Identification and Expression Profiles of 14 Odorant-Binding Protein Genes From *Pieris rapae* (Lepidoptera: Pieridae)

Mao-Ye Li,1,* Xiu-Yun Jiang,1,* Yu-Zhe Qi,1 Yuan-Jie Huang,2 Shi-Guang Li,1 and Su Liu1,3*

1Anhui Province Key Laboratory of Integrated Pest Management on Crops, Key Laboratory of Biology and Sustainable Management of Plant Diseases and Pests of Anhui Higher Education Institutes, College of Plant Protection, Anhui Agricultural University, 130 West Changjiang Road, Hefei, Anhui 230036, China, 2People’s Government of Fenshui Town, Tonglu County, Hangzhou 311519, China, 3Corresponding author, e-mail: suliu@ahau.edu.cn

*These authors contributed equally to this work.

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Abstract

The small white butterfly, *Pieris rapae* (L.), is an important insect pest of *Brassica* crops. This species utilize olfactory cues to find their hosts and mates. However, the molecular mechanism underlying the olfactory perception in this species remains unclear. Here, we identified 14 odorant-binding proteins (OBP) genes—essential for insect olfaction—in *P. rapae* by exploring a previously published transcriptome dataset. Proteins encoded by all of these genes contain N-terminal signal peptides and six positionally conserved cysteine residues, which are characteristic of insect OBPs. These OBPs displayed high amino acid identity with their respective orthologs in other lepidopterans, and several conserved motifs were identified within these OBPs. Phylogenetic analysis showed that these OBPs were well segregated from each other and clustered into different branches. PrapOBP1 and PrapOBP2 were clustered into the ‘general odorant-binding protein’ clade, and PrapOBP3 and PrapOBP4 fall into the ‘pheromone-binding protein’ clade. The 14 OBP genes were located on seven genomic scaffolds. Of these, PrapOBP1, 2, 3, and 4 were located on scaffold332, whereas PrapOBP5, 6, 7, 8, and 9 were located on scaffold116. Ten of the 14 genes had antenna-biased expression. Of these, PrapOBP1, 2, 4, and 13 were enriched in male antennae, whereas PrapOBP7 and PrapOBP10 were female-biased. Our findings suggest that these OBPs may be involved in olfactory communication. To the best of our knowledge, this is the first report on the identification and characterization of *OBP* genes in *P. rapae*, and our findings provide a solid foundation for studying the functions of these genes.

Key words: small white butterfly, OBP, olfaction, genomic distribution, expression pattern

Odorant-binding proteins (OBPs) are a class of small, water-soluble proteins that play a critical role in olfaction in various insect species (Pelosi et al. 2018, Sun et al. 2018). According to a proposed model for insect olfactory process, odorants enter the antennal sensilla through small pores in the sensillar wall and bind to OBPs; then, OBPs transport these hydrophobic compounds through the aqueous sensillum lymph to reach specific odorant receptors (ORs) located in the dendritic membrane of the olfactory sensory neurons (Leal 2013, Fleischer et al. 2018). Thus, the recognition of odorants by OBPs was considered to be the initial step in olfactory perception (Vogt et al. 1985, Bito et al. 2016).

The first OBP was identified in the wild silk moth, *Antheraea polyphemus* (Cramer) (Lepidoptera: Saturniidae) and was named as pheromone-binding protein (PBP) owing to its pheromone-binding function (Vogt and Riddiford 1981). Since then, a growing number of *OBP* genes and proteins have been identified from various insect species, and their functions in odorant detection have been elucidated (Pelosi et al. 2014). In lepidopteran insects, there are two subgroups of OBPs: PBP and general odorant-binding protein (GOBP) (Vogt et al. 2015). The PBPs are believed to recognize the pheromone constituents, whereas members in the GOBP group are considered to recognize ‘general’ odorants such as host plant volatiles (Zhou 2010). However, many studies have also demonstrated that PBPs are able to recognize volatiles from host plants and GOBPs have a strong affinity for sex pheromone constituents (Liu et al. 2015a, Khuhro et al. 2017, Yu et al. 2018, Sun et al. 2019). PBPs and GOBPs are both belong to ‘classic OBP’ group, and the remarkable feature of classic OBPs is the presence of six positionally conserved cysteine residues (Zhou 2010, Pelosi et al. 2014, Bito et al. 2016). Crystal structure studies have revealed that the six cysteines form three disulfide bridges, which are essential for the protein stability (Sandler et al. 2000, Li et al. 2014, Pelosi et al. 2018). Besides classic OBPs,
there are other OBP groups with divergent cysteine motif, including plus-C OBPs (have two additional conserved cysteines plus one proline), minus-C OBPs (lost two conserved cysteines), dimer OBPs (having two six-cysteine motifs), and atypical OBPs (having 9 or 10 cysteines and a long C-terminus) (Zhou 2010).

