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Identification and Expression Pattern of Putative Odorant-Binding Proteins and Chemosensory Proteins in Antennae of the *Microplitis mediator* (Hymenoptera: Braconidae)

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Abstract

The parasitoids of Cotton Bollworm *Microplitis mediator* (Hymenoptera: Braconidae) find their hosts through the odor released by stressed plants. In this study, preliminary characterization and isolation of cDNAs from male *M. mediator* antennal libraries identified 8 putative odorant-binding proteins (OBPs). Real-time polymerase chain reaction method was used to study the expression pattern of these isolated genes. Their gene expression profiles under a wide range of conditions indicated that only 4 OBP genes in *M. mediator* were antenna specific. The remaining 4 genes are either expressed ubiquitously or strictly regulated in specialized tissues or during different developmental stages. Some OBP genes were gender specific. These findings support that OBPs play dynamic roles during development of *M. mediator* and are likely to be involved in broader physiological functions.

Key words: antennal cDNA library, expression pattern, *Microplitis mediator*, odorant-binding proteins

Introduction

Insects have an enormous impact on global agriculture both as pests and beneficial organisms. Olfaction is an important sensory input that regulates their behavior. Therefore, understanding the interplay of molecular components of the olfactory system becomes crucial. In insects, the olfactory neurons are located mainly on their antennae. Odorant-binding proteins (OBPs) are thought to carry lipophilic odorants to the olfactory receptor cells through hydrophilic surroundings and are involved in the initial biochemical step of odor reception (Vogt et al. 1985; Prestwich et al. 1995; Vogt 1995; Steinbrecht 1996, 1999; Ziegelberger 1996; Kaissling 1998; Wang et al. 2003; Tsuchihara et al. 2005; Xu et al. 2005; Pelosi et al. 2006; Matsuo et al. 2007; Laughlin et al. 2008).

Initially, OBPs were identified in insects as pheromone-binding proteins (PBPs; Vogt and Riddiford 1981). Currently, a large number of OBPs are being isolated from insects using different methods (Vogt et al. 1991; Pikielny et al. 1994; Robertson et al. 1999; Galindo and Smith 2001; Biessmann et al. 2002; Forêt and Maleszka 2006). OBPs are present at extraordinarily high concentrations in the sensillum lymph of insects' antennae (Vogt and Riddiford 1981). By establishing and characterizing the antennal cDNA libraries, various OBPs were isolated from *Bombyx mori*, *Manduca sexta*, and *Anopheles gambiae* (Krieger et al. 1996; Robertson et al. 1999; Biessmann et al. 2002).

In different species, the OBPs have special expression patterns. In the paper wasp *Polistes dominulus*, OBPs are equally expressed in antennae, wings, and legs of all castes and ages, whereas chemosensory proteins (CSPs) are often specifically present in antennae and in some cases also in the legs. In the vespine species, V*espa crabro* CSP is antennal specific, whereas OBPs are expressed in legs and wings. Six CSPs and 5 OBPs of *Apis mellifera* show a complex pattern of expression, where both classes of proteins include proteins specifically expressed in antennae and also present in other parts of the body (Calvello et al. 2005; Forêt and Maleszka 2006). In *Drosophila melanogaster*, most OBPs are expressed in both olfactory and gustatory sensilla (Galindo and Smith 2001). In

mosquitoes (*A. gambiae* and *Anopheles arabiensis*), many of the OBPs are expressed mainly in head tissue and a subset of these show the highest expression in female heads. AgCP1588 was expressed only in heads of *A. gambiae* and *A. arabiensis*, and at a higher level in female heads. AgCP3071 and AgCP15554 are expressed only in female heads and AgC15554 also showed higher expression levels in *A. gambiae* than *A. arabiensis*. (Li, Lu, et al. 2005; Li, Pickett, et al. 2005). Most OBPs are slightly downregulated 24 h after blood feeding, but some, especially those with higher expression levels in males are upregulated in blood-fed females (Biessmann et al. 2005).

In *Sesamia nonagrioides*, the PBP is present specifically in the antennae of both sexes, with males having approximately 3-fold the quantity found in females. OBP 2 was present in the antennae of both sexes of *M. sexta* (Vogt et al. 2002). The levels of *SnonPBP* and *SnonGOBP* were compared between scotophase and photophase periods in insects raised under L16:D8 or under constant light. The level of GOPB2 was significantly lower in both sexes during photophase and continuous light, whereas the level of the PBP was significantly lower in the antennae of females during the scotophase. However, in males, it remained at the same level as in the light phase as that found during the scotophase (Konstantopoulou et al. 2006). The expressions of PBPs in *Lymantria dispar* were examined during the 11-day development of the adult antenna. PBP1 and PBP2 were first detected 3 days before adult eclosion. On the 3rd day prior to adult eclosion, the levels for both proteins increased, reaching a plateau on the 1st day preceding adult eclosion that continued into adult life, and remained at a high level into adult life (Vogt et al. 1989). The expression of *SexigPBP1* was higher compared with *SexigPBP2* expression, regardless of sex in the Beet Armyworm, *Spodoptera exigua* Hübner (Xiu and Dong 2007).

Microplitis mediator (Haliday) (Hymenoptera: Braconidae) is a solitary endoparasitoid, widely distributed in the Palaearctic region (Slovak 1985; Arthur and Mason 1986; Mason et al. 2001). It is polyphagous using about 40 lepidopteran species as hosts (Shenefelt 1973). *Microplitis mediator* has evolved a perfect olfaction system to locate its host. In this study, to understand the molecular mechanisms of olfaction, we isolated cDNA clones encoding putative OBPs from the antennae of the *M. mediator* and analyzed sex-, tissue-, and development-specific OBP gene expressions.

