GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genome-wide identification and expression profiling of the fatty acid desaturase gene family in the silkworm, *Bombyx mori*

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Genet. Mol. Res. 13 (2): 3747-3760 (2014)
Received August 21, 2013
Accepted December 6, 2013
Published May 13, 2014
DOI http://dx.doi.org/10.4238/2014.May.13.2

ABSTRACT. Fatty acid desaturases exist in all living organisms and play important roles in many different biologic processes, such as fatty acid metabolism, lipid biosynthetic processes, and pheromone biosynthetic processes. Using the available silkworm genome sequence, we identified 14 candidate fatty acid desaturase genes. Eleven genes contain 3 conserved histidine cluster motifs and 4 transmembrane domains, but their N-terminal residues exhibit obvious diversity. Phylogenetic analysis revealed that there are 6 groups; *Bmdesat1* and *Bmdesat5-8* were clustered into group 2, which is involved in Δ11 desaturation activity, and *Bmdesat3-4* were grouped in group 1, which is involved in Δ9 desaturation activity. Twelve of the 14 genes have expressed sequence tag evidence. Microarray data and reverse transcription polymerase chain reaction analysis demonstrated that *Bmdesat3-4* and *Bmdesat10* were expressed from the larval to moth stages and in multiple tissues on day 3 of 5th instar larvae. *Bmdesat9*, *Bmdesat11*, and *Bmdesat14* were expressed during the pupal and late-embryonic stage, suggesting that they may take part in fatty acid metabolism to provide energy. These results provide some insights into

the functions of individual fatty acid desaturases in silkworm.

Key words: Reverse transcription polymerase chain reaction; *Bombyx mori*; Fatty acid desaturase genes; Multiple sequence alignment; Expression profiles

INTRODUCTION

Fatty acid desaturases exist in bacteria, fungi, plants, and animals, and they can convert a C-C single bond to a C=C double bond at specific positions in a fatty acyl chain. They are classified into 3 categories according to diverse substrates, namely acyl-CoA, acyl-lipid, and acyl-acyl carrier protein (ACP) desaturases (Los and Murata, 1998). Acyl-CoA desaturases are present in animals, yeast, and fungal cells; acyl-lipid desaturases are in plants and/or cyanobacteria; and acyl-ACP desaturases are in the plastids of plant cells. Acyl-CoA and acyl-lipid desaturases are hydrophobic proteins with 4 transmembrane domains, whereas acyl-ACP desaturases are soluble proteins (Shanklin et al., 1994; Murata and Wada, 1995).

Fatty acid desaturase was first characterized from rat and consists of 358 amino acid residues (Thiede et al., 1986). Recently, fatty acid desaturases have been broadly analyzed in many species. Acyl-CoA desaturase genes from vertebrates, including *Siganus canaliculatus* (Li et al., 2010), *Danio rerio* (Hastings et al., 2001) and *Homo sapiens* (Li et al., 1994) have been cloned. In insects, 3 and 2 acyl-CoA desaturases were identified in *Drosophila melanogaster* (Wicker-Thomas et al., 1997; Dallerac et al., 2000; Chertemps et al., 2006) and *Tribolium castaneum* (Horne et al., 2010), respectively. In moths, many fatty acid desaturase genes were cloned from pheromone glands, including Δ11 and/or Δ9 desaturase in *Trichoplusia ni* (Knipple et al., 1998), *Helicoverpa zea* (Rosenfield et al., 2001), *Bombyx mori* (Yoshiga et al., 2000; Moto et al., 2004), *Helicoverpa assulta* (Jeong et al., 2003), *Manduca sexta* (Matouskova et al., 2007), and *Ostrinia* spp (Roelofs et al., 2002); Δ14 desaturase in *Ostrinia* spp (Roelofs et al., 2002); and Δ10 desaturase in *Planotortrix octo* (Hao et al., 2002).

Fatty acid desaturases of insects are structurally and biochemically homologous to the Δ9 acyl-CoA desaturases from fungi and animals, suggesting that they originate from a common ancestor (Stukey et al., 1990; Shanklin and Cahoon, 1998). They are a class of integral proteins residing in the endoplasmic reticulum that contains non-heme iron atoms at their active site (Strittmatter et al., 1974), and they function as component of a multienzyme complex consisting of Δ9 acyl-CoA desaturases, NADH-cytochrome b_5 reductase (a flavoprotein) and cytochrome b_5 (a hemoprotein) (Holloway, 1971; Spatz and Strittmatter, 1971; Rogers and Strittmatter, 1973). In most insects, fatty acid desaturases that are involved in sex pheromone biosynthetic pathways were widely studied; however, there is little research on the fatty acid metabolism, regulation of cell membrane fluidity to adapt to temperature fluctuations, and production of beneficial materials (such as pupal oil). Further studies will help us comprehensively understand the multiple functions of insect fatty acid desaturases.