Because OBPs are critical for insect olfaction, they have been used in the reverse chemical ecology approach to screen natural or synthetic attractants (Leal et al. 2008, Kröber et al. 2018, Venthur and Zhou 2018). For instance, two active attractants (trimethylamine and nonanal) for the mosquito Culex quinquefasciatus Say (Diptera: Culicidae) have been identified by using an OBP (CquiOBP1; Leal et al. 2008), and effective repellents have been discovered for Anopheles gambiae Giles (Diptera: Culicidae) by using the same approach (Kröber et al. 2018). In addition, OBP-based high-throughput screening of behaviorally active semiochemicals was successfully performed for Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) (Jayanthi et al. 2014). Recently, OBPs are considered potential molecular targets for developing RNA interference (RNAi)- and genome editing-based strategies for pest management. For instance, knockdown of OBP genes by RNAi impairs olfactory sensitivity in Adelphocoris lineolatus (Goeze) (Hemiptera: Miridae) and Helioconger armiger (Hubner) (Lepidoptera: Noctuidae) (Dong et al. 2017, Zhang et al. 2017), and deletion of OBPs by CRISPR/Cas9 technology significantly reduces the olfactory response in H. armigera and Spodoptera littoralis Fabricius (Lepidoptera: Noctuidae) (Ye et al. 2017, Zhu et al. 2019).

The small white butterfly, Pieris rapae (L.), is a worldwide pest that infests cruciferous vegetables (Huang et al. 2018b). Outbreak populations of this insect pest can completely consume all the leaves on a Brassicaceae plant, thereby causing a significant loss in yield (Kingsolver 2000). Pieris rapae can be controlled with large doses of chemical pesticides; however, this practice often leads to insecticide resistance in this pest (Peng et al. 1996). Furthermore, the extensive spraying of insecticides leaves pesticide residues on the crops and pollutes the environment (Liu et al. 2014). In this case, non-insecticidal methods must urgently be developed to control P. rapae.

Materials and Methods

Insects

The P. rapae individuals used in this study were reared in our laboratory under the conditions of 26 ± 1°C, 65% relative humidity, and a 16:8 (LD) h photoperiod, as described previously (Jiang et al. 2018). Two-day-old virgin adults were sampled, and different tissues were dissected, including 100 male antennae, 100 female antennae, 60 heads (without antennae; 30 from males and 30 from females, pooled together), 60 abdomens (30 from males and 30 from females, pooled together), and 200 legs (100 from males and 100 from females, pooled together). The samples were frozen in liquid nitrogen immediately and stored at −80°C until RNA extraction was carried out.

RNA Extraction and cDNA Synthesis

Total RNA was extracted using RNAiso Plus reagent (Takara, Dalian, China) following the manufacturer’s protocol. Each RNA sample was treated with RNase-free DNase I (Takara, Dalian, China) to eliminate genomic DNA contamination. The quality of the RNA was determined by electrophoresis using a 1% (w/v) agarose gel, and the concentration of RNA was assessed with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). First-strand cDNA was synthesized using the ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan).

Identification of OBP Genes

OBP genes were identified from a previously published transcriptome dataset of P. rapae (BioProject number: PRJNA285028, available at NCBI’s SRA database; Qi et al. 2016). The TBLASTN algorithm in the Basic Local Alignment Search Tool (BLAST) program was used for the search (Altschul et al. 1997). The annotated OBP protein sequences from other lepidopteran species, including Danaus plexippus (L.) (Lepidoptera: Nymphalidae), Heliconius melpomene L. (Lepidoptera: Nymphalidae), Bombyx mori (L.) (Lepidoptera: Bombycidae), H. armigera, Manduca sexta (L.) (Lepidoptera: Sphingidae), C. suppressalis, Plutella xylostella L. (Lepidoptera: Plutellidae), and S. littoralis (Boisduval) (Lepidoptera: Noctuidae), were used as queries. The cutoff e-value was set as 10–5. All the output OBP sequences were manually checked, and duplicate and redundant candidates were removed. To confirm that these transcripts are not chimeric, gene-specific primers (Supp Table S1 [online only]) were designed and used to amplify full or near-full open reading frames (ORFs) from the antennal cDNA of P. rapae. Polymerase chain reaction (PCR) products were cloned into pMD18-T vector (Takara, Dalian, China) and sequenced.

