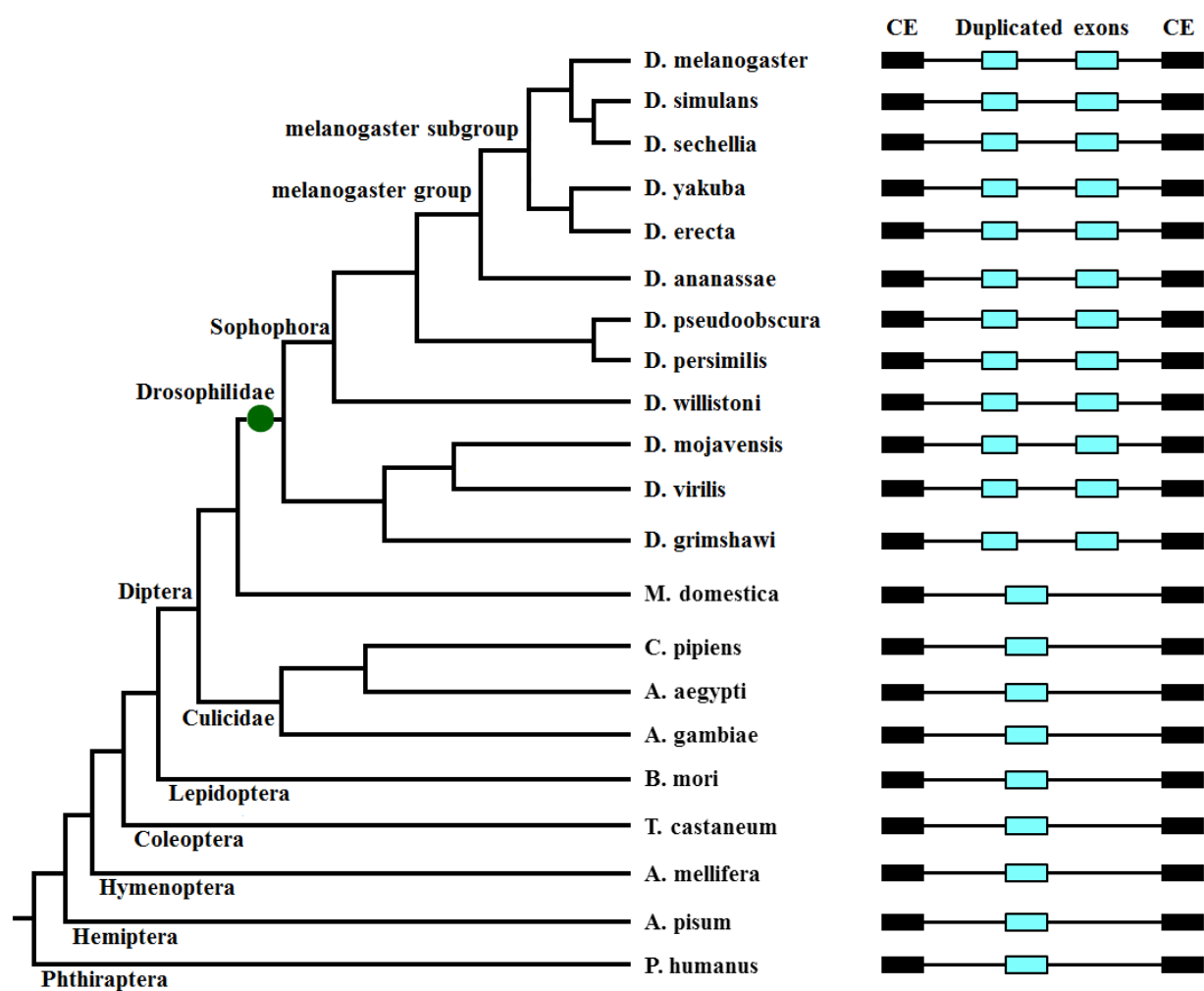


Figure S1. Phylogenetic analysis of the duplicated exons of *srp* in insect species. Phylogenetic tree was modified from FlyBase web (http://flybase.org/blast/species_tree.png). Exons that are duplicated in tandem or their orthologues are shown in color boxes while constitutive exons (CE) flanking the duplicated exons or their orthologues are shown in black box. The introns are represented with the lines, and not drawn to scale. The exon duplication is indicated by solid circles.

Exon 3

5' splice site

Exon 4

Ud

Exon 5

3' splice site

Exon 6

5' splice site

Us

Ds

Exon 7

3' splice site

Exon 8

5' splice site

Exon 9

Exon 10

Exon 11

Exon 5

3' splice site

Figure S2. Sequence alignment of *srp* from *Drosophila* species. Dme: *D. melanogaster* (AE014297.3); Dsi: *D. simulans* (CM000364.1); Dse: *D. sechellia* (CH480815.1); Dya: *D. yakuba* (CM000160.2); Der: *D. erecta* (CH954181.1); Dan: *D. ananassae* (CH902629.1); Dps: *D. pseudoobscura* (CM000070.3); Dpe: *D. persimilis* (CH479185.1); Dwi: *D. willistoni* (CH964272.1); Dmo: *D. mojavensis* (CH933806.1); Dvi: *D. virilis* (CH940650.1); Dgr: *D. grimshawi* (CH916369.1). Sequences aligned are from the upstream constitutive exon 3 to the downstream constitutive exon 5. Constitutive exon 3 and 5 are marked in orange, while alternative exon 4.1 and 4.2 are marked in cyan. The asterisk (*) indicates invariant positions. Four highly conserved intronic elements [Upstream and downstream docking sites (Ud and Dd) and downstream or upstream selector sequence (Us and Ds)] and splice sites are indicated. Upstream and downstream docking sites (Ud and Dd) were reverse-complementary to a downstream or upstream selector sequence (Ds and Us), respectively.