The silkworm, *B. mori*, is an important economical insect and a model organism for Lepidoptera (Goldsmith et al., 2005). To date, only 4 desaturase genes were reported; one of them, originally named *desat1*, possesses both Z11 desaturation and Δ10,12 desaturation activities, which are responsible for the 2 desaturation steps in the biosynthesis pathway of bombykol, (E,Z)-10,12-hexadecadien-1-ol (Moto et al., 2004). The functions of other desaturases are still not well known, and in the past decades, the main focus was on the molecular mechanisms underly-

ing sex pheromone production in the silkworm (Matsumoto et al., 2007; Matsumoto, 2010). Thus, in this study, we first identified the fatty acid desaturase genes based on the complete silkworm genome sequence; then, multiple sequence alignment of silkworm desaturases and phylogenetic analysis were performed, and the spatio-temporal expression of these genes was also investigated via microarray data and reverse transcription polymerase chain reaction (RT-PCR) analysis.

MATERIAL AND METHODS

Identification of fatty acid desaturase genes in silkworm and other insects

Five desaturase sequences from silkworm were downloaded from GenBank and were used as queries against the silkworm database using basic local alignment search tool (BLAST), with an E-value threshold of 10^{-6}. Predicted sequences were validated by aligning them to the non-redundant dataset and the expressed sequence tag (EST) database (threshold E value $<10^{-30}$, identities > 90%, and match lengths > 100 bp). All of the identified fatty acid desaturases were further checked for the 3 conserved histidine tracks, which were used to characterize the fatty acid desaturases (Los and Murata, 1998). The protein sequences of *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera*, and *Tribolium castaneum* were downloaded from the National Center for Biotechnology Information (NCBI). We used fatty acid desaturases from silkworm as queries in a homology search for the 4 insects fatty acid desaturases.

The chromosome distribution of fatty acid desaturase genes in silkworm and the 4 other surveyed insects was analyzed by SilkMap (http://silkworm.swu.edu.cn/silksoft/silkmap.html) and the Map View tool on http://www.ncbi.nlm.nih.gov/projects/mapview/ and http://genome.ucsc.edu/, respectively.

Prediction of silkworm fatty acid desaturase characteristics

Predictions of a transmembrane helix of silkworm fatty acid desaturases were performed by TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and TMpred (http://www.ch.embnet.org/software/TMPRED_form.html). Endoplasmic retention signals were predicted by PSORT (http://psort.hgc.jp/form2.html). The protein domains were predicted by InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan/).

Phylogenetic analysis

Amino acid sequences of fatty acid desaturases were aligned using 2 methods, Clustal X v1.83 and Muscle v3.6. Using a conserved core domain, we reconstructed the phylogenetic tree by the neighbor-joining (NJ) methods in the program MEGA5. The evolutionary distance was assessed by the JTT amino acid matrix using complete deletion option, and the tree was tested by bootstrapping with 1000 resampling replicates.

Sample preparation

We collected individuals of the silkworm strain Dazao at 27 different time points from day 1 to day 10 after oviposition (E1-E10) and from day 3 of the 5th instar stage (V3) to moths, including V3, V5, V7, and W12 (12 h after wandering); W24, W36, and W48 (completion

of spinning); W60 (pupation); W72, W96, and W120 (formation of egg); and W144, W168, W192, W216, W240, and adult (moths) for both sexes; all samples were immediately frozen with liquid nitrogen and stored at -80°C until use.

Expression profiling of silkworm fatty acid desaturase genes

Total RNA was extracted using Trizol reagent (Invitrogen, USA), which was followed by treatment with RNase-free DNaseI (Promega, USA) for 30 min at 37°C to eliminate the contaminating genomic DNA. The first strand of cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen, USA) for 1 h at 42°C. All cDNA samples were normalized using the silkworm cytoplasmic actin A3 gene as an internal control (forward primer: 5'-AACACCCCGTCCTGCTCACTG-3'; reverse primer: 5'-GGGCGAGACGTGTGATTTCCT-3'). All gene-specific primers for semi-quantitative RT-PCR detection are listed in **Table S1**. PCR amplification was performed in a total reaction volume of 25 µL containing normalized cDNA, 10 pmol each primer, 2 mM $MgCl_2$, 0.25 mM dNTP, 1X buffer, and 2.5 units Taq DNA polymerase. RT-PCR were performed using the following program: initial incubation at 94°C for 4 min; 25 cycles at 94°C for 40 s, 40 s of annealing (temperatures listed in **Table S1**), and 40 s to 1 min extension at 72°C (the time depends on the length of the gene); and final extension at 72°C for 10 min. PCR products were separated on 1.5% agarose gels and stained with ethidium bromide. We also analyzed the expression patterns of silkworm fatty acid desaturase genes from microarray data that were described in previous studies (Xia et al., 2007). Microarray-based gene expression was visualized using GeneCluster 2.0.