Biological Analyses

Searching for orthologs was performed using BLASTX online program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The theoretical molecular weight (Mw) and isoelectric point (pI) were obtained using an ExPASy tool (http://web.expasy.org/compute_pi/). Putative signal peptides were predicted with SignalP 5.0 (http://www.cbs.dtu.dk/services/SignalP). Multiple alignment of OBP protein sequences was performed using Clustal Omega (http://www.ebi.ac.uk/tools/msa/clustalo/). Phylogenetic trees were constructed in MEGA7 software using the neighbor-joining method with 1,000-fold bootstrap resampling (Kumar et al. 2016). The GenBank accession numbers of the OBP protein sequences used in the phylogenetic analysis are listed in Supp Table S2 [online only]. Motif pattern analysis was performed using the online program MEME (http://meme-suite.org/tools/meme); the lepidopteran OBPs used in this analysis are listed in Supp Table S3 [online only]. The parameters were as follows: minimum width = 6, maximum width = 10, and maximum number of motifs to find = 8. Genomic localization and exon–intron structure of each OBP gene was analyzed by mapping cDNA with the P. rapae genomic DNA (Shen et al. 2016) using the Splign program (https://www.ncbi.nlm.nih.gov/sutils/splign/splign.cgi).
Quantitative Reverse Transcription-PCR

Quantitative reverse transcription-PCR (qRT-PCR) was carried out using SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan). Each reaction mixture (20 µl) contained 10 µl SYBR Green Master Mix, 1 µl (10 ng) cDNA template, 0.4 µl (0.2 µM) of each primer, and 8.2 µl nuclease-free water. Primers for qRT-PCR are listed in Supp Table S1 (online only). 18S rRNA was used as the internal reference. The amplification efficiencies of all the primers range between 90 and 110%. Reactions were performed in 96-well plates in a CFX96 Real-time System (Bio-Rad, Hercules, CA). The thermal cycle parameters are: one cycle of 95°C for 2 min, followed by 40 cycles of 95°C for 5 s and 60°C for 25 s. To confirm that only a single gene was amplified, a heat dissociation protocol was set at the end of each thermal cycle. A no-template control and no-reverse-transcriptase control were both included on each reaction plate to detect possible contamination. The qRT-PCR reactions were performed in three biological replicates, each with three technical replicates. Relative expression levels of genes were calculated using the 2^ΔΔCT method (Livak and Schmittgen 2001).

Statistical Analysis

Data were analyzed using Data Processing System (DPS) software (version 9.5; Tang and Zhang 2013). To analyze the differences in gene expression levels among multiple samples, one-way analysis of variance (ANOVA) with Tukey’s post-hoc test were performed. The level of significance was set at \( P < 0.05 \).

Results

Identification of OBP Genes in \textit{P. rapae}

By searching the \textit{P. rapae} transcriptome dataset, we identified 14 putative OBPs (PrapOBP1 to PrapOBP14; Table 1). The names of these genes have been designated according to the order of discovery. These sequences were verified by PCR amplification and DNA sequencing (data not shown). All of the OBP genes had complete ORFs, and the length of the deduced proteins ranged from 130 to 188 amino acid residues (Table 1). The predicted Mw of these proteins ranged from 14.5 to 21.9 kDa, and the pI ranged from 4.5 to 8.8 (Table 1). BLASTX results showed that these OBPs shared 45–87% amino acid identities with their respective orthologs from other lepidopteran species (Table 1). The percentage of amino acid identity among all \textit{P. rapae} OBPs ranged between 11% and 46% (Supp Table S4 [online only]). Signal peptide regions were predicted to be at the N-terminus of all the deduced PrapOBP protein sequences, and six positionally conserved cysteine residues were present in all the deduced proteins (Fig. 1). In addition, the cysteine patterns of the 14 PrapOBPs are C1–X2–C2–X3–C3–X36–X43–C4–X8–C5 (X represents any amino acid), indicating these proteins are classic OBPs (Fig. 1).