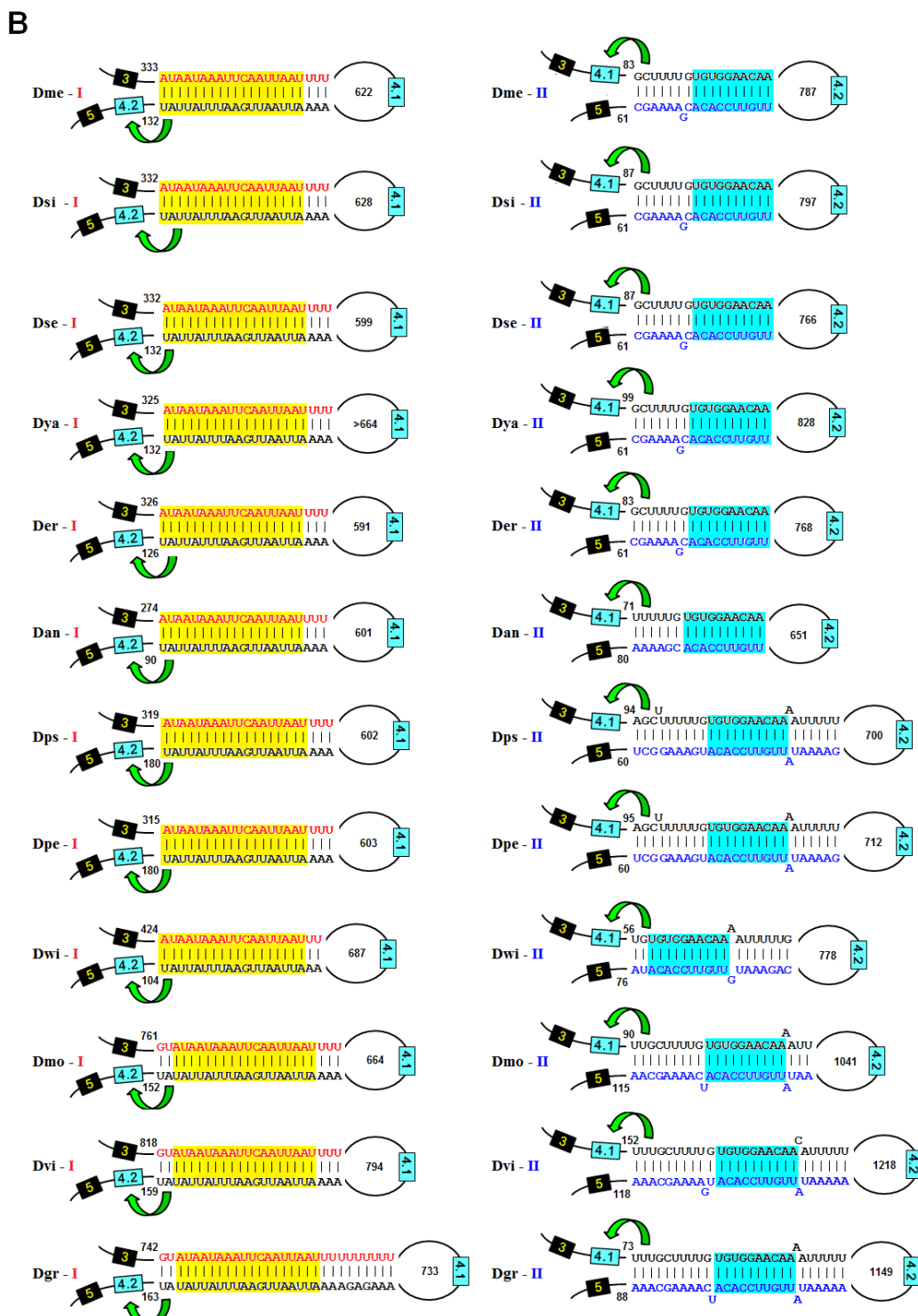
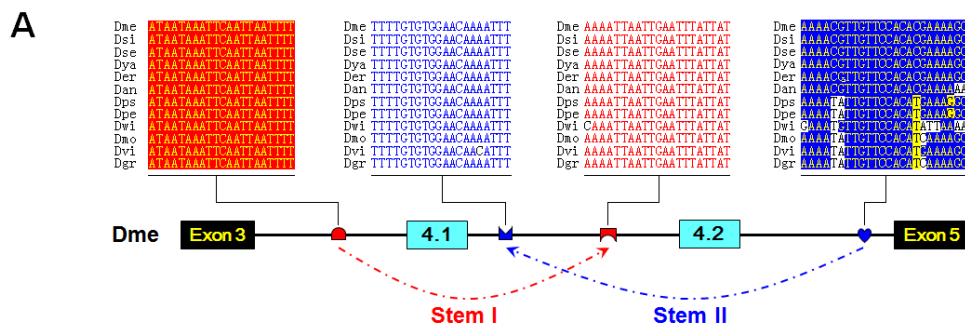


