

mse-miR-2b*	Alk, Atg4LP, Atg6, Dredd/Caspase 6, Dsor1, FADD, galectin-4, Hdd1, hemocyte specific integrin α 1, HP14, HP4, HP5, IKK β , Integrin β 1, JAK/Hopscotch, Jra, leureptin-1, PPBP2, Pelle, PGRP-L5, PGRP-SA, Ras85D, Serrate, tetraspanin, Tollip
mse-miR-8*	ANKRD54, Atg2, hemolectin, HP14, HP19, integrin related 1, TAK1, tyrosine hydroxylase, Vrille
mse-miR-9a*	Dscam, ECSIT, ERK, Hdd1, IML3, JAK/Hopscotch, Nimrod A, Pellino, PGRP-L2, protein phosphatase type 2c, Rac1, Rel2A, Rel2B
mse-miR-9b*	ERK, HP22, JAK/Hopscotch, Nimrod A, Pellino, PGRP-L2, protein phosphatase type 2c, Rac1, Rel2A, Rel2B
mse-miR-14*	serpin4
mse-miR-31*	Aos1, β GRP2, Domeless, HP6, HP8, leureptin-1, PGRP-L2, secreted peptide 30
mse-miR-34*	Atg12, Dsor1, ERK, hemocyte specific integrin α 1, integrin related 1, PPBP2, Pelle, PI6, MASK, Rel2B, tetraspanin, Tube
mse-miR-71*	Domeless, IML3, lebecinD, Licrone/MKK3, MLK1, MASK, Sickie, tetraspanin
mse-miR-79*	HP12, JNK, Rac1, serpin6
mse-miR-279a*	Caspar, cdc42, lysozyme, serpin4, Serrate
mse-miR-281*	Domeless, hemolectin, Misshapen, Nuclear transport factor 2, serpin4
mse-miR-965*	Atg4LP, hemocyte specific integrin α 2, JNK, Rel2B, tyrosine protein kinase
mse-miR-2755*	Atg4LP, Dscam, ERK, FADD, Hdd13, HP6, serpin6, SPH4
mse-miR-2766*	Dsor1, Licrone/MKK3

Table 5. miRNA:mRNA pairs with reverse profiles involved in extracellular signal transduction and melanization

Target gene	Putative regulatory miRNA
HP2	mse-miR-79
HP5	mse-miR-9a, -2b*
HP6	mse-miR-12, -279d, -2766, -31*
HP7	mse-miR-9b, -316, -2766, -2767
HP8	mse-miR-308, -31*
HP14	mse-miR-263a, -2763, -2767, -2b*, -8*
HP17	mse-miR-8, -9a
HP19	mse-miR-307, -8*
HP21	mse-miR-9a
HP22	mse-miR-9b, -9b*
PAP2	mse-miR-9a, -71, -252
PAP3	mse-miR-12, -2763
SPH1	mse-miR-308
Serpin3	mse-miR-12, -263a, -970
Serpin4	mse-miR-7, -9b, -11, -79, -263a, -308, -989, -let-7a, -281*
Serpin6	mse-miR-11, -79*
Serpin7	mse-miR-308
tyrosine hydroxylase	mse-miR-8*
dopa decarboxylase	mse-miR-6100
Phe hydroxylase	mse-miR-10a, -2763
Punch	mse-miR-9a, -71, -6100

Table S1. List of novel miRNA candidates*

Serial ID	Mature miRNA sequence	CF	IF	CH	IH	Predicted precursor				
						Scaffold	Strand	Start	End	Energy**
t357780	AAGCGGUAAUUGUUGUUGAG	0	1	0	0	00061	minus	736483	736583	-30.6
t391850	AAGUGUCAGGACGAGUUCUAGC	1	0	8	3	00045	minus	682268	682370	-31.1
t454580	AAUCGGACAAGCAGUGCGAACG	1	0	3	1	00639	plus	98314	98415	-38.5
t614965	ACAUAGGACUUUGUAAUUGCCA	1	1	1	0	00349	plus	245361	245463	-33.8
t721312	ACGUAGGGAUGAGAUUGUGG	0	0	1	1	00431	minus	3699	3802	-29.4
t1480635a	AUGCGCUGUAGCACGCGUGC	1	0	0	0	00152	minus	47489	47588	-49.3
t1480635b	AUGCGCUGUAGCACGCGUGC	1	0	0	0	00152	minus	410572	410671	-49.1