RESULTS

Identification of fatty acid desaturase genes in silkworm and other insects

We used the sequences of previously reported fatty acid desaturases as queries to search against the silkworm genome sequence by BLAST and identified a total of 14 putative fatty acid desaturase genes (Table 1). *Bmdesat1* and *Bmdesat2* (GenBank accession No. AF182405) shared 98% identity in their proteins; therefore, they were considered to be alleles from hybrid strains (Yoshiga et al., 2000; Moto et al., 2004). *Bmdesat13-15* lacked the N-terminal region containing the first histidine box; this may be because of gaps in the silkworm genome or pseudogenes.

Bmdesat5 and *Bmdesat13* are located on nscaf2959 and scaffold 798, respectively; they could not be mapped to chromosomes in the current version of the silkworm genome sequence. The other 12 genes reside on 7 chromosomes in a nonhomogeneous manner. Among them, there are 5 genes on chromosome 12, 2 genes on chromosome 24, and only 1 gene on each of the other chromosomes. *Bmdesat10*, *Bmdesat12*, and *Bmdesat15* are tandemly arranged in the middle region of chromosome 12, whereas *Bmdesat4* is separately located on the same chromosome apart from the tandem set (**Figure S1**).

The inventories of fatty acid desaturase genes in the other 4 insects were also identified, including *D. melanogaster*, *A. gambiae*, *A. mellifera*, and *T. castaneum*. There are 7 candidate fatty acid desaturase genes in *D. melanogaster*, 6 in *A. gambiae*, 5 in *Apis mellifera*, and 15 in *T. castaneum* (Table 2). All of the fatty acid desaturase genes in *D. melanogaster* and *A. gambiae* are distributed on chromosome 3 and chromosome 2R, respectively. Most of

the fatty acid desaturase genes in *T. castaneum* are located on group 6, whereas only 1 fatty acid desaturase gene in *A. mellifera* could be mapped to LG12 based on the current genome assembly (Table 2 and **Figure S1**). The number of introns in fatty acid desaturase genes varied from 0 to 6 in the 5 insect species. Only *D. melanogaster desatF* (CG7923), which originated from a single retrotransposition event, has no intron (Bai et al., 2007).

Table 1. Desaturase genes in silkworm, *Bombyx mori*.

Gene	Gene ID	Chromosome	Scaffold and interval	Intron	Protein length	EST No.	Probe	Accession No.
Bmdesat1/2	*BGIBMGA011563*	23	nscaf3027 (+):2884323..2886490	2	330	21	sw19398	NM-001043552
Bmdesat3	*BGIBMGA010681*	12	nscaf2998 (+):1408999..1415629	3	353	20	sw13636	NM-001043553
Bmdesat4	*BGIBMGA010365*	12	nscaf2993 (-):7934254..7930909	3	352	15	sw13468	NM-001043506
Bmdesat5	*BGIBMGA009556*	Unknown	nscaf2959 (+):49138..52253	4	339	1	sw19913	NM-001043449
Bmdesat6	*BGIBMGA008171*	24	nscaf2891 (+):570250..580115	3	440	4	sw09764	
Bmdesat7	*BGIBMGA009568*	24	nscaf2961 (+):58496..62721	2	319	No	sw14550	
Bmdesat8	*BGIBMGA004868*	25	nscaf2818 (+):2092666..2093711	2	326	20	sw22861	NM-001046676
Bmdesat9	*BGIBMGA005471*	8	nscaf2828 (+):3852852..3859845	6	411	10	sw18173	
Bmdesat10	*BGIBMGA010611*	12	nscaf2998 (-):1203091..1215839	5	360	1	sw20866	
Bmdesat11	*BGIBMGA006471*	6	nscaf2853 (+):1887484..1891043	5	377	2	sw19842	
Bmdesat12	*BGIBMGA010614*	12	nscaf2998 (-):1120075..1122566	4	361	4	sw06159	
Bmdesat13	*BGIBMGA014550*	Unknown	scaffold798 (+):17961..22613	3	-	1	sw22551	
Bmdesat14	*BGIBMGA001534*	21	nscaf2136 (+):4097030..4099447	2	-	No	sw13905	
Bmdesat15	*BGIBMGA010676*	12	nscaf2998 (+):1285801..1292180	4	-	3	sw12032	

(-) The corresponding desaturase gene may be incomplete.

Characteristics of the silkworm fatty acid desaturases

Multiple sequence alignment of silkworm fatty acid desaturases showed a substantial conservation in the region of the fatty acid desaturase core domains (delimited by GAHR and EGFH motifs) (Figure 1). In contrast, great diversity was noted in sequence length and order at the N-terminus of silkworm fatty acid desaturases. Three histidine clusters (labeled H1, H2, and H3) are common features of fatty acid desaturases and might be essential for desaturase catalytic activity (Shanklin et al., 1994). All silkworm fatty acid desaturases with complete sequences have 4 transmembrane domains, which are marked TM1-TM4. Eight silkworm fatty acid desaturases comprise the proposed endoplasmic retention signal.