Figure S3. Dual RNA pairings are evolutionarily conserved in *Drosophila srp* pre-mRNAs. **(A)** Genomic organization of *D. melanogaster srp* gene is shown, and symbols used are the same as in Figure 1. Nucleotide variations that are structurally silent are shaded in yellow. **(B)** Upstream and downstream docking sites are shown in red and blue, respectively. The intronic RNA pairings between the selector sequences and the docking site were predicted using Mfold program (Zuker 2003). The distances from exon 3 to the 5' boundary of the upstream RNA stem and the distances from the 3' boundary of the upstream RNA stem to exon 4.2 are indicated for each structure. Similarly, the distances from exon 4.1 to the 5' boundary of the downstream RNA stem and the distances from the 3' boundary of the downstream RNA stem to exon 5 are indicated. In addition, the size of the loop is indicated. The sequences that make up the core of the upstream and downstream stem are highlighted in yellow and cyan, respectively.

A			B		
	Stem I	ΔG		Stem II	ΔG
WT	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA	-16	WT	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G	-20
T1	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA	-4.9	U1	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G SUGGA	-3.1
T2	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA	-8.9	U2	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G SAA	-9.2
T3	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA	-10.2	U3	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G AAC	-12
T4	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA	-20.1	U4	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G CA	-14.6
T5	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUG	-24.5	U5	GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G	-24.7
T6	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAG	-32.4	U6	CAAAACGCUUUUGUGUGGAACAA GUUGCGAAACACACCUUGUU	-32.3
T7	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAGAA	-36.5	U7	CAAAACGCUUUUGUGUGGAACAAUUUU GUUGCGAAACACACCUUGUU G AAA	-39.2
T8	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAGAACCCUG	-49.8	U8	GCUUUUGUGUGGAACAAUUUUUGUGUGCAACA CGAAACACACCUUGUU G UAAAGACACACGUUGU	-50.6
C					
	Stem I			Stem II	$\Delta G_{\text{Stem I}} / \Delta G_{\text{Stem II}}$
C1	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA			GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G	0.74
C2	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAA			GCUUUUGUGUGGAACAA CGAAACACACCUUGUU G	0.80
C3	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUG			CGCUUUUGUGUGGAACAA GCGAAACACACCUUGUU U	0.90
C4	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAG			CAAAACGCUUUUGUGUGGAACAAUUUU GUUGCGAAACACACCUUGUU G AAA	0.88
C5	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAGAA			CAAAACGCUUUUGUGUGGAACAAUUUU GUUGCGAAACACACCUUGUU G AAA	0.93
C6	AUAAUAAUUCAAUUAUUUUU UAUUUUUAAGUUAUUAAAAAGUGAGAGAACCCUG			GCUUUUGUGUGGAACAAUUUUUGUGUGCAACA CGAAACACACCUUGUU G UAAAGACACACGUUGU	0.98

Figure S4. Overview of the minigene constructs used to change the upstream or/and downstream RNA pairing. (A) The minigene constructs used to change the upstream RNA

pairing. Predicted RNA pairing for the wild-type (WT) and a series of mutants (T1-T8, point mutations are shaded in green), with the estimated equilibrium free energies (given in kcal/ mol). The intronic RNA pairings between the selector sequences and the docking site and free energies were predicted using Mfold program (Zuker 2003). **(B)** Predicted downstream RNA pairing for the wild-type (WT) and a series of mutants (U1-U8). **(C)** Schematic diagrams of the combined mutations on dual pairings (C1-C6).