Phylogenetic Analysis

To better understand the relationship between the \textit{P. rapae} OBPs and OBPs from other lepidopteran species, we performed a phylogenetic analysis (Fig. 2). The phylogeny of OBPs in this study is consistent with the findings reported by Vogt et al. (2015). In this tree, PrapOBP1 and PrapOBP2 were clustered into the GOBP1 and GOBP2 clades, respectively (Fig. 2), it is possible that the two proteins may be potential GOBPs. PrapOBP3 fell into the PBP-D clade and PrapOBP4 into PBP-C clade, implying that these proteins may belong to PBP group (Fig. 2). Other PrapOBPs were well segregated
from each other and clustered into different branches with high bootstrap support (Fig. 2).

Motif Pattern Characterization
We used the MEME program to identify the motifs of OBPs identified in *P. rapae*. Eight motifs were found by comparing the protein sequences of *P. rapae* OBPs with other lepidopteran OBPs (Fig. 3A). PrapOBP1 and PrapOBP2 (potentially GOBPs, according to phylogenetic analysis) showed the same motif pattern 4-3-1-5-6-2; PrapOBP3 was similar to PrapOBP1 and PrapOBP2 but with an additional seventh motif at its C-terminus (Fig. 3B). Surprisingly, the motif pattern differed considerably between PrapOBP3 and PrapOBP4 (two potential PBPs): PrapOBP4 lacked motif 6 compared with PrapOBP3 (which was replaced by motif 4; Fig. 3B).

Among the 14 *P. rapae* OBPs, the most conserved motif pattern was 4-1-2, which was observed in six OBPs (PrapOBP5, 6, 9, 10, 12, and 14). PrapOBP7 and PrapOBP8 showed the same motif order (8-4-1-2), and PrapOBP11 and PrapOBP13 only showed the motifs 1 and 2 (Fig. 3B).

Genomic Organization and Exon–Intron Structure of *P. rapae* OBPs
We analyzed the genomic organization of *P. rapae* OBPs and found that the 14 genes were distributed among seven scaffolds (scaffold51, 116, 240, 283, 332, 569, and 1007; Table 1). Of these, PrapOBP1, 2, 3, and 4 (potentially GOBPs and PBPs) were located on scaffold332, and PrapOBP5, 6, 7, 8, and 9 were located on scaffold116 (Table 1; Fig. 4A). The remaining five genes (PrapOBP10, 11, 12, 13, and 14) were located individually on a single scaffold (Table 1). Remarkably, PrapOBP2, 3, and 4 were in a tight cluster spanning 11.1 kb of the genome, whereas PrapOBP1 was located 109.5 kb downstream of PrapOBP2 (Fig. 4A).

We also investigated the exon–intron structure of *P. rapae* OBPs. The results showed that the size of the 14 OBP genes ranged from 393 to 14371 bp (Table 1). Among the 14 OBPs, PrapOBP10 and PrapOBP11 were intronless genes, whereas PrapOBP13 contained the maximum number (six) of exons (Table 1; Fig. 4B). PrapOBP1, 2, 3, and 4 showed a common structure containing three exons and two introns; the other OBP genes had four or five exons (Table 1; Fig. 4B). Notably, we found that PrapOBP1, 2, 3, and 4 have conserved intron insertion sites; intron 1 was inserted between two codons, and intron 2 split a codon between nucleotides 1 and 2 (Supp Table S5 [online only]). Moreover, the length (181 bp) of the second exon of the four genes was equal (Supp Table S5 [online only]).

Expression Profiles of *P. rapae* OBPs
We investigated the expression profiles of *P. rapae* OBP genes in different tissues using qRT-PCR. The results showed that the size of the 14 OBP genes ranged from 393 to 14371 bp (Table 1). Among the 14 OBPs, PrapOBP10 and PrapOBP11 were intronless genes, whereas PrapOBP13 contained the maximum number (six) of exons (Table 1; Fig. 4B). PrapOBP1, 2, 3, and 4 showed a common structure containing three exons and two introns; the other OBP genes had four or five exons (Table 1; Fig. 4B). Notably, we found that PrapOBP1, 2, 3, and 4 have conserved intron insertion sites; intron 1 was inserted between two codons, and intron 2 split a codon between nucleotides 1 and 2 (Supp Fig. S1 [online only]). Moreover, the length (181 bp) of the second exon of the four genes was equal (Supp Table S5 [online only]).
tissues. For example, PrapOBP5 and PrapOBP6 were mainly expressed in the abdomen, and PrapOBP9 and PrapOBP11 were enriched in all the tested tissues including male and female antennae, head, abdomen, and legs (Fig. 5).