It is obvious that the fatty acid desaturases of silkworm display distinct signature motifs based on a group of 4 amino acids that are upstream of H3. The signature motifs of Bmdesat1, Bmdesat5, Bmdesat6, and Bmdesat7 are xxxQ; that of Bmdesat3 is NPVE; and that of Bmdesat4 is KPSE. The amino acid sequences of fatty acid desaturases containing the xxxQ signature motif encode Δ11 desaturases, and the sequences containing the NPVE and KPSE signature motifs encode Δ9 desaturases (Knipple et al., 2002).

Table 2. Summary of fatty acid desaturase genes in other four insects.

Gene name gene ID	Accession No.	Chromosome location	Intron	Protein length
Drosophila melanogaster				
Desat1 CG5887	NM_169468	3R(+):8270531..8272660	3	383
Desat2 CG5925	NM_141944	3R(+):8262143..8263478	3	361
CG8630	NM_169500	3R(+):9108127..9109672	3	408
Fad2 CG7923	NM_143709	3L(+):11016639..11017703	0	355
CG9747	NM_143522	3R(-):26011569..26016463	3	461
CG15531	NM_143523	3R(-):26020608..26021890	3	334
CG9743	NM_143524	3R(-):26022362..26024615	4	420
Anopheles gambiae				
AGAP001713	XM_321375	2R(+):8873957..8875611	3	355
AGAP003049	XM_001237392	2R(-):31439418..31452934	4	405
AGAP003050	XM_311821	2R(-):31465070..31466450	3	363
AGAP003051	XM_311819	2R(-):31474690..31476162	3	381
AGAP003418	XM_311704	2R(+):39199869..39204460	3	394
AGAP004572	XM_313877	2R(+):59481796..59490161	5	415
Apis mellifera				
GB11969	XM_395629	Unknown	4	347
GB11596	XM_623922	Unknown	5	368
GB15513	XM_624791	Unknown	4	291
GB17206	XM_624554	Unknown	4	368
GB18070	-	LG12(-):3184837..3186686	4	336
Tribolium castaneum				
GLEAN_00549	XM_968940	LG2(-):852711..851338	4	335
GLEAN_03656	NM_001143734	LG3(+):188726..189951	3	358
GLEAN_15108	EFA05022	LG6(-):54316..50512	3	323
GLEAN_15349	XM_963735	LG6(-):116035..115397	4	321
GLEAN_15382	XM_963804	LG6(+):116215..117271	2	318
GLEAN_15383	XM_963877	LG6(+):117625..118971	2	320
GLEAN_15338	XM_965427	LG6(-):242416..241459	2	290
GLEAN_15395	EFA05245	LG6(+):263878..264992	2	329
Z9desA GLEAN_11471	NM_001195235	LG10(+):607006..608123	1	353
GLEAN_16399	EFA12397	Unknown	3	277
Z9desB GLEAN_16415	NM_001193649	Unknown	1	350
GLEAN_14821	EFA12799	Unknown	3	389
GLEAN_14820	EFA12798	Unknown	5	414
GLEAN_14819	XM_962850	Unknown	4	356
GLEAN_16414	EFA12412	Unknown	4	288

Phylogeny of the insect fatty acid desaturase family

According to multiple sequence alignments of the conserved core region of insect fatty acid desaturases (**Figure S2**), we constructed the NJ tree using the program MEGA5.02 (Figure 2). The phylogeny showed that the deep internal branches were very short, suggesting that the duplication events causing the insect fatty acid desaturase gene families occurred within a narrow period. The phylogeny also showed that the internal branches within different clusters were very short but the terminal branches were much longer, suggesting that the changes that correlated with speciation occurred quickly. The tree could be divided into 6 groups (group 1-6). Group 1 had 2 subgroups, Δ9 (16C>18C) and Δ9 (18C>16C) lineages, based on the catalytic activity of previously reported fatty acid desaturases. Fatty acid desaturases containing the xxxQ signature motif were in group 2; these were identified only from Lepidoptera, suggesting that the group may be Lepidoptera-specific. Other desaturases that clustered into groups 3-6 were distantly related to the functionally known fatty acid desaturases in group 1 and group 2, suggesting that they are likely to evolve new functions by an independent route, such as the Δ14 desaturases in *Ostrinia* spp (Roelofs et al., 2002) and Δ12 desaturase of