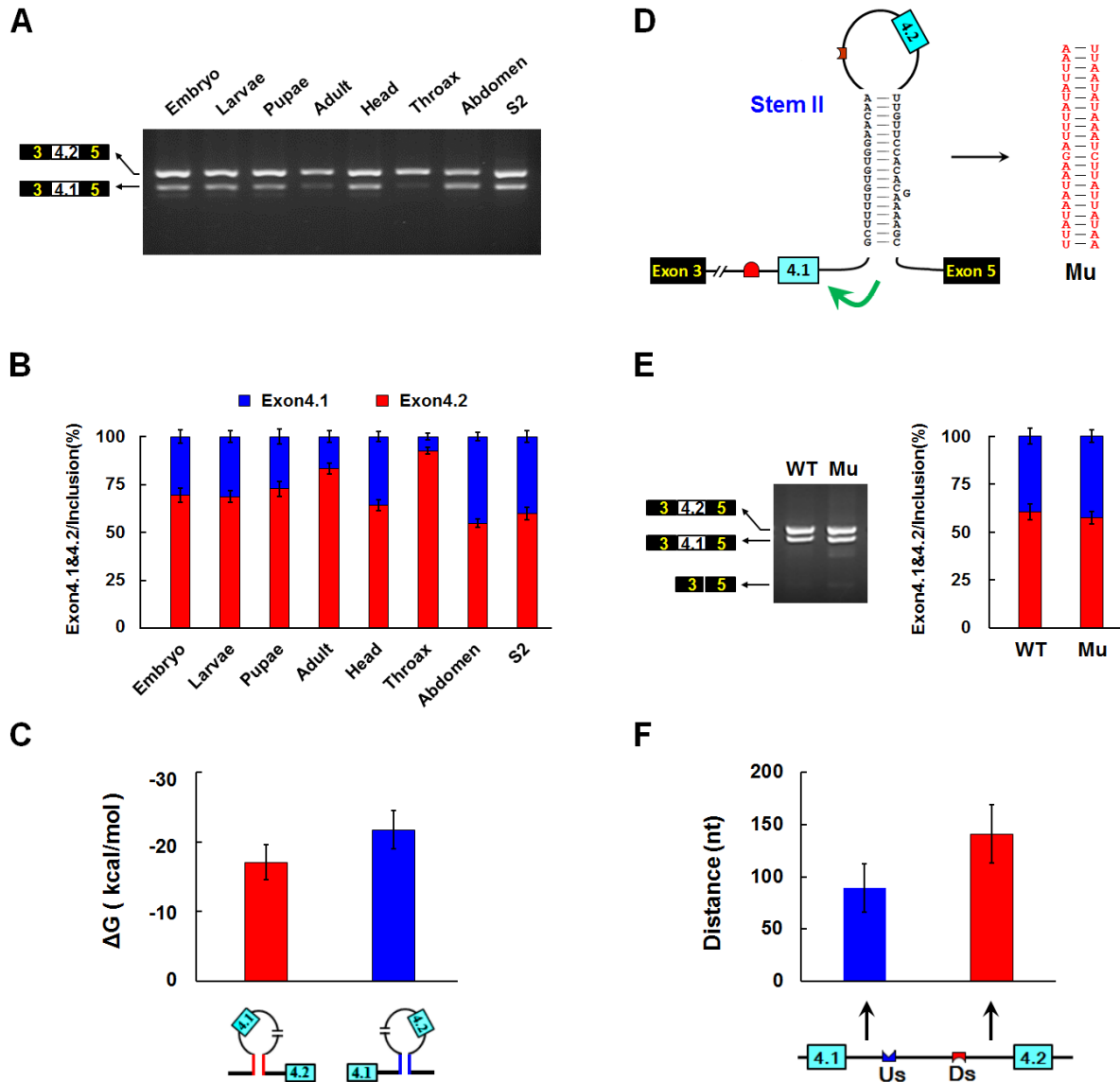


Figure S5. Analysis of the inclusion frequency of splice variant and other characteristics of *srp* gene. **(A)** Analysis of the selection frequency of splice variant in various tissues of *D. melanogaster*. **(B)** Quantization of the data in panel A. Data are expressed as percentages of the mean $\pm$ SD from three independent experiments. **(C)** The predicted free energy of the upstream and downstream pairing were investigated across *Drosophila* species using Mfold program (Zuker 2003). **(D)** Schematic diagrams of mutating the pairing strength of stem II to be identical to that of stem I. **(E)** The inclusion analysis of alternative exon 4. **(F)** The distances between the selector and the splice site were investigated across *Drosophila* species.

