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# Identification and tissue distribution of odorant binding protein genes in the beet armyworm, *Spodoptera exigua*

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ABSTRACT

Odorant binding proteins (OBPs) contribute to the remarkable sensitivity of the insect's olfactory system and play an important role in insect chemical communication. In this study, we identified 11 putative cDNAs encoding OBPs (namely SexiOBP1-11) from the antennal full length cDNA library of the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) and examined their expression profiles in different adult body tissues (antennae, heads, thoraxes, abdomens, legs and wings) by real-time quantitative PCR (qPCR). All SexiOBPs had the characteristic typical features of the OBP family, with the exception of SexiOBP11, which lacked the predicted signal peptide sequence at the N-terminus. qPCR revealed that all of these genes were highly transcribed in the antennae. SexiOBP1-4 and SexiOBP10 were dominantly restricted to antennae. Within antennae, SexiOBP2-4 and SexiOBP10 exhibited female-biased expression patterns, while the expression of SexiOBP7 was male-biased, indicating that they might be involved in interacting with sex pheromones. In general, these OBPs were mainly expressed in chemosensory-specific tissues, although some displayed non-chemosensory or ubiquitous tissue expression. The data is helpful for further determining the potential physiological functions of *S. exigua* OBPs, and paves the way towards a better understanding of the chemosensory perception of this pest, which may help to uncover new targets for behavioral interference used as a control strategy.



## 1. Introduction

In the insect olfactory system, odorant-binding proteins (OBPs) are small (about 120–150 amino acids), globular, water-soluble proteins highly concentrated in the aqueous sensillar lymph (Pelosi and Maida, 1995). To date, a large wealth of structural and biochemical information is available (Zhou, 2010). With respect to the physiological function, it is now recognized that OBPs solubilize ligands, help transport hydrophobic semiomolecules through the aqueous environment of the sensillar lymph towards the dendritic membrane of olfactory sensory neurons, where the olfactory receptor proteins are located, and contribute to the sensitivity of the insect olfactory system, thus initiating the first biochemical step in odorant reception (Leal, 2013). Yet, the full range of their physiological functions is still not well understood; recent data have indicated that they can serve multiple functions, such as specific binding with pheromone compounds and odorant molecules, activation of odorant-responsive chemosensory neurons, work as selective filters in odor recognition or even participate in signal termination by inactivating odorant molecule (Du and Prestwich, 1995; Pelosi et al., 2006; Fan et al., 2011; Tunstall and Warr, 2012).

Since the first discovery of insect OBP from the male antenna of the moth, *Antheraea polyphemus* (Vogt and Riddiford, 1981), OBPs have been identified in many insects from at least eight orders, namely, Lepidoptera, Orthoptera, Isoptera, Diptera, Hymenoptera, Hemiptera, Coleoptera and Anoplura (Pelosi et al., 2006). The gene number of OBPs varied in different orders or species. For instance, detailed genomic analysis has revealed the presence of 51 genes encoding OBP-like proteins in *Drosophila melanogaster* (Hekmat-Scafe et al., 2002), 21 in *Apis mellifera* (Forêt and Maleszka, 2006), and 83 in *Anopheles gambiae* (Vieira and Rozas, 2011), whereas only 15 are present in *Acyrthosiphon pisum* (Zhou, 2010) and only five in *Pediculus humanus*, which appear to possess the smallest set of OBPs (Kirkness et al., 2010). So far, a large number of OBP genes (more than 300) have been registered at the National Center for Biotechnology Information (NCBI) (Zhou et al., 2010a). Recent genomic projects led to a major effort in identifying insect OBP genes, which has revealed the full repertoire of OBPs in a wide range of insect species (Sánchez-Gracia et al., 2009). However, we still lack a global view of the OBP genes in important species for which no genomic data are yet available.

The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is a serious pest of numerous agricultural crops worldwide,

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and causes severe losses in agricultural production, especially in vegetables (Burris et al., 1994). As this pest has developed resistance to a broad-spectrum of chemical pesticides, the effectiveness of most of insecticides used has been declining (Lai and Su, 2011). Thus, development of new environmentally friendly strategies for controlling beet armyworm is urgently needed to supplement or replace chemical control programs. Sex pheromones and volatile compounds produced by host plants are expected to be efficient biological agents to control pests through population forecasting, communication disruption or the mass trapping strategy (Mcbbrien et al., 1996). Synthetic pheromones and host volatiles of beet armyworm have been thoroughly investigated, and appear to offer effective control of this pest in both the open field and greenhouse (Bau et al., 2002; Deng et al., 2004; Acín et al., 2010). However, the related molecular mechanisms have been considered only recently. A better knowledge of the molecular basis of olfactory reception of *S. exigua* will undoubtedly contribute to facilitate the design and implementation of novel tools for control of the beet armyworm. Currently, 15 nucleotide sequences of *S. exigua* OBPs have been deposited to NCBI, but only three of them have been partially characterized (Wang et al., 2001; Xiu and Dong, 2007; Zhang et al., 2012a). In this study, we have identified 11 candidate OBPs (designated as SexiOBPs) from the antennae cDNA library of *S. exigua* and analyzed their tissue gene expression patterns.