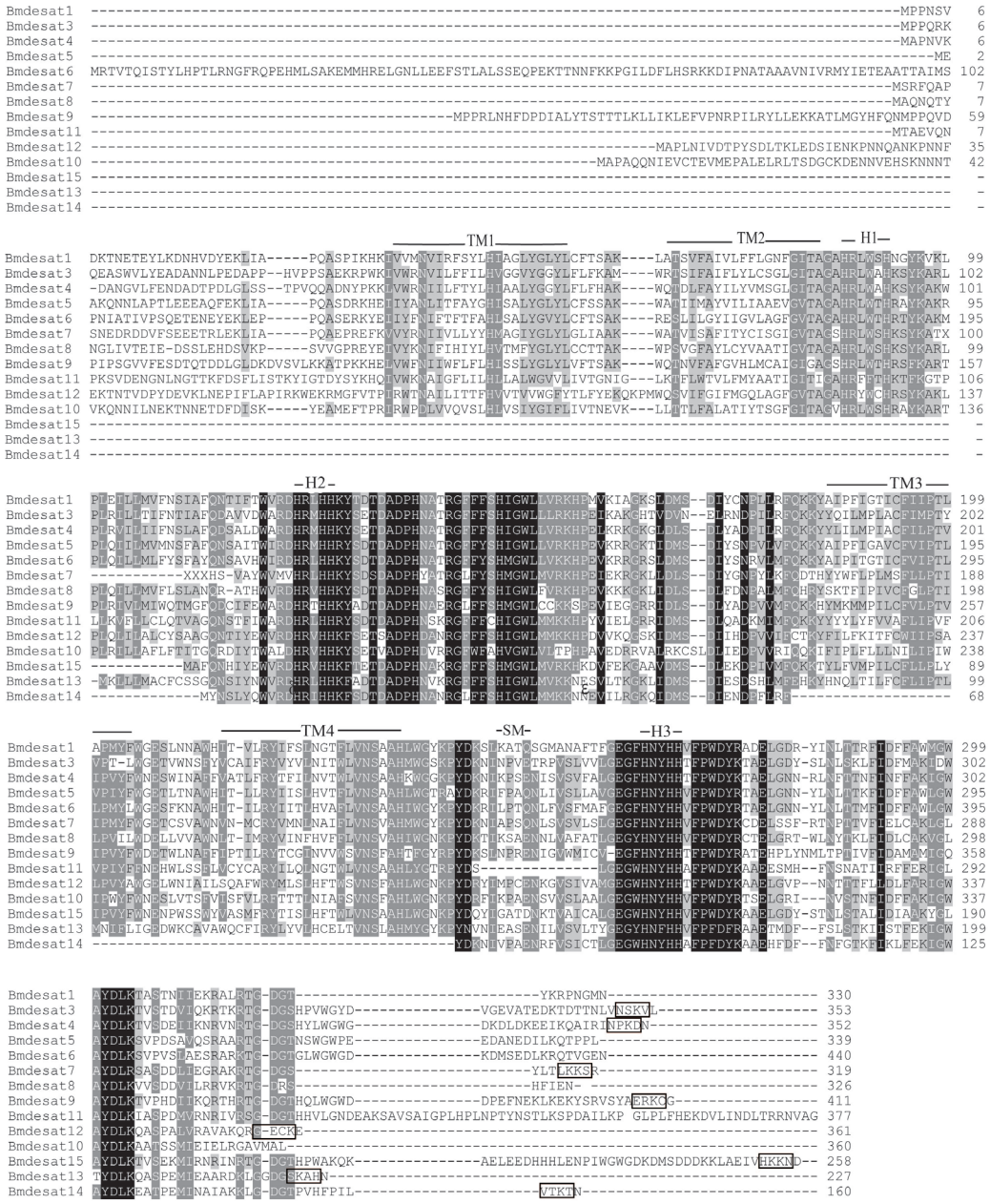

Figure 1. Aligned amino acid sequences encoded by silkworm putative desaturase genes. Black and grey backgrounds indicate amino acid identities and conservative substitutions, respectively. Three conserved histidine clusters of desaturases (H1-H3), the four transmembrane domains (TM1-TM4) and the signature motif (SM) are overlined. Boxed regions indicate the proposed ER retention signal.

T. castaneum (Zhou et al., 2008). It is noteworthy that TcEFA12397, AgAGAP003050, and DmCG15531 formed a clade with a 99% bootstrap value; their 3rd histidine motif differed from the conserved 3rd histidine domain (**Figure S2**), indicating fast evolution.