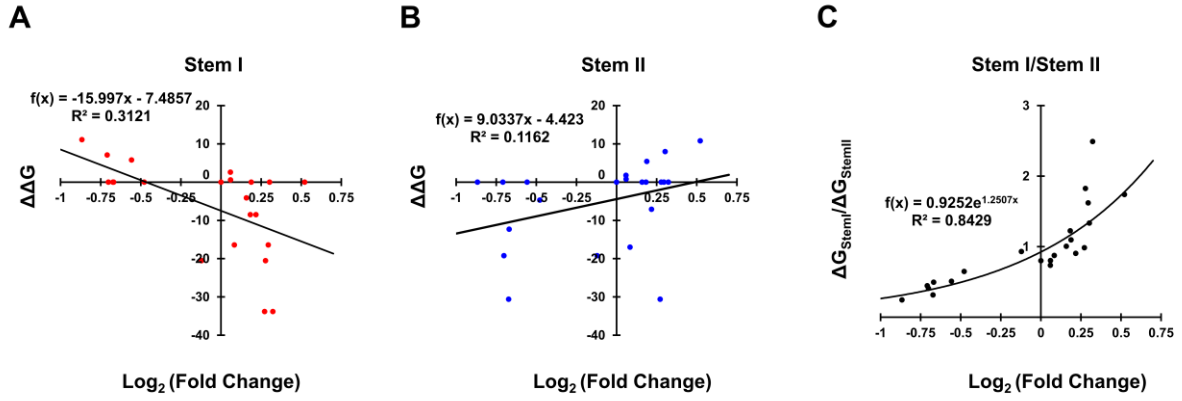
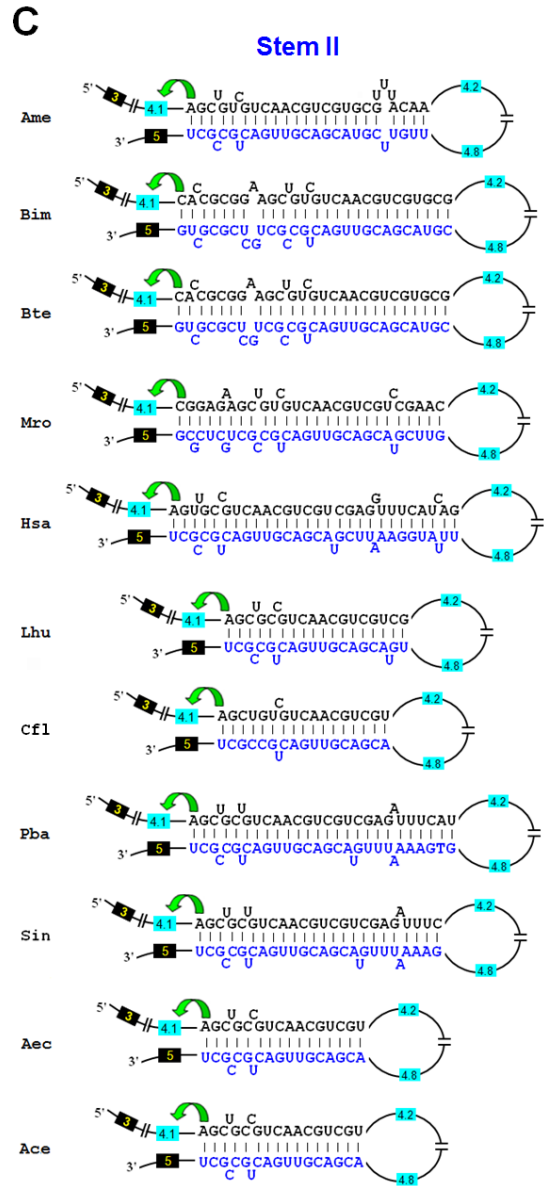
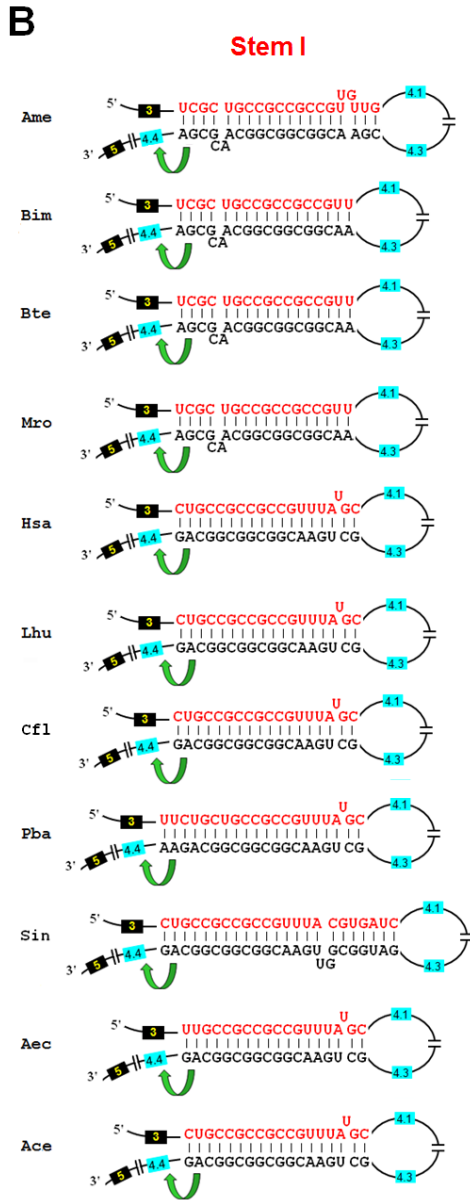
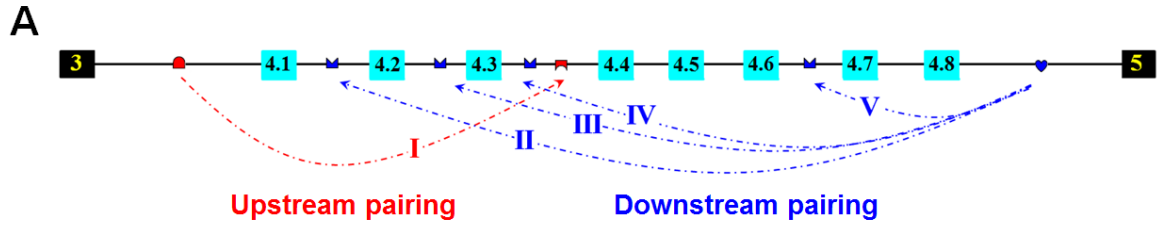


Figure S6. Regulation of mutually exclusive splicing in relative pairing strength-dependent mode. Correlation of upstream RNA pairing strength (**A**), downstream RNA pairing strength (**B**), and their relative strength (**C**) with the exon 4.2 inclusions is analyzed. The change in the strength of the predicted docking site-selector sequence interactions ($\Delta\Delta G$) is plotted as a function of the $\log_2$ fold change in the frequency of exon 4.2 inclusion for WT and mutant constructs.



(ADTU000000000.1); Nvi *N. vitripennis* (AAZX000000000.1). (**B-F**) The inter-intronic pairings are shown for each hymenopteran species. Nucleotide covariations that maintain the structural integrity of the dsRNA were shaded in green.