**Discussion**

To date, OBP gene families have been identified in various insect species, through genomic and/or transcriptomic analyses (reviewed by Venthur and Zhou 2018). These studies have greatly contributed to the research on the molecular mechanisms underlying insect olfaction (Venthur and Zhou 2018). However, information on the OBPs in *P. rapae* remains limited, which restricts the understanding of olfactory signal pathways in this insect species. In the present study, we identified 14 OBP genes from *P. rapae* by searching a previously published transcriptome dataset. To the best of our knowledge, this is the first report on the identification and characterization of OBPs in *P. rapae*, and our findings pave the way for studying the function of these genes.

The deduced protein sequences of the *P. rapae* OBPs contain N-terminal signal peptides and six positionally conserved cysteine residues, which are the hallmark of insect OBPs (Pelosi et al. 2018, Sun et al. 2018). The motif pattern analysis showed that the motif pattern varied in different OBPs; PrapOBP1 and PrapOBP2 (two potential GOBPs) have a similar motif pattern (4-3-1-5-6-2), and they lack motif 7 at the C-terminus, unlike PrapOBP3 (potentially PBP). This difference implies a possible functional difference between them. In most lepidopterans, GOBPs and PBPs show distinct binding affinities for plant volatiles and sex pheromone constituents (Liu et al. 2015b, Khuhro et al. 2017, Huang et al. 2018a,
The most noteworthy finding was that the two potential PBPs, PrapOBP3 and PrapOBP4, displayed different motif patterns. In most lepidopteran species, including *S. litura*, *Dendrolimus houi* Lajonquiere (Lepidoptera: Lasiocampidae), and *De. kikuchii* Matsumura (Lepidoptera: Lasiocampidae), the motif patterns between PBPs are quite similar (Zhang et al. 2014, Gu et al. 2015); however, in *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), PBP1 displayed distinct motif pattern with PBP2 (Zhang et al. 2016). It is possible that PrapOBP3 and PrapOBP4 may have an affinity for different kinds of pheromone constituents and/or plant odorants. PBPs that selectively bind different types of odorants have also been reported in other moth species. For example, PBP1 in *H. armigera* strongly binds and sex pheromone components, whereas PBP2 specifically binds alcohols, and PBP3 preferably binds acetates (Guo et al. 2012). A similar phenomenon was also observed in *Helicoverpa assulta* (Guenné) (Lepidoptera: Noctuidae) and *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) (Guo et al. 2012, Jin et al. 2014).

We found that several *P. rapae* OBP s were located on the same genomic scaffold and formed gene clusters, for example, PrapOBP1, 2, 3, and 4 on scaffold132, and PrapOBP5, 6, 7, 8, and 9 on scaffold116 (Fig. 4A). This phenomenon is also observed in other insect species, such as *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), *Apis mellifera* L. (Hymenoptera: Apidae), *B. mori*, and *Myzus persicae* (Sulzer) (Homoptera: Aphididae) (Hekmat-Scafe et al. 2002, Forêt and Maleszka 2006, Gong et al. 2009, Wang et al. 2019). Among insect OBP s, GOBP s and PBP s are lepidopteran specific, and the location of GOBP and PBP genes on the same genomic scaffold has been found in several lepidopterans, including *B. mori*, *M. sexta*, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), and *S. frugiperda* (Smith) (Lepidoptera: Noctuidae) (Gong et al. 2009, Yasukochi et al. 2018). Vogt et al. (2015) analyzed the GOBP and PBP genes in lepidopterans and suggested that GOBP s and PBP s are derived by duplication events from a common ancestor, based on the following evidence: 1) GOBP s and PBP s contain three exons, and the length of the second exon is identical; 2) introns in GOBP s and PBP s have conserved insertion sites and phase (positioned between codons or within a codon); and 3) in the phylogenetic tree, the PBP/GOBP clade forms a well-supported lineage, which excludes other OBP s (Vogt et al. 2015). In the present study, we found that PrapOBP1, 2, 3, and 4 have the same exon–intron structures and share conserved intron positions (Supp Fig. S1 [online only]; Supp Table S5 [online only]), suggesting that they originated by duplication of an ancestral gene. Further analysis of the exon–intron structure in OBP s will provide new insights into the evolution of this gene family in *P. rapae*.

