Identification of chemosensory genes from the antennal transcriptome of Indian meal moth *Plodia interpunctella*

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Abstract

Olfaction plays an indispensable role in mediating insect behavior, such as locating host plants, mating partners, and avoidance of toxins and predators. Olfactory-related proteins are required for olfactory perception of insects. However, very few olfactory-related genes have been reported in *Plodia interpunctella* up to now. In the present study, we sequenced the antennae transcriptome of *P. interpunctella* using the next-generation sequencing technology, and identified 117 candidate olfactory-related genes, including 29 odorant-binding proteins (OBPs), 15 chemosensory proteins (CSPs), three sensory neuron membrane proteins (SNMPs), 47 odorant receptors (ORs), 14 ionotropic receptors (IRs) and nine gustatory receptors (GRs). Further analysis of qRT-PCR revealed that nine OBPs, three CSPs, two SNMPs, nine ORs and two GRs were specifically expressed in the male antennae, whereas eight OBPs, six CSPs, one SNMP, 16 ORs, two GRs and seven IRs significantly expressed in the female antennae. Taken together, our results provided useful information for further functional studies on insect genes related to recognition of pheromone and odorant, which might be meaningful targets for pest management.

Introduction

Indian meal moth, *Plodia interpunctella* (Hübener) (Lepidoptera: Pyraloidea, Pyralidae), is a notorious stored-product pest worldwide [1]. The larvae infest a variety of processed foods, including fruits, nuts, cereals, powdered milk, chocolate, birdseed, and pet food [2], causing extensive damage by impairing dry weight, germination, nutritional value, and quality grade. It is difficult to control *P. interpunctella* by conventional insecticides, because it often inhabits our kitchen, closet and warehouse, and its larvae are mixed with our processed foods. Accordingly, several novel strategies have been developed to monitor and control *P. interpunctella*. Among these novel methods, sex pheromone is widely acceptable due to its safety and efficiency. Meanwhile, host volatiles have been thought to affect the oviposition behavior of *P.*
However, the underlying molecular mechanisms of olfactory recognition of *P. interpunctella* remain largely unexplored.

An accurate olfactory system plays crucial roles in survival, reproduction, and chemical communication for most insects [4]. Using the olfactory system in antennae, when peripheral odorants are detected, insects will activate olfactory sensory neurons (ORNs) and translate the signals into nerve impulses to the brain [5]. At least six gene families are involved in the olfactory sensory procedure, including three sensory protein families: odorant-binding proteins (OBPs), chemosensory proteins (CSPs), and sensory neuron membrane proteins (SNMPs); and three major chemosensory receptor families: odorant receptors (ORs), ionotropic receptors (IRs) and gustatory receptors (GRs). Additionally, odorant degrading enzymes (ODEs) are also classified in olfactory system, due to their integral roles in the rapid inactivation of semiochemicals [6–7, 4].

Sensory proteins, functioning as molecular actors, are considered to play crucial roles in detection of semiochemicals. They participate in the initial transduction of olfactory signals. When the odorants are detected, binding proteins (OBPs, CSPs and SNMPs) will specifically bind the hydrophobic odorants, and transport them to cross the aqueous sensillum lymph that embeds olfactory neuron dendrites. Subsequently, the odorants interact with membrane-bound chemosensory receptors (ORs IRs, and GRs) in the receptor neuron membrane, in which the odorant signals are transformed into electric signals. Finally, signal termination is inactivated by ODEs, which prevent the continuous accumulation of stimulants and subsequent sensory adaptation, and allow insects to rapidly respond to changes in environmental odorants [8–9].

During the past decade, the emergence of next generation sequencing (NGS) technology has dramatically improved the efficiency of gene screening. Meanwhile, the entomological research has also benefited from the development of NGS technology [10]. With the improvement of high-throughput sequencing methods, olfactory-related genes have been identified from antennal transcriptomes in numerous Lepidoptera species, including several notorious agricultural pests [11–22]. Such technology has been widely used to identify genes involved in olfaction of insects. However, little information is available about the function of olfactory-related genes of *P. interpunctella* due to the deficiency of the genomic data for this species.