Materials and methods

Insects

Microplitis mediator was obtained from the Institute of Plant Protection, Hebei Academy of Agriculture and Forestry. The parasitoids emerge from the host as mature larvae, and spin a silk cocoon inside which they pupate. Emerged adult parasitoids were fed on a 10% honey solution and mated in culture cages (20 × 20 × 10 cm) at 26 ± 1 °C, 14:10 light:dark (Wang et al. 1984). An antennal cDNA library was created from the antennae dissected from 1- to 3-day-old females. Real-time polymerase chain reaction (PCR) was performed using the reverse transcribed RNA isolated from, antennae, head (without antennae), and the body (including thorax, abdomen, legs, and the wing) dissected at 4 stages prior to emergence, and antennae, head (without antennae), thorax, abdomen, legs, and wings from 1- to 5-day-old adults (1st–5th days). The 4 juvenile stages selected (stages 1–4) were as follows: 24 h before emergence (black thorax and abdomen); 16 h before emergence (darker thorax with slime); 8 h before emergence (wing can be seen); and 4 h before emergence (able to move freely). The tissues were immediately frozen in liquid nitrogen after the dissection. All tissues were stored at –70 °C prior to use.

RNA manipulation and characterization of antennal cDNA library

Total RNA was extracted from the homogenized antennae or other tissues using Trizol reagent (Invitrogen) following the manufacturer's instructions. The isolated total RNA from female antennae was reverse transcribed to double-stranded cDNA using a modification of the SMART™ cDNA method (Clontech). Briefly, a primer containing an oligo dT and a unique *Sfi* I site at the 3′ end was used to prime the first cDNA strand. A second oligonucleotide was added that anneals to the 5′ cap resulting in a second unique *Sfi* I site at the 5′ end at the end of the first-strand synthesis. After second-strand synthesis, the resulting cDNA was PCR amplified using primers complementary to the sequences that have been added to the 5′ and 3′ ends. The amplified cDNA was cut using *Sfi*I and size selected using column chromatography. Fractions containing fragments above 400 bp were pooled, ethanol precipitated, and directionally cloned into a modified pDNR-LIB plasmid using the asymmetric *Sfi* I sites. The ligated vector–cDNA mixture was electroporated into DH5α*E. coli* cells (Invitrogen) and transformed colonies were selected on ampicillin plates.

RNA was extracted from antennae and the above-mentioned tissues were reverse transcribed into single-strand cDNA using oligo (dT) 18 primers and Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Promega). The reverse transcription reaction was performed at 42 °C for 30 min and stopped by heating at 95 °C for 5 min.

Clone detection and sequencing

The sizes of cDNA inserts were determined using PCR. A pair of sequence-specific primers was designed based on the sequence of the pDNR-LIB plasmid. The clones that contained the cDNA insert greater than 400 bp were chosen and sequenced using ABI3730 sequencer. Nucleotide sequences were subjected to BLASTX, TBLASTX, and BLASTN database for homology searches (Altschul et al. 1997).

Real-time quantitative PCR and data analysis

Real-time PCR was performed using the iCycler iQ Real-Time PCR Detection System (Bio-Rad). Taqman primers and probe were designed and synthesized by Genecore. The TaqMan probes were labeled at the 5′ end with the FAM reporter dye and were labeled at the 3′ end with the quencher dye TAMRA. As an endogenous control, 18SrRNA was used to normalize the target gene expression and to correct for sample-to-sample variation (Table 1). A 25-μL real-time PCR reaction mix contained 2.5 μL of 10× PCR buffer (Mg^{2+}free), 2.5 μL of $MgCl_2$ (25 mM), 2 μL of deoxyribonucleotide triphosphate (10 mM), 1 μL of each primer (10 μM), 0.5 μL of probe (10 mM), 1 μL of sample cDNA, 0.2 μL of rTaq (5 U/μL), and 14.3 μL sterilized ultrapure H_2O (Millipore). A "no sample control" in triplicates served as the negative control. Cycling parameters included 94 °C for 3 min, 40 cycles at 94 °C for 30 s, and 68 °C for 1 min. Relative quantification was performed using the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) to identify the amount of OBP mRNA in the female tissues relative to male tissues. The expression quantity of MmedOBP5 in the 1st day female antenna sample, which was one of the experimental samples, was used for calibrating the relative fold changes (Mittapalli et al. 2006). Thus, the relative fold change was assessed by comparing the expression level of MmedOBPs in female moths with that of males.

The amplification efficiencies of the target and references were found to be similar. To confirm this, a pilot experiment was conducted to examine the variation of ΔCT (CT, Target – CT, 18SrRNA) with template dilution. Briefly, 4 serial 10-fold dilutions of cDNA from each sample were amplified. For each dilution, amplifications were performed in triplicate by using primers for OBPs and 18SrRNA. Mean CT was calculated for both OBPs and 18SrRNA, ΔCT was

Table 1 Oligonucleotide primers used for expression analysis of the OBPs in *Microplitis mediator*