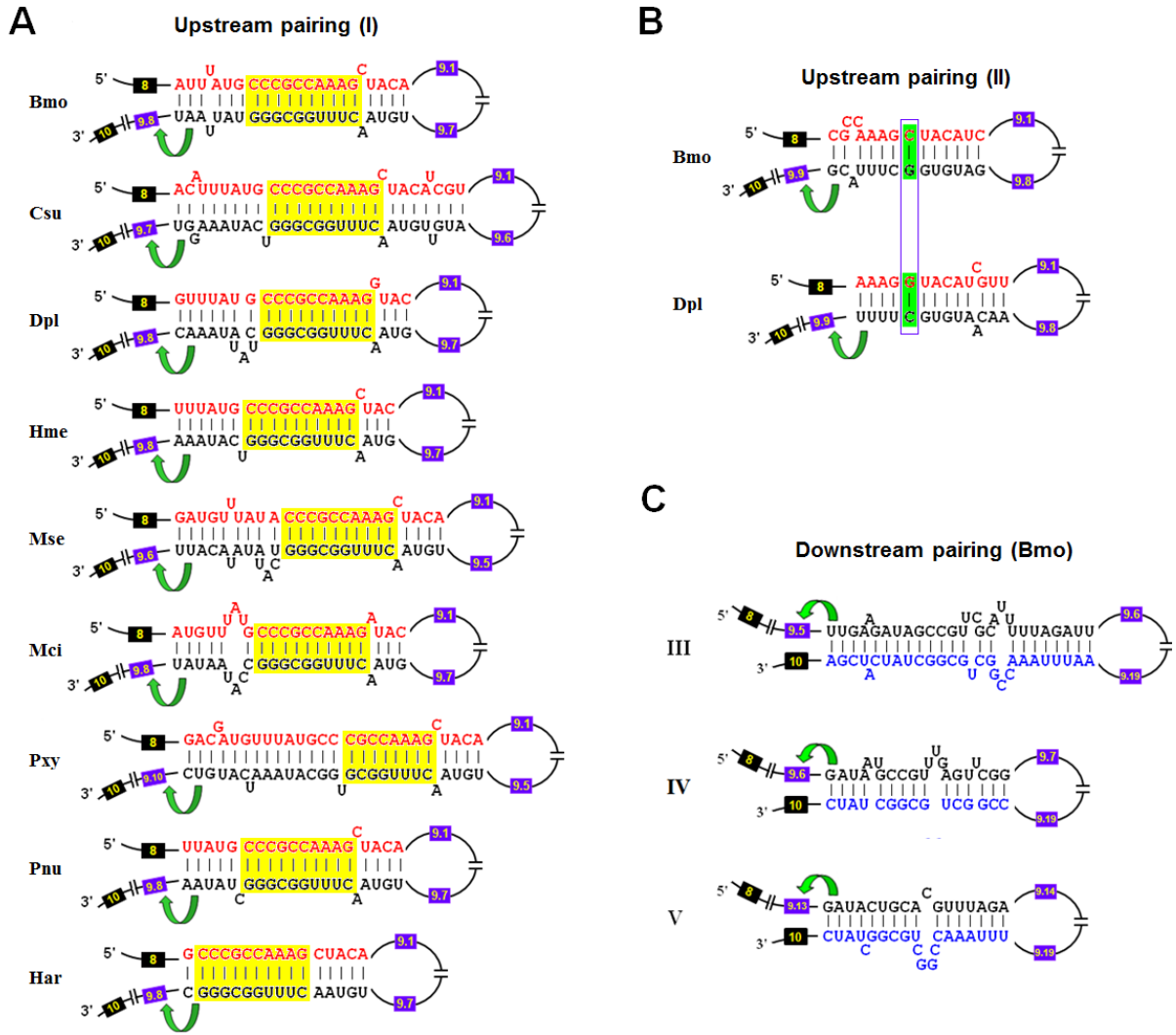


Figure S8. Bidirectional RNA pairings are evolutionarily conserved in the lepidopteran *Dscam* exon cluster 9. **(A)** The upstream intronic pairings are conserved for each lepidopteran species. The sequences that make up the core of the dsRNA stem are highlighted in yellow. Bmo: *B. mori* (BABH000000000.1); Csu: *C. suppressalis* (KJ776429); Dpl: *D. plexippus* (AGBW000000000.1); Hme: *H. melpomene* (CAEZ000000000.1); Mse: *M. sexta* (AIXA01009061.1); Mci: *M. cinxia* (APLT01027263.1); Pxy: *P. xylostella* (BAGR000000000.1); Pnu: *P. nubilalis* (KJ776441); Har: *H. armigera* (KJ776436). **(B)** The upstream intronic pairings are shown for *B. mori* and *D. plexippus*. Nucleotide covariation that maintains the structural integrity of the dsRNA was shaded in green. **(C)** The downstream pairings are shown within *Dscam* exon cluster 9 of *B. mori*.