t1480635c	AUGCGCUGUAGCACGCGUGC	1	0	0	0	00060	minus	15843	15942	-49.1
t1566101	AUGGAUGAAUUCAUAAAAU	42	1	1	0	00039	minus	465551	465649	-23.8
t1820768	CAAUUGACUAGAGGAGAGUC	14	18	75	85	00034	plus	1201689	1201787	-39
t1943004	CAGCGAAUUGUUUGCGGCA	1	1	9	4	00001	plus	1535457	1535556	-69.5
t2543141	CGUACUGUGCCAUUGGAUAAUA	17	22	15	14	00123	minus	407746	407845	-25.6
t3057689	GACAGAGAUUGUGGCAAGUCGUC	0	1	0	0	00045	plus	1412717	1412819	-32.5
t3301296	GAUGUACUGACUGUUUCAUCGC	0	2	12	5	00355	minus	375346	375447	-42.75
t3923601	GUAUGUCGGCUUUUGAAAUUGA	1	0	1	0	00201	plus	540249	540350	-44.2
t4479723	UACGUUCGCACUCGUUGCCGA	0	0	2	5	00639	minus	98333	98435	-43.6
t4577038	UAGGAAGAUUACACACUCUGUGU	0	1	0	1	00013	minus	1566602	1566702	-29.67
t4589761	UAGGCGUUUCGCGACGACAAU	1	0	2	0	00030	plus	1711190	1711291	-25.1
t4593803	UAGGGCUAAUUAUCUCUCGC	0	0	1	0	00274	plus	387655	387754	-58.3
t4695037	UAUCUUCGUUGUAAUUGUCAU	5	3	3	2	00332	plus	56774	56877	-29.8
t5253999	UGAACUUUUUGCUUGGUAGAGG	0	2	0	0	08414	plus	7	107	-37.4
t5758083	UGUUGGAACGCAGGACAUACU	0	1	2	0	00097	plus	500482	500584	-34.34
t5815605	UUAAUGUAGUGAGAUUCGCGA	1	0	0	0	02545	plus	1146	1247	-34.8
t5865555	UUAGGAUUCCGAUGACUACUA	1	2	0	5	00079	plus	311949	312049	-34.8
t5939672	UUCAGACGCAUACAUAUUUU	0	0	0	1	00097	minus	639888	639989	-54
t6224666	UUUACAAGUACGUCCGCCUUAAG	0	0	0	1	00185	plus	355167	355270	-27
t6233600a	UUUAGAGAUUUUUGAGCCAAA	0	0	0	2	00351	minus	25078	25179	-40
t6233600b	UUUAGAGAUUUUUGAGCCAAA	0	0	0	2	00351	plus	25057	25158	-40

* The read counts are absolute values in the control (C) and induced (I) fat body (F) and hemocyte (H) libraries; ** Calculated free energy of fold back precursors.

Table S2. Abundance of conserved miRNA variants with no precursors identified*

miRNA name	Mature miRNA sequence	CF	IF	CH	IH	IF/CF	IH/CH
miR-1	UGGAAUGUAAAAGAAGUAUGUAG	84	26	8	2	0.31	0.25
miR-1	UGGAAUGUAAAAGAAGUAUGUAU	39	25	3	2	0.64	0.67
miR-1	UGGAAUGUAAAAGAAGUAUGUGA	39	24	3	2	0.62	0.67
miR-1	UGGAAUGUAAAAGAAGUAUGCGU	12	20	3	1	1.67	0.33
miR-2	UAUCACAGCCAGCUUUGAUGAGU	57	22	78	49	0.39	0.63
miR-2	UAUCACAGCCAGCUUUGAUGUGC	6	3	9	4		
miR-7	UGGAAGACUAGUGAUUUUAUUGUU			2	2		
miR-8	UAAUACUGUCAGGUAAUGAUGUC	24	17	3	10	0.71	3.33
miR-8	UAAUACUGUUAGGUAAAAGUCC		2		1		
miR-13	UAUCACAGCCAUUUUUGACGAGU	6	4	5	6		
miR-13	UAUCACAGCCACUUUGAUGUG		2	1			
miR-31	GGCAAGAAGUCGGCAUAGCUG	105	14	87	56	0.13	0.64
miR-31	AGGCAAGAUGUCGGCAUAGCUGA	36	4	52	26	0.11	0.50
miR-33	GUGCAUUGUAGUUGCAUUGCAU	165	79	93	45	0.48	0.48
miR-33	GUGCAUUGUAGUUGCAUUGC	36	33	28	10	0.92	0.36
miR-33	GUGCAUUGUAGUUGCAUUG	12	19	14	8	1.58	0.57
miR-34	UGGCAGUGUGGUUAGCUGGUAGU	24	15	15	35	0.63	2.33
miR-71	UGAAAGACAUGGGUAGUGAGAUU	504	192	637	750	0.38	1.18
miR-71	AGAAAGACAUGGGUAGUGAGAU	15	8	16	15	0.53	0.94
miR-71	UGAAAGACAUGGGUAGUGAGACG	51	35	99	125	0.69	1.26
miR-79	UAAAGCUAGAUUACCAAAGCAU		11	8	6		0.75
miR-100	AACCCGUAGAUCGGAACUUGUGU	84	39	69	22	0.46	0.32
miR-124	UUAAGGCACGCGGUGAAUGCCA		2				
miR-133	UUGGUCCCCUUAACACAGCUGC		1				
miR-133	UUGGUCCCCUUAACACAGCUA		1				
miR-133	UUUGGUCCCCUUAACACAGCUAU		1				
miR-184	UGGACGGAGAACUGAUAAAGGGU	10672	3093	9647	8586	0.29	0.89
miR-184	UGGACGGAGAACUGAUAAAGGCU	2745	1093	2476	1737	0.40	0.70