Primer name	Sequence (5′–3′)	Position (bp)
OBP2-FP	GCGACCAAGTTTACGGAGTTG	384–404
OBP2-RP	TTAGATGAAACTTCGCAATTGTTTG	438–462
OBP2-probe	FAM-ACTGTGCCCCTCAAGCCGAAAAAGG-TAMRA	411–435
OBP3-FP	GAGAAGATCCCTGCTGATTTACCA	312–335
OBP3-RP	CGTTTGTTCGCTGCATTTTG	364–383
OBP3-probe	FAM-ATCGCGAAAGAATTATTGAAGCCG-TAMRA	337–360
OBP4-FP	GCGTCGAAGAAGCCAATAAGG	365–385
OBP4-RP	TTTGTACGCATGCCTCGAAA	475–494
OBP4-probe	FAM-TGAGAATGAATGTGACGTAGCAGCC–TAMRA	448–472
OBP5-FP	ACTCACTACATTGAAAACAAGAAGACACT	216–244
OBP5-RP	TTGACGTTCCATCAAACATGCT	273–294
OBP5-probe	FAM-AGACAATCCAGAAATTCGGTGTTTCA-TAMRA	246–271
OBP6-FP	TGGTAGACAAAGCAAAAACAGCTT	377–400
OBP6-RP	CAACTCGGTCTACAACCCTCAGT	430–452
OBP6-probe	FAM-TCAGGTTGCGCCGATGAAGTTACAGC-TAMRA	403–428
OBP7-FP	GGGTGCCTGCAGAAATAATTG	241–261
OBP7-RP	CTGTTGTTCCGTGCTCAGACA	292–312
OBP7-probe	FAM-ATGGCACAAGGAGAGAAGGGTAGATGT-TAMRA	264–290
CSP-FP	TGGCTTTGGCTGCGACTACT	89–108
CSP-RP	TCACTGCCTAAAATACCATCAACGT	140–164
CSP-probe	FAM-AAACCTACACCAGTAAATTCGATGATGTT-TAMRA	110–138
18S-FP	CGGAGAGGGAGCCTGAGAA	215–233
18S-RP	CCGGGAGTGGGTAATTTGC	263–281
18S-probe	FAM-TACCACATCCAAGGAAGGCAGCAGG-TAMRA	235–259

calculated, and log cDNA dilution versus ΔCT was plotted (Xiu and Dong 2007).

Results

Isolation and characterization of OBP cDNAs from female *M. mediator* antennae

About 1000 colonies were sampled randomly from the antennal cDNA library. PCR results revealed that ~50% of all colonies had cDNA insertions ranging from 0.3 to 2 kb in size. About 415 cDNAs from the female antennal libraries with inserts greater than 0.5 kb were sequenced. Among these sequences, 95 (22%) showed no similarity to any sequence in the database, 145 (35%) had significant similarity to known proteins from a variety of species, 10 (12%) had similarity to conceptual translations of genomic sequences from the *Drosophila* and other genome projects with unknown functions, and 35 (8%) had strong similarity to OBPs from a variety of insect species.

We identified 36 putative OBP (or CSP) cDNAs in *M. mediator*–encoding proteins with significant similarity to antennal proteins from other insects. These were named MmedOBPs. The 35 putative MmedOBPs cDNAs represented 8 unique OBP genes, denoted as MmedOBP1–7 (GenBank accession numbers: EF141513–EF141518 and EF397517), and MmedCSP1 (GenBank accession numbers: EF397518) (Figure 1). Among these cDNAs, we found similarity to OBP2 in 24 sequences, OBP3 in 3 sequences, OBP4, OBP5, and OBP6 each in 2 sequences, whereas OBP1, OBP7, and CSP1 were each found only in a single sequence. By alignment of deduced proteins with OBPs from other insect species, the OBPs were classified into 5 clades (Table 2).

By BLASTN/BLASTP in Genbank with MmedOBPs and MmedCSP1, many OBPs and CSPs were found belonging to Diptera, Hymenoptera, and Lepidoptera. A phylogenetic tree was constructed using the amino acid sequences of MmedOBPs, MmedCSPs, and the similar OBPs (CSPs) (Figure 2). The cladogram indicates that MmedOBPs were divided into several clusters; MmedOBP1 and MmedOBP6 belonged to a single cluster and were similar to NvitOBP1. MmedOBP2, MmedOBP4, and MmedOBP5 belonged to another cluster and were distant from the other OBPs. MmedOBP3, MmedOBP7, and MmedCSP1 belonged to a different cluster.

Expression of MmedOBPs in different tissues

Real-time PCR was used to investigate the presence of MmedOBPs in different tissues and compare the transcript levels of MmedOBPs. For each sex, cDNA was synthesized from mRNA isolated from different tissues in 4 developing stages. For sample analysis, the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) was used to quantify the results obtained for MmedOBP transcripts using real-time PCR. All samples were normalized with reference to 18SrRNA. The absolute values of the slope of all lines from template dilution plots (log cDNA dilution vs. ΔCT) were close to zero (data not shown). Therefore, the efficiencies of the target and reference genes were similar in our analysis, and the ΔΔCT calculation method was used for relative quantification.

The MmedOBP2, MmedOBP4, MmedOBP5, and MmedOBP7 were expressed only in the antennae; the others were expressed in all parts of the body. MmedOBP4 (~99%) and MmedOBP5 (~94%) were dominantly expressed in female antennae. The expression level of MmedOBP4 was 18.8 times higher in female than that in male at stage 4 and this ratio was increased by about 3911 times on the 5th day. The MmedOBP5 expression level in female compared with male was at its highest on the 4th day. In general, the expression level of MmedOBP2 was higher in males (64%) compared with females (36%) up to about 8 times higher in male than female at stage 4. The expression levels of MmedOBP2 were found to be similar on the 1st day and 5th day. The expression level of MmedOBP7 is given in Table 3, and the total expression in the female and male was 44.98% and 55.02%, respectively. Although MmedOBP3 and