OBPs that are mainly expressed in the insect antennae are considered to have an olfactory function. By contrast, OBP s enriched in non-olfactory tissues are thought to be involved in other physiological processes (Pelosi et al. 2018). Therefore, the potential function of OBP s could be predicted by analyzing their expression profiles in different tissues. Our qRT-PCR results showed that ten *P. rapae* OBP s displayed antenna-specific or
antenna-enriched expression, indicating that these genes may play important roles in olfaction. Among these genes, four (PrapOBP1, 2, 4, and 13) were mainly expressed in male antennae. These genes may encode proteins involved in the detection of sex pheromones released from females. In many other lepidopteran species such as C. suppressalis, Se. inferens, and Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), male antenna-biased OBPs displayed a strong affinity for sex pheromone components (Gu et al. 2013, Jin et al. 2014, Chang et al. 2015). We observed that PrapOBP7 and PrapOBP10 showed female antenna-biased expression. Previous studies have shown that the P. rapae females use chemicals emitted from host plants to locate oviposition sites (Renwick et al. 1992, Sato et al. 1999). Furthermore, mate recognition behavior in P. rapae females largely relies on the perception of volatiles released by males (McQueen and Morehouse 2018). Thus, it is possible that PrapOBP3 and PrapOBP6 are involved in these female-specific functions.

We also found that PrapOBP5 and PrapOBP6 were enriched in the abdomen, and PrapOBP9 and PrapOBP11 were highly abundant in all the tested tissues, including male and female antennae, head, abdomen, and legs. These OBPs may have important functions in physiological pathways other than olfaction, e.g., gustatory function. In Ad. lineolatus, Apolygus lycors (Meyer-Dür) (Heteroptera: Miridae), Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae), and Meteorus pulchricornis (Wesmael) (Hymenoptera: Braconidae), a large number of OBPs are expressed in the mouthparts, ovipositor, and tarsi. These OBPs are considered to have a potential gustatory function (Sun et al. 2016, Sheng et al. 2017, Sun et al. 2017, Dou et al. 2019, Li et al. 2020). Another potential function of these genes is as transporters that bind xenobiotic compounds, especially insecticides. In Apis cerana Fabricius (Hymenoptera: Apidae), Athetis lepigone (Möschler) (Lepidoptera: Noctuidae), Ectropis obliqua Prout (Lepidoptera: Geometridae), and S. littura, OBPs can interact with various insecticides and may contribute to defense against these harmful xenobiotic compounds (Li et al. 2015, 2017; Zhang et al. 2020).

It should be noted that, although the findings discussed above lead us to predict the potential functions for OBPs in P. rapae, we measured the transcription levels of the genes in adult tissues, but did not measure the expression patterns of these genes in larval tissues. It is known that OBPs in larval antennae are essential for chemosensation and behavior guidance (Jin et al. 2015, Zhu et al. 2016). Determining the OBP expression profiles in larval tissues will provide additional supporting evidence that these genes may be playing important roles in chemosensory perception.

In conclusion, we successfully identified 14 putative OBPs from P. rapae by searching the transcriptomic dataset. Phylogeny, sequence motif, genomic localization, and expression profile analyses suggested that some of these genes are involved in olfaction. The results of this work will not only lead to a better understanding of the olfactory system in this lepidopteran species but also contribute to the development of sustainable pest management strategies using OBPs as targets to disrupt insect behavior.
Fig. S1. Alignment of amino acid sequences of four odorant-binding proteins (PrapOBP1 to PrapOBP4) from Pieris rapae. Signal peptides have been removed from the sequences, and six positionally conserved cysteines are highlighted in red. Two introns are identified in each gene, and the conserved intron insertion sites are marked with boxes. In each box, nucleotide sequences are in lowercase letters, followed by the respective amino acid residues (capitalized). The slash indicates the intron insertion site. Intron 1 is inserted between two codons and intron 2 splits a codon between nucleotides 1 and 2.

Fig. 5. Relative expression levels of OBP genes in different tissues of Pieris rapae. mA: male antennae; fA: female antennae; H: head (without antennae); Ab: abdomen; L: legs. Data are presented as mean (n = 3) ± SE. Different lowercase letters indicate significant differences (P < 0.05; one-way ANOVA with Tukey’s test).

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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