miR-184	UGGACGGAGAACUGAUAAAGGA	21	7	13	6	0.33	0.46
miR-184	UGGACGGAGAAUUGAUAGGGU	57	18	48	65	0.32	1.35
miR-184	UGGACGGAGAACUGAUAGGGC	24	5	13	13	0.21	1.00
miR-252	AUAAGUACUAGUGCCGCAGGAG	30	3	3	7	0.10	2.33
miR-252	CUAAGUACUAGUGCCGCAGGUGU	6	11	1	9	1.83	9.00
miR-263	AAUGGCACUGAAAGAAUUCACGGG	3	1				
miR-274	GUUUGUGACCGUCACUAACGGGCAGU	66	58	14	10	0.88	0.71
miR-276	UAGGAACUUAUACCGUGCUCU	3					
miR-305	AUUGUACUUAUCAGGUGCUCUGG	90	71	63	202	0.79	3.21
miR-305	AUUGUACUUAUCAGGUGCUCUG	81	69	55	197	0.85	3.58
miR-305	GGCGCUUGUUGGAGUACACUU		3		8		
miR-306	CAGAGCCGCCUCGUGCCUCAG		3	1	1		
miR-317	UGAACACAGCUGGUGGUUAUACAGU		4	21	7		0.33
miR-750	AGUUGGACAGGGGAUCUUGACA				2		
miR-927	UUUUAGAAUCCUACGCUUUACC	39	64	29	17	1.64	0.59
miR-932	UCAAUUCCGUAGUGCAUUGCAG		1	2	1		
miR-970	UCAUAAGACACACGCGGCUAU	1233	2509	4491	13088	2.03	2.91
miR-989	UGUGAUGGACGUAGUGGAACA	3		2			
miR-989	UGUGAUGGACGUAGUGGUAC	3					
miR-1175	UGAGAUUCAACUCCUCCAACUGC		1				
miR-1175	UGAGAUUCAACUCCUCCAACUUA	9	3	13	3	0.33	0.23
miR-1959	GGGAUGUAGCUCAGUGGAG	24	2	3	1	0.08	0.33
miR-2478	GUAUCCACUUCUGACACCA	15	7	33	8	0.47	0.24
miR-2795	CAAGUUUGGUGAUACGCGGGCGC	15	15	59	89	1.00	1.51
mse-bantam-b	UGAGAUCAUUGUGAAAGCUAU	12	18	13	7	1.50	0.54
mse-bantam-c	UGAGAUCAUUGUGAAAGCUGAUU		8	8	4		
mse-let-7b	UGAGGUAGUAGGUUGUGUGGUU	3	1		1		
mse-let-7c	UGAGGUAGUAGGUUGUAUGGUU	141	93	220	117	0.66	0.53
mse-let-7d	AGAGGUAGUAGGUUGCAUAGU				1		
mse-let-7e	UGAGGUAGGAGGUUGUAUAGU			5	1		
mse-let-7f	UGAGGUAGUUGGUUGUAUAGU		2	5	6		
mse-let-7g	UGAGGUAGUAGUUUGUAUAGUU		6	2	3		
mse-let-7h	UGAGGUAGGAGGUUGUAUAGUU			5	1		
mse-let-7i	AGAGGUAGUAGGUUGCAUAGUU				1		
mse-let-7j	UGAGGUAGUAGGUUGUUUAGUU	3	2	2	4		

* Abundance is shown with normalized read numbers (reads per million) in the control (C) and induced (I) fat body (F) and hemocyte (H) libraries. Read numbers are not shown for those either non-detectable or whose values are below 0.5 after normalization. Up ($I/C > 1.25$) and down ($I/C < 0.80$) regulated ones are shaded orange and green, respectively.

Figure legends

Fig. 1. Size distributions of numbers of the total (red bars, left y-axis) and unique (black bars columns, right y-axis) reads in the four libraries combined.

Fig. 2. Predicted stem-loop structures of novel *M. sexta* miRNAs. The precursor sequences are retrieved from the genome based on the loci of mature and star strands (Section 2.2), with the mature ones shown in bold red capital letters.

Fig. 3. One cluster of novel miRNAs. A) Alignment of the mature miRNA sequences with identical residues labeled “|”. B) alignment of the miRNA precursor sequences with different