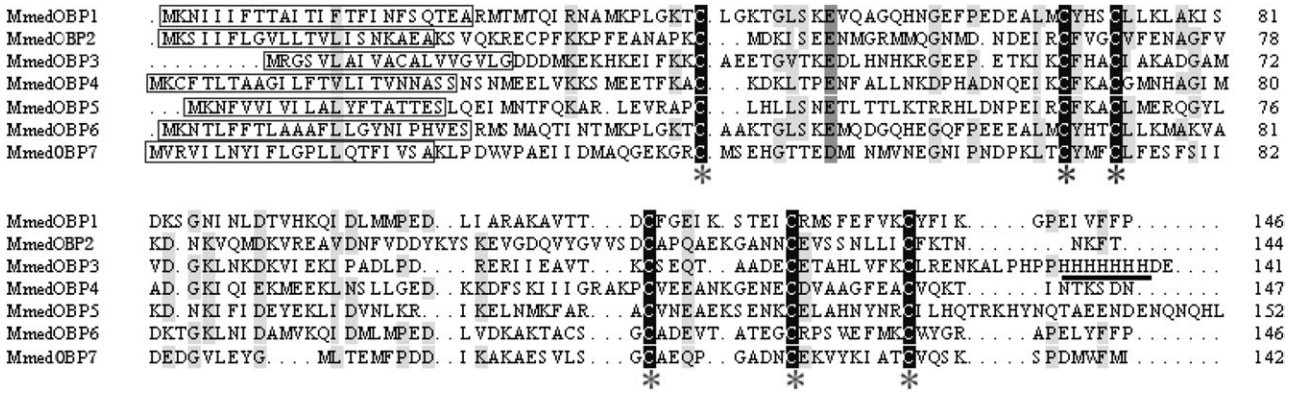

Figure 1 Alignment of amino acid sequences of MmedOBPs. Full-length amino acid sequences of MmedOBPs are aligned. Predicted signal peptides are boxed. (/SignalP/), conserved cysteine residues were marked "*" and ployH was marked "—".

Table 2 Ratio of identities of amino acid residues

	MmedOBP1	MmedOBP6	NvitASP5	MmedASP5	MmedOBP2	MmedOBP4	MmedOBP5	AaegOBP56a	AgamOBPjj111	MmedOBP3	NvitOBP3	MmedPBP1	MmelASP1	NvitASP1	MmedCSP1	AmelASP3c	HvirCSP2
MmedOBP1	100.00																
MmedOBP6	55.48	100.00															
NvitASP5	32.65	31.76	100.00														
MmedASP5	29.45	29.05	25.58	100.00													
MmedOBP2	/	/	/	/	100.00												
MmedOBP4	/	/	/	/	29.33	100.00											
MmedOBP5	/	/	/	/	25.93	26.25	100.00										
AaegOBP56a	/	/	/	/	22.44	17.53	17.68	100.00									
AgamOBPjj11	/	/	/	/	29.14	19.21	13.46	18.95	100.00								
MmedOBP3	/	/	/	/	/	/	/	/	/	100.00							
NvitOBP3	/	/	/	/	/	/	/	/	/	36.81	100.00						
MmedPBP1	/	/	/	/	/	/	/	/	/	/	/	100.00					
MmelASP1	/	/	/	/	/	/	/	/	/	/	/	40.28	100.00				
NvitASP1	/	/	/	/	/	/	/	/	/	/	/	47.18	46.53	100.00			
MmedCSP1	/	/	/	/	/	/	/	/	/	/	/	/	/	/	100.00		
AmelASP3c	/	/	/	/	/	/	/	/	/	/	/	/	/	/	56.15	100.00	
HvirCSP2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	51.18	42.13	100.00

Pairwise comparison putative *Microplitis mediator* OBP sequences with those from other insects (%).