Although several transcriptomic studies related to *P. interpunctella* have been performed [23–25], antennal transcriptome analysis of olfactory system has not been conducted in previous studies. To identify the olfactory-related genes, we described the antennal transcriptome analysis of *P. interpunctella* in the present study. The expression levels of olfactory-related genes were investigated using quantitative real-time PCR. Taken together, our study successfully identified olfactory-related genes of *P. interpunctella* and provided useful information for further studies on pheromone and host volatile recognition.

### Materials and methods

#### Insects material and RNA extraction

*Plodia interpunctella* was the laboratorial population which was reared for more than 20 generations in our laboratory. The larvae were reared on crushed grains of wheat under constant conditions (28±1°C, 60±5% RH and 14:10 L:D photoperiod). Mature larvae were sorted by sex according to the black spot in the middle of male back. Antennae were excised from 3-day-old unmated moths, immediately frozen in liquid nitrogen and ground with a pestle. Total RNA was extracted from 100 antennae for each sex. The evaluation of RNA purity, RNA concentration and RNA quality were conducted following our previous method [13].
cDNA library preparation for transcriptome sequencing

cDNA libraries were constructed following previous method [17]. Briefly, 3 μg RNA per sample was used as input material for the RNA sample preparation. Sequencing libraries were generated using NEBNext™ Ultra™ RNA Library Prep Kit for Illumina™ (NEB, USA) following manufacturer’s instructions. Newly isolated mRNA was further purified using with Oligo (dT) magnetic beads and sheared into 200–700 nucleotides sections using fragmentation buffer. The fragmented mRNA was used as templates for first-strand cDNA synthesis using random hexamer primers. Subsequently, second-strand cDNA was synthesized using DNA polymerase I and RNaseH. All remaining overhangs were passivated via polymerase. After adenylation of 3’ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure was ligated for hybridization. In order to select cDNA fragments of preferentially 150~200 bp, the library fragments were purified using an AMPure XP system. Then 3 μL USER Enzyme (NEB, USA) was incubated with size-selected, adaptor-ligated cDNA at 37˚C for 15 min followed by incubation at 95˚C for 5 min before PCR reaction. Subsequently, PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Amplicons were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. The cDNA library of *P. interpunctella* was sequenced on Illumina Hiseq™ 2500 using paired-end technology in a single run by Beijing Biomake Company (Beijing, China).

Clustering and sequencing

Following a previous report [17], clustering and sequencing were performed on a cBot Cluster Generation System and an Illumina Hiseq 2500 platform, respectively.

Sequence analysis and assembly

Raw reads of fastq format were firstly processed through in-house perl scripts. In this step, clean reads were obtained by removing reads containing adapter, reads containing ploy-N and low quality reads. At the same time, Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. Cleaned reads shorter than 60 bases were removed because the short reads might represent sequencing artifacts [26]. The qualified reads were assembled into unigenes using short reads assembling program-Trinity [10].

The obtained contigs were annotated against the NCBI non-redundant protein (NR) database using BLASTn (E-value<10^-5) and BLASTx (E-value<10^-5) programs [11]. To annotate the assembled sequences with Gene Ontology (GO) terms, the Swiss-Prot BLAST results were imported into BLAST2GO, a software package that retrieves GO terms, allowing determination and comparison of gene functions [27]. The unigene sequences were also aligned to the Clusters of Orthologous Groups of proteins (COG) database to predict and classify the unigene sequences [28]. Pathway annotations for unigenes were determined using Kyoto Encyclopedia of Genes and Genomes (KEGG) ontology [29]. Finally, the best matches were used to identify coding regions and determine the sequence direction [30].

Olfactory gene identification and phylogenetic analysis

The annotations of OBP, CSP, SNMP, OR, IR and GR genes in *P. interpunctella* were verified by BLASTx and BLASTn programs NCBI. The complete coding region was predicted using the open reading frame (ORF) finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) based on the results given by BLASTx. After completing the alignments of the candidate chemosensory genes using ClustalX (2.1), phylogenetic reconstruction for the analysis of OBPs, CSPs, ORs, IRs and GRs was performed by MEGA5.0 software using the neighbor-joining method with
1000 Bootstrap iterations [31]. In addition, the evolutionary distances were assumed by using the Poisson correction method [11].