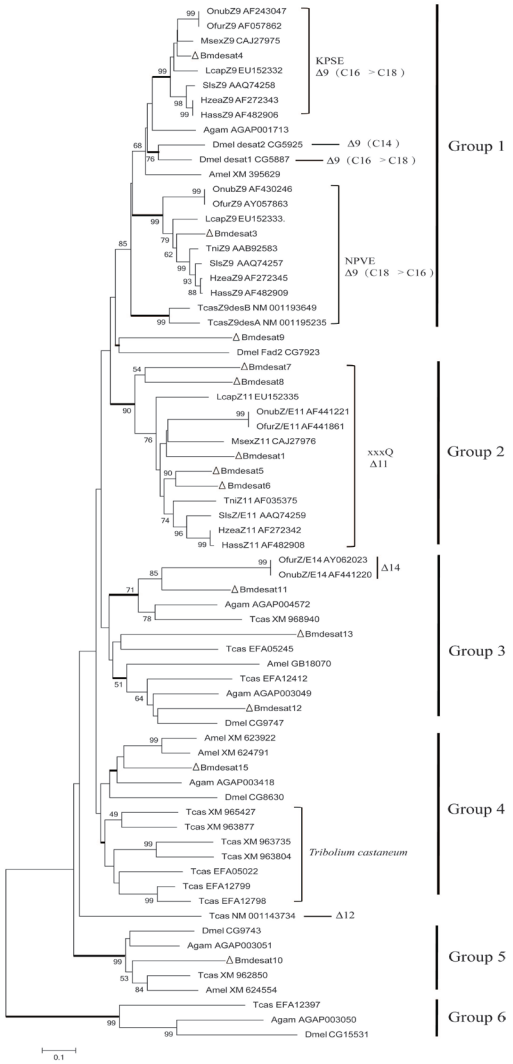

Figure 2. A neighbor-joining unrooted tree of fatty acid desaturase core domains (delimited by GAHR and EGFH motifs) among *Bombyx mori*, *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera*, *Tribolium castaneum* and other reported fatty acid desaturases, the result of multiple sequence alignment is showed in **Figure S2**. Numbers along branches indicate bootstrap support from 1000 replicates. The names for all sequences are composed of abbreviated species name and accession numbers (Dmel = *Drosophila melanogaster*: Agam = *Anopheles gambiae*: Amel = *Apis mellifera*: Tcas = *Tribolium castaneum*: Tni = *Trichoplusia ni* (Knipple et al., 1998, Liu et al., 1999); Onub/Ofur, *Ostrinia species* (Roelofs et al., 2002); Msex = *Manduca sexta* (Matouskova et al., 2007); Lcap = *Lampronia capitella* (Lienard et al., 2008); Sls = *Spodoptera littoralis* (Rodriguez et al., 2004, Serra et al., 2006); Hzea = *Helicoverpa zea* (Rosenfield et al., 2001); Hass = *Helicoverpa assulta* (Jeong et al., 2003), biochemical activities are indicated in connection to the species name. The desaturases from silkworm are indicated by a triangle.

Spatio-temporal expression profiles of silkworm fatty acid desaturase genes

ESTs of silkworm fatty acid desaturase genes were identified. Twelve fatty acid desaturase genes matched at least 1 EST, and the coding regions of 4 fatty acid desaturase genes had complete ESTs. ESTs corresponding to *Bmdesat1* and *Bmdesat5* were only found in the pheromone gland. ESTs for *Bmdesat12* and *Bmdesat15* were detected in follicle cells and the compound eye, respectively. ESTs for *Bmdesat3*, *Bmdesat4*, and *Bmdesat9* were from unfertilized embryos. Intriguingly, one EST for *Bmdesat6* was present in the larval maxillary galea, which contains taste sensilla.

We further investigated the expression profiles of silkworm fatty acid desaturase genes in 9 different silkworm tissues on day 3 of the 5th instar larvae based on microarray data (Xia et al., 2007). *Bmdesat4* and *Bmdesat10* were widely expressed in all surveyed tissues; they were particularly highly expressed in the midgut and Malpighian tubule (Figure 3). *Bmdesat13* was specifically detected in the anterior/median silk gland (A/MSG), meaning that it may have beneficial effects in sericin production. *Bmdesat3*, *Bmdesat7*, *Bmdesat9*, and *Bmdesat15* were expressed in multiple tissues, such as head, testis, midgut, and Malpighian tubule. The expression patterns of these genes were similar in males and females except *Bmdesat3*. However, the other 7 fatty acid desaturase genes were not detected in any tissues on day 3 of the 5th instar larvae.

We also profiled the expression of fatty acid desaturase genes during silkworm metamorphosis using microarray data (unpublished data) and semi-quantitative RT-PCR (Figure 4). Most fatty acid desaturase genes showed distinct expression patterns at 19 sequential