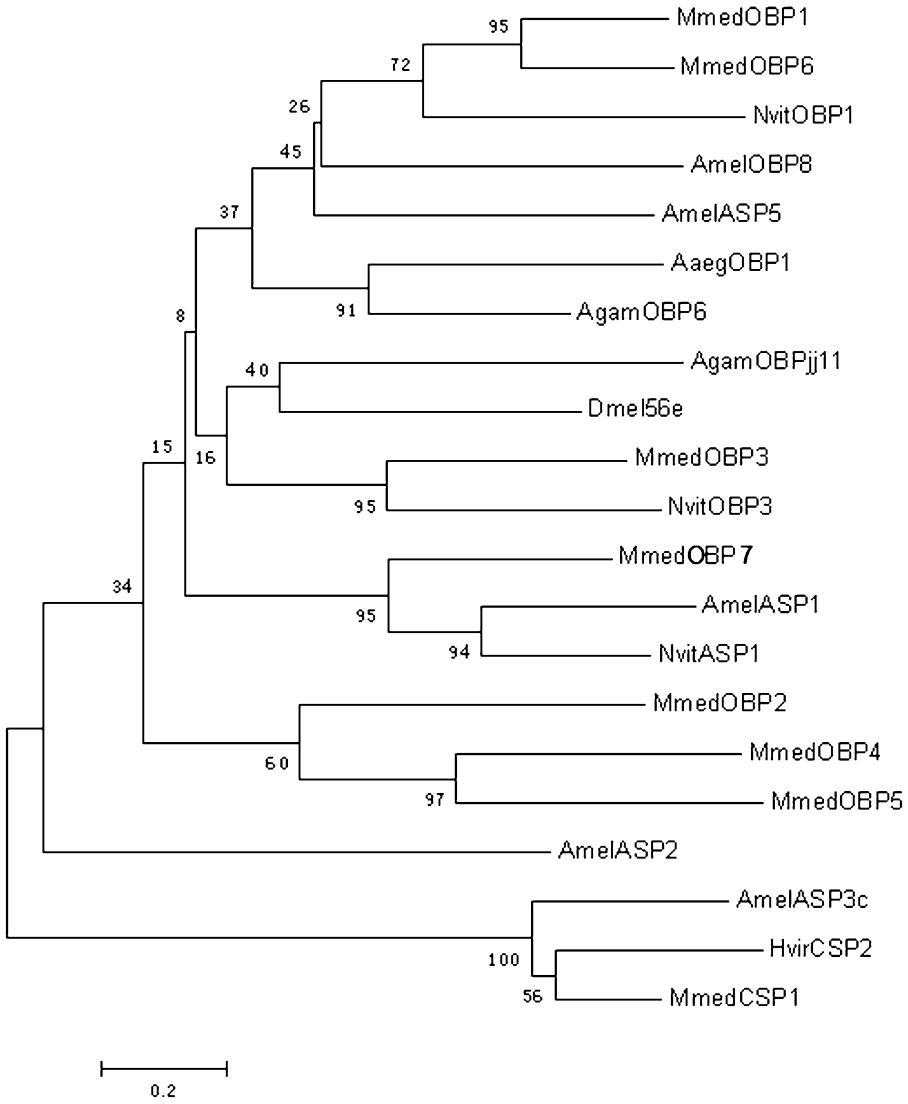

Figure 2 Phylogenetic tree of *Microplitis mediator* OBPs (MmedOBPs) and sensory-specific proteins (MmedCSPs) and the similar OBPs (CSPs) from *Apis mellifera*, *Heliothis virescens*, *Aedes aegypti*, *Anopheles gambiae*, *Nasonia vitripennis*, and *Drosophila melanogaster*. The tree was constructed using the neighbor joining method. The Arabic numerals at the branching points are bootstrap values (%) (Tamura 2007).

MmedOBP6 are ubiquitously expressed, the expression levels were low in all tissues except antennae, with 86.34% and 94.43% expression in antennae of MmedOBP3 and MmedOBP6, respectively. The MmedCSP1 was also expressed ubiquitously, with high levels observed in antennae, abdomen, legs, and wings (Table 4).

The expression of MmedOBP in antennae increased on 2 occasions, during the emergence day and on the 4th day. MmedOBP2 had the highest expression level during the 1st day with about 27-fold increase in the female and 25-fold increase in the male. MmedOBP3 had the highest expression level during the 1st day, about 15-fold higher in the females. MmedOBP4 had the highest expression level on the 4th day, about 7-fold higher in females. MmedOBP5 had the highest expression level during the 1st and 4th days, about 2-fold higher in the females. MmedOBP6 had the highest expression level during the 1st day, about 27-fold higher in females. MmedOBP7 expression was high on the 4th day, about a 31-fold increase in males. The expression quantity of MmedCSP1 was stable with a peak expression on the 1st day in the female antennae. However, in the male antennae, the MmedCSP1 expression was stable before emergence and increased gradually up to 11-fold after emergence until the 4th day. The expression levels of MmedCSP1 in other tissues were stable. The highest level of MmedOBPs was observed during the 1st or 4th day. MmedCSP1 had the highest expression level on the 4th day (Figure 3).

Table 3 Ratio of *Microplitis mediator* OBP gene expression levels in female compared with male adult antennae determined by quantitative real-time PCR

	Stage 1	Stage 2	Stage 3	Stage 4	1st day	2nd day	3rd day	4th day	5th day
OBP2	/	/	/	0.125	1.1	0.345	0.322	0.63	1.1
OBP3	/	/	/	1.6	36.8	17.1	11.6	18.8	75.2
OBP4	/	/	/	18.8	615.9	161.3	99.3	397.1	3911.0
OBP5	0.055	28.5	1.9	4.1	50.7	9.2	29.9	68.6	16.4
OBP6	0.095	189.6	8.6	0.053	2.2	0.455	0.467	0.466	2.6
OBP7	/	/	/	2.0	0.66	1.5	0.233	0.574	1.4
CSP1	0.095	37.6	2.5	0.097	1.7	0.222	0.161	0.203	2.0

Table 4 The percentage of *Microplitis mediator* OBP gene expression quantity in different parts determined by quantitative real-time PCR

	MmedOBP2	MmedOBP3	MmedOBP4	MmedOBP5	MmedOBP6	MmedOBP7	MmedCSP1
Female antennae	35.57%	81.83%	99.57%	93.75%	41.95%	44.98%	13.45%
Male antennae	64.43%	4.50%	0.43%	6.25%	52.48%	55.02%	30.74%
Female head	/	1.43%	/	/	1.90%	/	0.03%
Male head	/	0.09%	/	/	0.52%	/	1.45%
Female thorax	/	0.53%	/	/	0.06%	/	2.10%
Male thorax	/	0.33%	/	/	0.03%	/	0.90%
Female abdomen	/	3.68%	/	/	2.28%	/	11.38%
Male abdomen	/	0.43%	/	/	0.02%	/	3.02%
Female legs	/	2.24%	/	/	0.13%	/	4.21%
Male legs	/	2.07%	/	/	0.07%	/	5.23%
Female wing	/	1.22%	/	/	0.25%	/	14.05%
Male wing	/	1.06%	/	/	0.19%	/	8.97%
Body	/	0.58%	/	/	0.11%	/	4.47%

Discussion

Characterization of female *M. mediator* antennal cDNA library

We established a female *M. mediator* antennal cDNA library and characterized 8 OBPs. The present sequencing data indicated high sequence homologies with the *A. gambiae* antennal cDNA libraries (Biessmann et al. 2002).

Four OBPs were cloned from *M. mediator*, with high amino acid similarities to OBPs from hymenopteran insects. In these OBPS, 3 had high amino acid similarities with OBPs from other insect species containing a domain with 6 cysteine residues, and 1 had high amino acid similarities with CSPs from other insect species containing a domain with 4 cysteine residues. MmedOBP3 contains a poly-H domain, which is typical of the OBPs from *Nasonia vitripennis*, released by the genome project from National Center for Biotechnology Information . A phylogenetic cladogram was constructed, and the functions of these OBPs are yet to be identified.