### Analysis of differentially expressed genes and qRT-PCR verification

To compare the differential expression of chemosensory genes between the male and female antennal transcriptomes of *P. interpunctella*, the read number of each olfactory-related gene was converted to FPKM (fragments per kilobase of exon model per million mapped reads) [32].

qRT-PCR was performed to quantify the expression levels of olfactory-related genes in male and female antennae. Total RNA was extracted from 100 antennae as above description. cDNA from antennae of both sexes was synthesized using the SMART™PCR cDNA synthesis kit (Clontech, Mountain View, CA, USA). The β-actin gene (SRP05157) was used as an internal control in each sample, and it was selected as a housekeeping gene in our qRT-PCR test. Real-time PCR was performed on an ABI 7500 using SYBR green dye binding to double-stranded DNA at the end of each elongation cycle. Primer sequences were designed using the Primer Premier 5.0 program (S1 Table). Real-time PCR was conducted with our previous method [13]. Briefly, 10.0 μL of 2×SYBR Green PCR Master Mix, 0.4 μL of primer, 2.0 μL of sample cDNA (100 ng μL⁻¹) and 7.2 μL of sterilized ultrapure water were mixed to form a 20 μL reaction system. After an initial denaturation step at 95℃ for 3 min, amplifications were carried out with 40 cycles at a melting temperature of 95℃ for 10 s and an annealing temperature of 60℃ for 30 s. To check reproducibility, qRT-PCR test for each sample was performed with three technical replicates and three biological replicates.

#### qRT-PCR analysis

Relative quantification was determined using the comparative 2⁻ΔΔCt method [33]. All data were normalized to endogenous β-actin levels from the same individual samples. The relative fold change was assessed by comparing the expression level in male moths to that in females [34]. The results were presented as the means of the fold change in three biological duplicates. The comparative analyses of chemosensory genes between sexes were determined by one-way analysis of variance (ANOVA) using SPSS 19.0, with p-value of 0.05 considered significant.

### Results

#### Sequence analysis and assembly

cDNA library of *P. interpunctella* was constructed using the TRINITY *de novo* assembly program, and short-read sequences were assembled into 150,633 transcripts with a mean length of 1,491 bp and an N50 of 3,567 bp. A total of 20,261 scaffolds (13.45%) were longer than 1,000 bp, and 36,148 scaffolds (24.00%) were longer than 2,000 bp. The scaffolds were subjected to cluster and assembly analyses. Subsequently, 87,300 unigenes were obtained with a mean length of 699 bp and an N50 of 1,282 bp (Fig 1, Table 1). The length distribution of unigenes revealed that 26,054 unigenes (29.84%) were longer than 500 bp and 12,485 unigenes (14.30%) were longer than 1,000 bp (Table 1). The raw reads of *P. interpunctella* transcriptome have been deposited into the NCBI SRA database (accession number: SRR6002827 and SRR6002828), and the Transcriptome Shotgun Assembly (TSA) project has been deposited at DDBJ/ENA/GenBank under the accession GFWQ0000000. The version described in this paper is the first version, GFWQ01000000. The detailed TSA sequences could be obtained from Genbank (https://www.ncbi.nlm.nih.gov/Traces/wgs/?val=GFWQ01&disp=contigs&page=1).
Sequence annotation

The unigene annotation showed that 27,920 unigenes (31.98%) significantly matched in the NR database and 15,815 unigenes (18.12%) had significant matches in the Swiss-Prot database. A total of 31,921 unigenes (36.56%) were successfully annotated in the NR, Swiss-Prot, KEGG, GO and COG databases (Table 2), whereas 55,379 unigenes (63.44%) were unmapped in those databases.

NR database queries revealed that a high percentage of *P. interpunctella* sequences had closely matched sequences in *Bombyx mori* (6,087, 21.84%), followed by *Danaus plexippus* (4,612, 16.54%), *Acyrthosiphon pisum* (4,329, 15.53%) and *Bactrocera dorsalis* (3,839, 13.77%) (Fig 2).