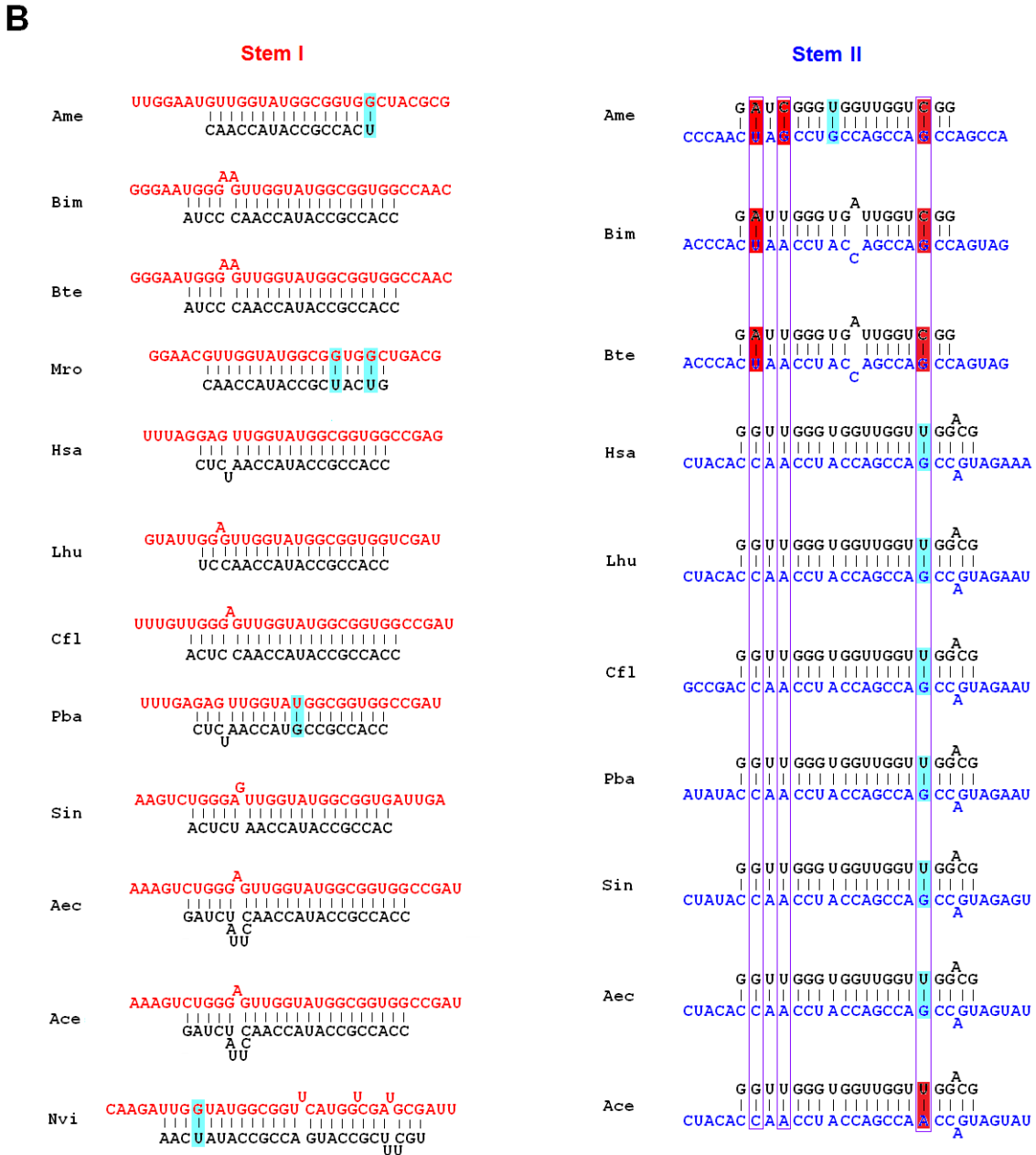
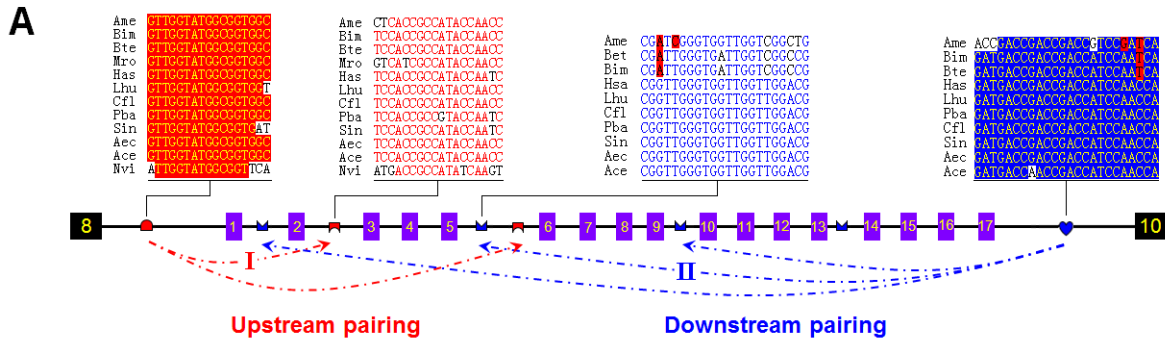
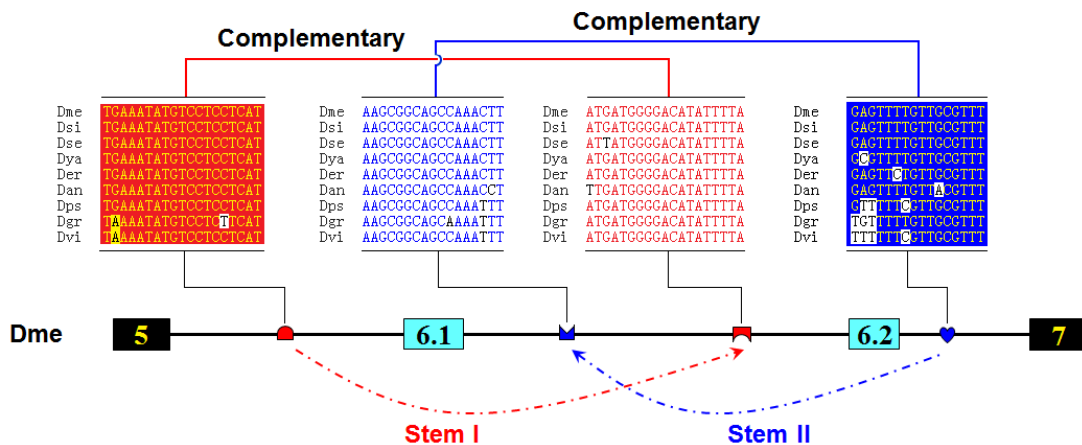


Figure S9. Similar structural architectures within *Dscam* exon cluster 9 of hymenopteran species.