Expression patterns of OBPs in *M. mediator*

Real-time PCR was used to determine the expression levels of the isolated OBPs and their role in various developmental stages and between genders. In *A. mellifera*, only 9 of 21 OBPs were antenna specific; the remaining genes were expressed either ubiquitously or strictly regulated in specialized tissues or during development (Forêt and Maleszka 2006). Here, we showed, in *M. mediator*, 4 of 8 identified OBPs were antenna specific. Three OBPs were expressed mainly in female antennae and MmedOB2 was expressed mainly in male antennae. Because female *M. mediator* has special behaviors like finding host and oviposition, the MmedOBPs expressed in female antennae could play important roles in these behaviors.

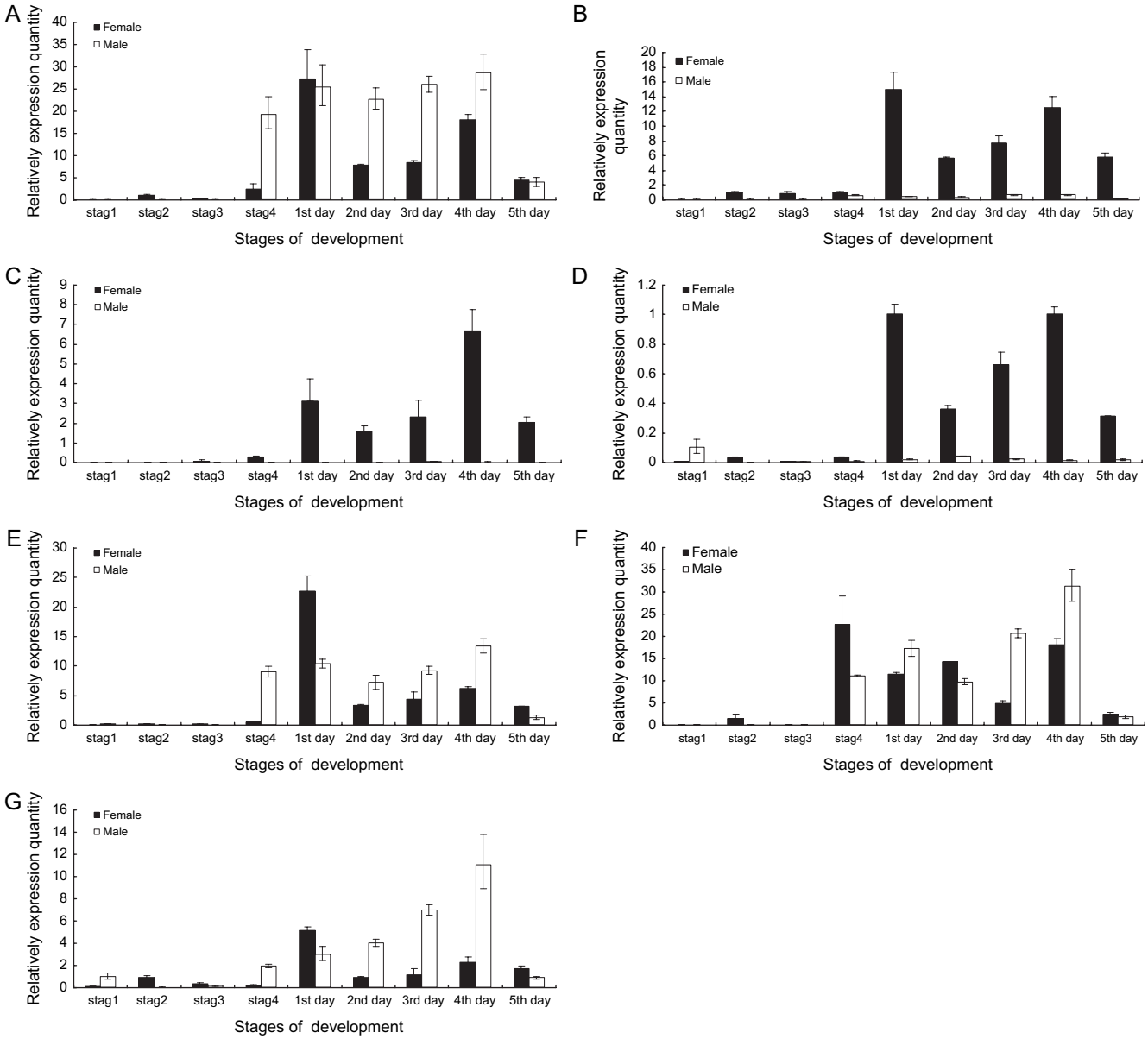

Figure 3 qPCR analysis of MmedOBPs expression levels in female and male moth antennae from different development stages. While the *X*-axis was for developmental stages, the *Y*-axis was the relative expression quantity (mean ± standard error of the mean), the expression of MmedOBP5 in the 1st-day female antennae sample was taken as the calibrator. *X*-axis: stage 1, about 24 h before emergence (black thorax and abdomen), stage 2, about 16 h before emergence (darker thorax with slime), stage 3, about 8 h before emergence (wing can be seen), and stage 4, about 4 h before emergence (able to move freely) and after the emergence 1st–5th days. **(A)** MmedOBP2 expression; **(B)** MmedOBP3 expression; **(C)** MmedOBP4 expression; **(D)** MmedOBP5 expression; **(E)** MmedOBP6 expression; **(F)** MmedOBP7 expression; and **(G)** MmedCSP1 expression.