For GO analysis, 15,893 unigenes (18.21%) could be assigned to three GO terms as follows: cellular components, molecular functions and biological process (Fig 3). The “cellular components” and “molecular functions” were most represented by 18.79% and 21.04% transcripts, respectively. In the “cellular components” ontology, the terms were mainly distributed in cell

| Length (bp) | Transcript | Unigene |
|-------------|------------|---------|
| 200–300     | 39,574(26.27%) (26.27%) | 35,516(40.68%) |
| 300–500     | 31,475(20.90%) (20.90%) | 25,729(29.47%) (29.74%) |
| 500–1000    | 23,172(15.38%) (15.38%) | 13,569(15.54%) |
| 1000–2000   | 20,261(13.45%) | 6,176(7.07%) |
| 2000+       | 36,148(24.00%) | 6,309(7.23%) |
| Total Number| 150,633     | 87,300 |
| Total Length| 224,546,425 | 61,027,187 |
| N50 Length  | 3,567       | 1,282  |
| Mean Length | 1490.69     | 699.05 |

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Identification of olfactory-related genes

In the present study, we identified 117 olfactory-related genes from antennal transcriptome of *P. interpunctella*, including 29 OBPs, 15 CSPs, three SNMPs, 47 ORs, nine GRs and 14 IRs. All genes were named according to a four-letter code (first letter of the genus name followed by the first three letters of the species name) + OR + number according to the ORF lengths. Analysis of differential expression of unigenes indicated that 1,031 genes showed differences between the antennal transcriptomes of male and female *P. interpunctella*, including 93 up-regulated and 938 down-regulated genes using female result as the reference standard.

Table 2. Functional annotation of the *Plodia interpunctella*.

| Annotated databases      | unigene | ≥300 bp | ≥1000 bp |
|--------------------------|---------|---------|----------|
| COG_annotation           | 10,106  | 4,383   | 3,554    |
| GO_annotation            | 15,893  | 6,734   | 5,269    |
| KEGG_annotation          | 15,016  | 6,404   | 4,654    |
| SwissProt_annotation     | 15,815  | 6,420   | 6,205    |
| nr_annotation            | 27,920  | 11,530  | 9,415    |
| Total                    | 31,921  | 13,492  | 9,548    |

COG = Cluster of Orthologous Groups of proteins; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; nr = nonredundant protein.

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(20.71%) and cell part (20.71%). In the “molecular functions” ontology, the terms of binding function and catalytic activity were the most represented (39.91% and 39.80%, respectively) (Fig 3).

To predict and classify the functional genes, all unigenes were searched against the COG database. A total of 10,106 unigenes could be assigned to 25 specific categories according to the COG classification results. “General function prediction” (2,494, 24.68%) was the largest group, and the categories of “cell motility” (20, 0.20%) and “nuclear structure” (11, 0.11%) were the smallest groups (Fig 4). In addition, 290 pathways were predicted in the KEGG database, representing 15,016 unigenes.

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**Fig 2.** Characteristics of homology search for *Plodia interpunctella* unigenes. The number of unigenes matching the top ten species using BlastX in the Nr database is indicated in square brackets.

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Candidate OBPs in antennae of *Plodia interpunctella*

In antennal transcriptomes of *P. interpunctella*, 29 OBP genes were annotated based on the tBLASTn results, including four pheromone-binding proteins (PBPs) and one general odor-ant-binding protein (GOBP) (Table 3). Among the 29 OBP genes, 17 had intact ORFs with lengths ranging from 291 bp to 1,014 bp. The BLASTx results indicated that 24 identified PinnOBPs shared relatively higher amino acid identities (>50%) with Lepidoptera OBPs in NCBI.

A neighbor-joining tree of 123 OBP sequences was constructed using OBPs of Lepidoptera species, including four species in Pyraloidea family (*P. interpunctella*, *Conogethes punctiferalis*, *Ostrinia furnacalis* and *Chilo suppressalis*), and *Bombyx mori*. Due to the lack of antennal transcriptome information of genus Plodia, we selected three closer relatives of *P. interpunctella* to
Table 3. The Blastx matches of *Plodia interpunctella* candidate OBP genes.