(A) The arrangement of *cis* intronic elements within hymenopteran exon 9 cluster. The *Dscam* gene sequences and symbols are the same as in Supplementary Figure S6. Notably, most of these differences are structurally silent, whereas nucleotide covariations that maintain the structural integrity of the dsRNA were shaded in red. (B) The inter-intronic pairings are shown for each hymenopteran species. Nucleotide covariation that maintains the structural integrity of the dsRNA was shaded in red, and their covariation intermediates (G-U, U-G) were shaded in blue.

A



B

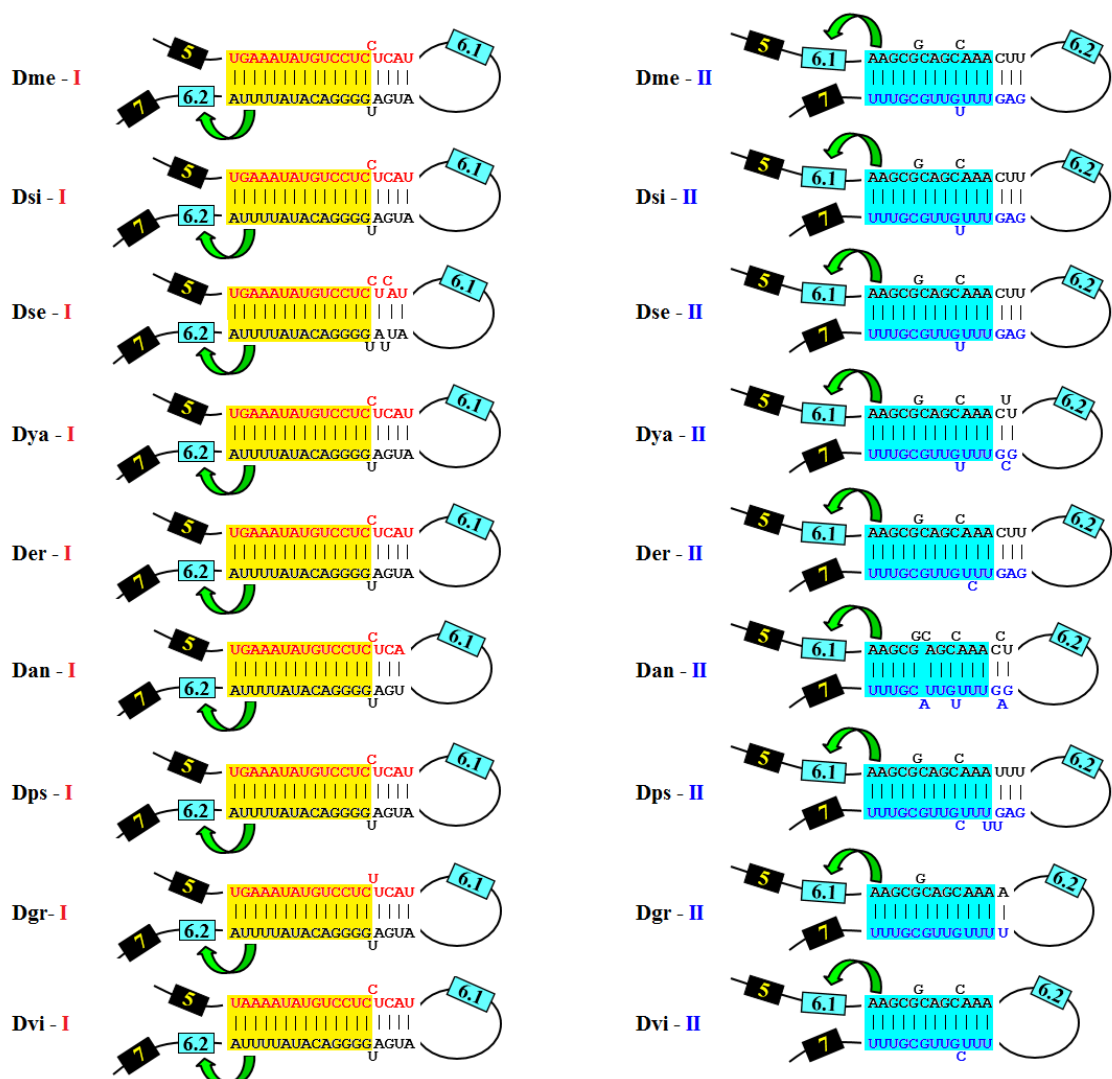


Figure S10. Similar bidirectional RNA pairings are embedded in *Drosophila* *RIC-3* pre-mRNAs.

(A) Partial genomic organization of *D. melanogaster* *RIC-3* gene. Dme: *D. melanogaster*

(AE013599.5); Dsi: *D. simulans* (CM002911.1); Dse: *D. sechellia* (CH480824.1); Dya: *D. yakuba* (CM000158.2); Der: *D. erecta* (CH954179.1); Dan: *D. ananassae* (CH902619.1); Dps: *D. pseudoobscura* (CM000071.3); Dgr: *D. grimshawi* (CH916367.1); Dvi: *D. virilis* (CH940648.1). Symbols used are the same as in Figure 1. Nucleotide variations that are structurally silent are shaded in yellow. **(B)** Upstream and downstream docking sites are shown in red and blue, respectively. The sequences that make up the core of the upstream and downstream stem are highlighted in yellow and cyan, respectively.

References

Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic acids research* **31**: 3406-3415.