The *M. mediator* mating takes place immediately after the adult emergence and the beginning of oviposition. The adult longevity of *M. mediator* is influenced by temperature and nutrition; the adults have the longest longevity of 9 days for female and 7 days for male at 28 °C with a 10% honey water supply. However, after the 5th day, the *M. mediator* became inactive; therefore, we studied the 5th day after emergence (Li, Lu, et al. 2005; Li, Pickett, et al. 2005). During different developmental stages, *M. mediator* had different behavior; therefore, *M. mediator* could be sensitive to different odors, which could be due to the changes in the expression of different OBPs.

CSPs were thought as the secondary classes of OBPs in chemical communication. The CSP expression patterns were different among species. The CSP showed a ubiquitous expression across the body in *Myzus persica*, *Schistocerca gregaria*, *Loucusta migratoria*, *B. mori*, and *Heliothis virescens*, with high levels in legs and antennae (Picimbon et al. 2001;

Jacobs et al. 2005). One *B. mori* CSP was found at a very high level in the pheromone gland (Angeli et al. 1999; Picimbon et al. 2000; Jin et al. 2005; Ghanim et al. 2006; Zhou et al. 2006). Calvello et al. (2005) found that some CSPs were specifically expressed in antennae of 3 social insect species *P. dominulus*, *V. crabro*, and *A. mellifera*. In this study, we found MmedCSP1 expressed ubiquitously across the body and had high levels in the male antennae. This suggests that although *M. mediator* belonged to Hymenoptera, it was not a social insect and the CSP gene expression pattern was similar to unsocial insects.

In this study, isolation and preliminary characterization of several cDNAs from female *M. mediator* antennal cDNA library coding for putative OBPs were determined. Future work will focus on the binding characteristics of these OBPs and their relationship with volatiles of cotton. Although several OBP genes were identified from the antennal cDNA library, the information obtained from this study could help us to understand the olfactory processes of parasitoids in finding its host.

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References

Altschul SF, Madden TL, Alejandro A, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Angeli S, Ceron F, Scaloni A, Monti M, Monteforti G, Minnocci A, Petacchi R, Pelosi P. 1999. Purification, structural characterization, cloning and immunocytochemical localization of chemoreception proteins from *Schistocerca gregaria*. Eur J Biochem. 262:745–754.

Arthur AP, Mason PG. 1986. Life history and immature stages of the parasitoid *Microplitis mediator* (Hymenoptera: Braconidae), reared on the bertha armyworm *Mamestra configurata* (Lepidoptera: Noctuidea). Can Entomol. 118:487–491.

Biessmann H, Nguyen QK, Le D, Walter MF. 2005. Microarray-based survey of a subset of putative olfactory genes in the mosquito *Anopheles gambiae*. Insect Mol Biol. 14:575–589.

Biessmann H, Walter MF, Dimitratos S, Woods D. 2002. Isolation of cDNA clones encoding putative odourant binding proteins from the antennae of the malaria transmitting mosquito, *Anopheles gambiae*. Insect Mol Biol. 11:123–132.

Calvello M, Brandazzaa A, Navarrinia A, Danib FR, Turillazzic S, Feliciolid A, Pelosi P. 2005. Expression of odorant-binding proteins and chemosensory proteins in some Hymenoptera. Insect Biochem Mol Biol. 35:297–307.

Galindo K, Smith DP. 2001. A large family of divergent *Drosophila* odorant-binding proteins expressed in gustatory and olfactory sensilla. Genetics. 159:1059–1072.

Ghanim M, Dombrovsky A, Raccah B, Sherman A. 2006. A microarray approach identifies ANT, OS-D and takeout-like genes as differentially regulated in alate and apterous morphs of the green peach aphid *Myzus persicae* (Sulzer). Insect Biochem Mol Biol. 36:857–868.

Forêt S, Maleszka R. 2006. Function and evolution of a gene family encoding odorant binding-like proteins in a social insect, the honey bee (*Apis mellifera*). Genome Res. 16:1401–1413.

Jacobs SP, Liggins AP, Zhou JJ, Pickett JA, Jin X, Field LM. 2005. OS-D-like genes and their expression in aphids (Hemiptera: Aphididae). Insect Mol Biol. 14:423–432.

Jin X, Brandazza A, Navarrini A, Ban L, Zhang S, Steinbrecht RA, Zhang L, Pelosi P. 2005. Expression and immunolocalisation of odorant-binding and chemosensory proteins in locusts. Cell Mol Life Sci. 62:1156–1166.

Kaissling KE. 1998. Pheromone deactivation catalyzed by receptor molecules: a quantitative kinetic model. Chem Senses. 23:385–395.

Konstantopoulou MA, Pratsinis H, Kletsas D, Mazomenos BE. 2006. Pheromone-binding protein and general odorant-binding protein of *Sesamia nonagrioides*: sex- and diel-dependent expression. Entomol Exp Appl. 119:129–136.

Krieger J, von Nickisch-Rosenegk E, Mameli M, Pelosi P, Breer H. 1996. Binding proteins from the antennae of *Bombyx mori*. Insect Biochem Mol Biol. 26:297–307.

Laughlin JD, Ha ST, Jones DNM, Smith DP. 2008. Activation of pheromone-sensitive neurons is mediated by conformational activation of pheromone-binding protein. Cell. 133:1255–1265.

Li JC, Lu ZY, Maimaiti Pan WL, Hun ZY, Wang ZP, Hamiti. 2005. The Influence of temperature and nutrition on adult longevity of *Microplitis Mediator* (Haliday) (Hymenoptera: Braconidae). J Hebei Agricul Sci. 19:6–9.

Li ZX, Pickett JA, Field LM, Zhou JJ. 2005. Identification and expression of odorant-binding proteins of the malaria carrying mosquitoes *Anopheles gambiae* and *Anopheles arabiensis*. Arch Ins Biochem Physiol. 58:175–189.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25:402–408.