**Table 1**
Primers used for expression analysis by qPCR in this study.

| Primer name | Sequence (5′ → 3′) | |
|---|---|---|
| SexiOBP1 | Forward | GACGGAGGAGAAAATGGAAGAG |
| | Reverse | CTCGGTGTTGTCGTGGTGC |
| SexiOBP2 | Forward | CAGGATGACACCAGGATGCA |
| | Reverse | TGAAGCAGGCAGCTACCTTC |
| SexiOBP3 | Forward | GGACCATGTCGGTGAGGG |
| | Reverse | TCTTCATGACATCTTGCGACG |
| SexiOBP4 | Forward | GTGATGACCGCCGTGTAGAC |
| | Reverse | GCCATAGCATTCTCGATTACAAG |
| SexiOBP5 | Forward | ACTAAGAAGAAGATGGACTGACG |
| | Reverse | CAAATCCAGGCTTTATTGCA |
| SexiOBP6 | Forward | ATCAACGCTTGCTTCCTACG |
| | Reverse | CATCGCTTACGGACTTTTCG |
| SexiOBP7 | Forward | GGTGTGACTGAAGATCAAGTTGG |
| | Reverse | GCTGATTCCTTTATATCAGGTGG |
| SexiOBP8 | Forward | GACGATGATGATACGGTGGATTA |
| | Reverse | TCTGGCATCTGTCTTTATCTGGT |
| SexiOBP9 | Forward | GAAGCACCGCACTGAATGTC |
| | Reverse | TTCTTGAACTTGCCCTCCTTG |
| SexiOBP10 | Forward | CGGCATAGTGTCTGAAGATGG |
| | Reverse | TCAGTGGCACACAGAGCAGTC |
| SexiOBP11 | Forward | AATACCAGAAGGACCAGGCG |
| | Reverse | GTGCAGAACGTGATTGTTGTGA |
| Actin | Forward | AGAGGGAAATCGTGCGTGAC |
| | Reverse | CCATACCCAAGAAGGAAGGC |

## 2. Materials and methods

### 2.1. Insect and tissue collection

*S. exigua* larvae were collected from the suburbs of Kunming, China and reared in plastic containers until emergence at 24 °C and 60% relative humidity with a 16:8 L:D cycle. Adults were provided with 10% honey solution. Antennae, head (without antennae), thorax, abdomen, legs and wings of male and female adults were removed from the moths after eclosion and were immediately stored in Trizol reagent (Invitrogen) at −80 °C until use.

### 2.2. cDNA library construction, sequencing and bioinformatics analysis

The total RNA of antennae was isolated using Trizol reagent (Invitrogen) following the manufacturer's instructions. The integrity of total RNA was routinely checked with 1% agarose gel electrophoresis. cDNA library was constructed using Creator SMART cDNA Library Construction Kit (Clontech) according to the user manual. SfiI-digested double strand cDNAs longer than 500 bp were cloned into the modified pUC19 vector (Takara). The products were transformed into electrocompetent *Escherichia coli* $DH5_{\alpha}$ host cells. The resulting colonies were randomly picked. The plasmid DNA was prepared using alkaline lysis preparation protocol and sequenced using an ABI PRISMR 377 DNA Sequencer (AppliedBiosystems). Sequences were assembled by SeqMan program (DNAstar, Madison, WI). Sequences with high homology were searched using BLASTX programs, provided by NCBI (http://www.ncbi.nlm.nih.gov/BLAST). Signal peptides were predicted using the online SignalP 4.0 server (Petersen et al., 2011). Amino acid sequence multiple alignments were created with ClustalX (Version 1.83) program (Thompson et al., 1997). The phylogenetic tree was constructed by MEGA version 5 (Tamura et al., 2011) using the neighbor-joining algorithms method at bootstrap 1000 with pairwise deletion.

### 2.3. Real-time quantitative PCR (qPCR)

Total RNA was extracted from the antennae, heads (without antennae), thorax, abdomen, legs and wings using Trizol reagent (Invitrogen) as described above. Each tissue was prepared in triplicate. cDNA was synthesized using the First Strand cDNA synthesis Kit (TaKaRa). The actin gene was used as the internal control. Primers used to amplify OBPs and actin are listed in Table 1. qPCR was performed using an FTC 2000 real-time PCR instrument (Funglyn) in a final volume of 50 μl reaction mixtures containing 25 μl 2× PCR buffer, 1 μl each of primers (25 pmol/μl), 0.5 μl Sybr Green (20×), 2 μl of template cDNA, and 20.5 μl DEPC $H_2O$. The PCR program was as follows: 94°C for 4 min, followed by 35 cycles of 94 °C for 20 s, 60 °C for 30 s and 72 °C for 30 s. The relative expression of genes was calculated using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001). Firstly, the expression of OBP genes were normalized to actin mRNA. Then, in each assay, expression levels were normalized to the expression level of SexiOBP6 in the male abdomen, which was arbitrarily set at one.

## 3. Results

### 3.1. Identification and characterization of SexiOBPs

Non-normalized full length cDNA library was constructed from antennae of female and male *S. exigua*. The capacity of the library was $1.6 \times 10^7$ cfu. The average length of cDNA inserts was approximately of about 1000 bp, ranging from 500 to 2500 bp. No sequence contamination was found from the random sequencing of 16 clones. A total of 500 clones were randomly selected and sequenced, which yielded 496 high quality sequences. They were subjected to a homology search out of which 64 clones were found to be putative OBPs that had the characteristic features of the OBP family (12.9% of all sequences). After assembly, 11 unique OBP genes were generated, which were denoted as SexiOBP1-11 (GenBank accession numbers: JX962784–JX962794).

The full-length sequences of these OBPs ranged from 520 to 1059 bp. Their cDNAs contained 402–522 bp open reading frames that encoded 134–174 amino acid proteins, with calculated molecular weights of 15.14–19.62 kDa and isoelectric points of 4.27–8.64 (Table 2). Except for SexiOBP11, signal peptides were predicted at the hydrophobic N-terminus of each. The deduced peptides were aligned, revealing the presence of the expected conserved