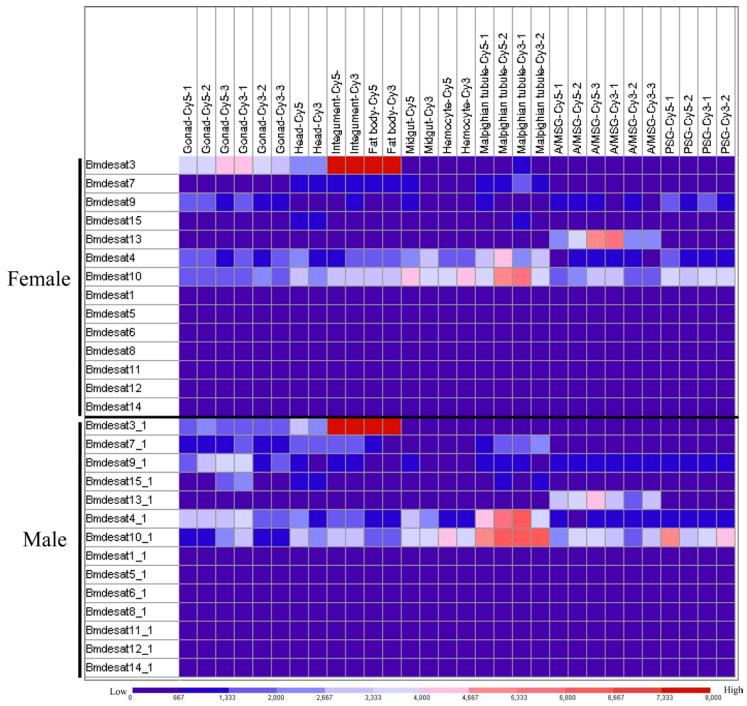

Figure 3. Expression profiles of desaturase genes in multiple larval tissues on day 3 of fifth instar of silkworm by microarray data. The expression levels are illustrated by red (higher expression) and blue (lower expression) boxes. A/MSG = anterior/median silk gland; PSG = posterior silk gland. F = female and M = male.

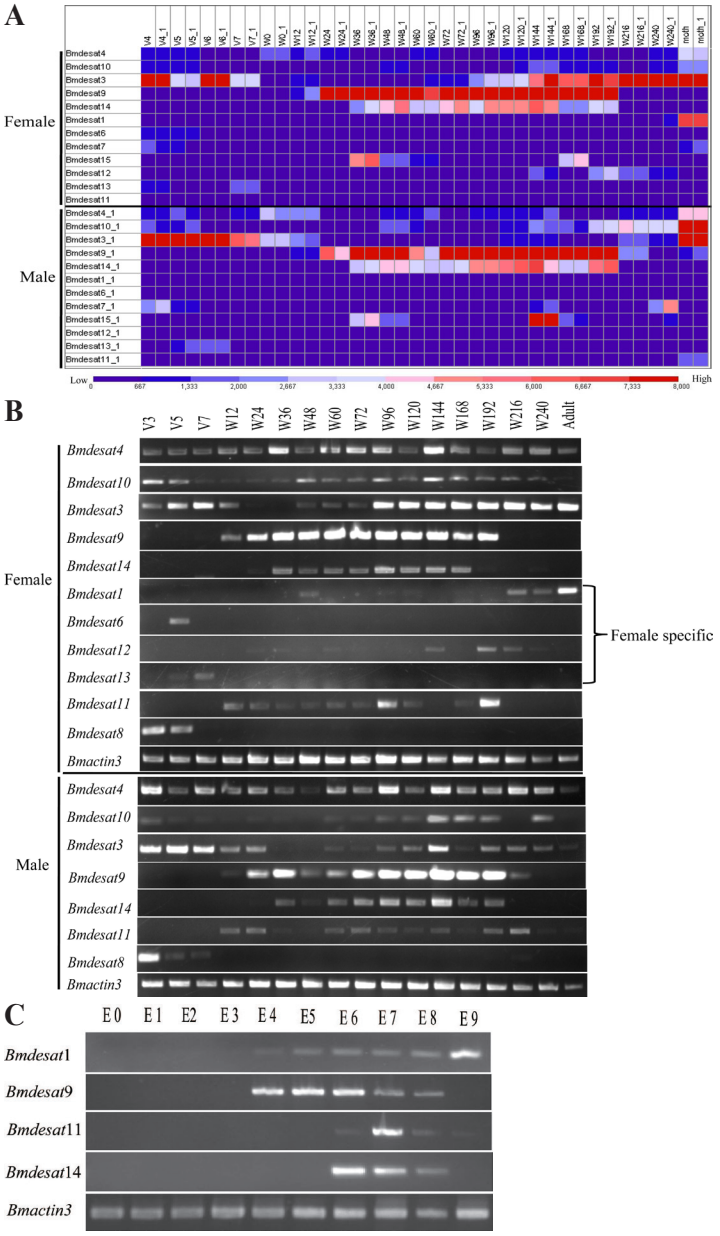

Figure 4. Expression profiles of fatty acid desaturase genes during silkworm metamorphosis based on microarray data (A) and RT-PCR (B, C). **A.** The expression levels are illustrated by red (higher expression) and blue (lower expression) boxes. The columns represents 19 different sample time points: V4 (day 4 of the fifth instar), V5, V6, V7, W0 (start of wandering), 13 different times after wandering (W12, W24, W36, W48, W60, W72, W96, W120, W144, W168, W192, W216, W240), and adult. **B.** Male and female individuals at 17 time points during silkworm metamorphosis (from V3 to moth) were selected for expression profiling using RT-PCR. The silkworm cytoplasmic actin A3 gene (GenBank accession No. U49854) was used as internal control, and denoted by *Bmactin3*. V means fifth larval instar; W means wandering. **C.** Expression profiling of embryonic development stages, E0-E9 means from day 0 to day 9 after oviposition.