Mason PG, Erlandson MA, Youngs BJ. 2001. Effects of parasitism by *Banchus flavescens* (Hymenoptera: Ichneumonidae) and *Microplits mediator* (Hymenoptera: Braconidae) on bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). J Hymenopt Res. 10:90–91.

Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y. 2007. Odorant-binding proteins OBP57d and OBP57e affect taste perception and host–plant preference in *Drosophila sechellia*. PLoS Biol. 5:985–996.

Mittapalli O, Wise IL, Shukle RH. 2006. Characterization of a serine carboxypeptidase in the salivary glands and fat body of the orange wheat blossom midge, *Sitodiplosis mosellana* (Diptera:Cecidomyiidae). Insect Biochem Mol Biol. 36:154–160.

Pelosi P, Zhou JJ, Ban LP, Calvello M. 2006. Soluble proteins in insect chemical communication. Cell Mol Life Sci. 63:1658–1676.

Picimbon JF, Dietrich K, Breer H, Krieger J. 2000. Chemosensory proteins of *Locusta migratoria* (Orthoptera: Acrididae). Insect Biochem Mol Biol. 30:233–241.

Picimbon JF, Dietrich K, Krieger J, Breer H. 2001. Identity and expression pattern of chemosensory proteins in *Heliothis virescens* (Lepidoptera, Noctuidae). Insect Biochem Mol Biol. 31:1173–1181.

Pickielny CW, Hasan G, Rouyer F, Rosbash M. 1994. Members of a family of *Drosophila* putative odorant-binding proteins are expressed in different subsets of olfactory hairs. Neuron. 12:35–49.

Prestwich GD, Du G, Laforest S. 1995. How is pheromone specificity encoded in proteins? Chem Senses. 20:461–469.

Robertson HM, Martos R, Sears CR, Todres EZ, Walden KKO, Nardi JB. 1999. Diversity of odourant binding proteins revealed by an expressed sequence tag project on male *Manduca sexta* moth antennae. Insect Mol Biol. 8:501–518.

Shenefelt RD. 1973. Part 9. Braconidae; 5. Macrogasterinae and Ichneutinae. In: van Vecht J, Shenefelt RD, editors. Hymenopterum catalogus nova edito. Gravenhage (Netherlands): W. Junk. p. 750–751.

Slovak M. 1985. Biological observations on *Microplitis mediator* Hal. (Hym., Braconidae). Biologia. 40:987–996.

Steinbrecht RA. 1996. Are odorant-binding proteins involved in odorant discrimination? Chem Senses. 21:719–727.

Steinbrecht RA. 1999. Olfactory receptors. In: Eguchi E, Tominaga Y, editors. Atlas of arthropod sensory receptors. Tokyo (Japan): Springer-Verlag. p. 155–176.

Tamura K. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol Biol Evol. 24:1596–1599.

Tsuchihara K, Fujikawa K, Ishiguro M, Yamada T, Tada C, Ozaki K, Ozaki M. 2005. An odorant-binding protein facilitates odorant transfer from air to hydrophilic surroundings in the blowfly. Chem Senses. 30:559–564.

Vogt RG. 1995. Molecular genetics of moth olfaction: a model for cellular identity and temporal assembly of the nervous system. In: Goldsmith MR, Wilkins AS, editors. Molecular model systems in the Lepidoptera. Cambridge (MA): Cambridge University Press. p. 341–367.

Vogt RG, Köhne AC, Dubnau JT, Prestwich GD. 1989. Expression of pheromone binding proteins during antennal development in the gypsy moth *Lymantria dispar*. J Neurosci. 9:3332–3346.

Vogt RG, Riddiford LM. 1981. Pheromone binding and inactivation by moth antennae. Nature. 293:161–163.

Vogt RG, Riddiford LM, Prestwich GD. 1985. Kinetic properties of pheromone degrading enzyme: the sensillar esterase of *Antheraea polyphemus*. Proc Natl Acad Sci. 82:8827–8831.

Vogt RG, Rogers ME, Franco MD, Sun M. 2002. A comparative study of odorant binding protein genes: differential expression of the *PBP1-GOBP2* gene cluster in *Manduca uca sexta* (Lepidoptera) and the organization of OBP genes in *Drosophila melanogaster* (Diptera). J Exp Biol. 205:719–744.

Vogt RG, Rybczynski R, Lerner MR. 1991. Molecular cloning and sequencing of general odorant-binding proteins GOBP1 and GOBP2 from the tobacco hawk moth *Manduca sexta*: comparisons with other insect OBPs and their signal peptides. J Neurosci. 11:2972–2984.

Wang DA, Nan LZ, Sun X, Li XZ. 1984. Study on a bionomics of *Microplitis* spp., larval parasitic wasp of *Helicoverpa armigera*. Nature Enemies Insect. 6:211–218.

Wang GR, Wu KM, Guo YY. 2003. Cloning, expression and immunocytochemical localization of a general odorant-binding protein gene from *Helicoverpa armigera* (Hubner). Insect Biochem Mol Biol. 33:115–124.

Xiu WM, Dong SL. 2007. Molecular characterization of two pheromone binding proteins and quantitative analysis of their expression in the beet armyworm, *Spodoptera exigua* Hübner. J Chem Ecol. 33:947–961.

Xu P, Atkinson R, Jones DN, Smith DP. 2005. Drosophila OBP LUSH is required for activity of pheromone-sensitive neurons. Neuron. 45:193–200.

Zhou JJ, Kan Y, Antoniw J, Pickett JA, Field LM. 2006. Genome and EST analyses and expression of a gene family with putative functions in insect chemoreception. Chem Senses. 31:453–465.

Ziegelberger G. 1996. The multiple role of the pheromone-binding protein in olfactory transduction olfaction in mosquito–host interactions. CIBA Found Symp. 200:267–280.

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