developmental time points from day 4 of the 5th instar larvae to the adult (moth). *Bmdesat1*, *Bmdesat6*, and *Bmdesat12* were exclusively expressed at 2 or 3 developmental time points in females, and *Bmdesat1* was expressed in the late embryonic stage, suggesting that the gene may have a function other than bombykol production. Transcription levels of *Bmdesat3* were higher in females than in males. *Bmdesat4* and *Bmdesat10* were expressed almost over the entire metamorphosis, and *Bmdesat8* was mainly detected in larvae. *Bmdesat9*, *Bmdesat11*, and *Bmdesat14* were expressed from spinning to the penultimate day before adult eclosion. Further investigation of their expression pattern during embryonic development stages revealed that they were expressed in the late embryo stages.

DISCUSSION

Using on genome dataset, we identified 14 fatty acid desaturase genes in silkworm. The deduced amino acid sequences of the fatty acid desaturase genes harbor 4 predicted transmembrane domains and 3 conserved histidine tracks, which are presumed to provide ligands to ferric iron in the catalytic center (Shanklin et al., 1994). Further analysis revealed that these motifs were localized at highly conserved positions of silkworm desaturases, and the location of the TM3 domain was well conserved in all of the fatty acid desaturase sequences of moths (Knipple et al., 2002). Although these genes have conserved functional domains and show high similarity, they have been clustered into different groups and display different expression profiles, indicating that they may have different functions.

Genes may be involved in pupal oil production

Silkworm pupae have a high nutritional value because of the presence of abundant fat, which constitutes about 30% of the total dry pupal weight; unsaturated fatty acid is abundant in pupal oil, reaching 66.8% of the total fat (Wei et al., 2009). The amount of the fatty acid in silkworm is far greater than that in mulberry leaves, indicating that many fatty acids in silkworm are the result of *de novo* synthesis *in vivo*. Polyunsaturated fatty acids were present in large amounts in silkworm eggs (Suzuki et al., 1970). Taken together, *Bmdesat9*, *Bmdesat11*, and *Bmdesat14* were expressed during pupal development stages and the late embryonic development stages; thus, we presumed that they have a role in silkworm pupal oil production, and they supply abundant nutrition for pupal and embryonic development.

Bmdesat3 may be involved in fatty acid metabolism

Bmdesat3 could be phylogenetically grouped within the clade of Δ9 (18C>16C). It displayed high similarity (>80%) to other moth Δ9 desaturases with a stearoyl-CoA preference, and it had an especially high similarity (86%) to the Δ9 desaturase from *Trichoplusia ni*, which was a component of the metabolic Δ9 desaturase complex (Liu et al., 1999). *Bmdesat3* mRNA was enriched in the W72 stage (72 h after wandering; namely, 12 h after pupation) through the adult stage of females, a period when the ovum was grown, and it was also highly expressed in fat bodies on day 3 of 5th instar larvae. Therefore, we suspected that Bmdesat3 may be involved in fatty acid metabolism.

Bmdesat4 may be important for silkworm life

Bmdesat4 is closely related to the other moth Δ9 desaturases with a 16-carbon substrate chain length preference, and it shared high sequence similarity to the fatty acid desaturase Dmdesat1 from *D. melanogaster*, which participates in *Drosophila* sex pheromone biosynthesis (Wicker-Thomas et al., 1997). However, in silkworm, Bmdesat1 was the only desaturase that was necessary to catalyze the 2 consecutive desaturation steps in sex pheromone biosynthesis (Moto et al., 2004). Therefore, Bmdesat4 may not be involved in the sex pheromone biosynthetic pathway. Interestingly, based on the genome resequencing of 40 domesticated and wild varieties, *Bmdesat4* was predicted to be a domestication-related candidate gene that was under artificial selection during domestication (Xia et al., 2009). Additionally, it was widely expressed in multiple tissues and constantly expressed from embryo to moth (Chen et al., 2012), suggesting that the gene may be important in silkworm, like housekeeping genes, but the concrete function of the gene is ambiguous.

ACKNOWLEDGMENTS

Research supported by grants from the National Hi-Tech Research and Development Program of China (#2011AA100306), the National Basic Research Program of China (#2012CB114600), and the National Natural Science Foundation of China (#31172157).

Supplementary material

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