| Gene ID | Gene name | Full length | ORF (aa) | Blastx annotation (Reference/Name/Species) | Score | E-value | Identity (%) | FPKM values |
|---------|-----------|-------------|----------|-----------------------------------------------|-------|---------|--------------|-------------|
| c34980. | OBP1      | Y           | 338      | ALD65883.1 | odorant binding protein 9 [Spodoptera litura] | 325   | 4e-107  | 56            | 40.51       |
| c20255. | OBP2      | Y           | 252      | ADD71058.1 | odorant-binding protein [Chilo suppressalis] | 376   | 5e-130  | 69            | 302.91      |
| c31451. | OBP3      | N           | 242      | BAV56797.1 | odorant binding protein 10 [Ostrinia furnacalis] | 320   | 4e-108  | 67            | 271.44      |
| c44096. | OBP4      | N           | 210      | ALT31639.1 | odorant-binding protein 9 [Cnaphalocrocis medinalis] | 309   | 6e-105  | 71            | 0.18        |
| c16901. | OBP5      | N           | 206      | BAV56794.1 | odorant binding protein 7 [Ostrinia furnacalis] | 156   | 7e-45   | 47            | 154.93      |
| c29670. | OBP6      | Y           | 197      | EHJ74351.1 | odorant-binding protein 2 [Danaus plexippus] | 293   | 7e-99   | 81            | 0           |
| c33870. | OBP7      | N           | 180      | BAV56800.1 | odorant binding protein 13 [Ostrinia furnacalis] | 242   | 1e-79   | 76            | 1.71        |
| c42887. | OBP8      | Y           | 180      | AII00998.1 | odorant binding protein [Dendrolimus kikuchii] | 130   | 2e-35   | 44            | 2443.01     |
| c16794. | OBP9      | Y           | 170      | JAV45894.1 | odorant binding protein 19 [Mythimna separata] | 194   | 6e-61   | 61            | 0.53        |
| c29465. | OBP10     | N           | 164      | AGK24577.1 | odorant-binding protein 1 [Chilo suppressalis] | 100   | 3e-24   | 36            | 2682.84     |
| c33892. | OBP11     | Y           | 149      | JAP86818.1 | OBP [Conogethes punctiferalis] | 155   | 5e-46   | 49            | 2747.16     |
| c34383. | OBP12     | N           | 146      | BAV56795.1 | odorant binding protein 8 [Ostrinia furnacalis] | 213   | 7e-69   | 83            | 898.18      |
| c55087. | OBP13     | Y           | 142      | JAI18227.1 | Antennal Binding Protein X [Epiphyas postvittana] | 209   | 2e-67   | 68            | 455.66      |
| c40570. | OBP14     | Y           | 142      | ANC68517.1 | odorant-binding protein 29 [Chilo suppressalis] | 135   | 3e-38   | 51            | 0           |
| c40388. | OBP15     | Y           | 142      | AFD34173.1 | odorant binding protein 5 [Argyresthia conjugella] | 235   | 8e-78   | 77            | 4746.92     |
| c20185. | OBP16     | Y           | 139      | BAV56799.1 | odorant binding protein 12 [Ostrinia furnacalis] | 245   | 1e-81   | 83            | 335.28      |
| c47744. | OBP17     | Y           | 137      | AGM38607.1 | odorant binding protein [Chilo suppressalis] | 207   | 5e-67   | 78            | 21184.85    |
| c25858. | OBP18     | N           | 135      | AGC82130.1 | odorant-binding protein 1 [Bactrocera dorsalis] | 275   | 1e-93   | 100           | 0.66        |
| c32612. | OBP19     | Y           | 134      | ALS03864.1 | odorant-binding protein 16 [Ectropis obliqua] | 248   | 3e-83   | 90            | 146.09      |
| c79234. | OBP20     | Y           | 114      | JAV45893.1 | odorant binding protein 20 [Mythimna separata] | 157   | 3e-47   | 61            | 3.76        |
| c37240. | OBP21     | N           | 104      | ALD65893.1 | odorant binding protein 19 [Spodoptera litura] | 87.8  | 2e-19   | 63            | 0.25        |
| c32421. | OBP22     | Y           | 97       | BAV56803.1 | odorant binding protein 16 [Ostrinia furnacalis] | 130   | 1e-36   | 61            | 27.47       |
| c36217. | OBP23     | N           | 71       | JAI18081.1 | Odorant Binding Protein [Epiphyas postvittana] | 81.6  | 4e-18   | 47            | 6.5         |
| c32145. | OBP24     | N           | 54       | JAV45888.1 | odorant binding protein 25 [Mythimna separata] | 108   | 1e-28   | 94            | 3.82        |
| c30184. | OBP25     | N           | 182      | AHZ89398.1 | pheromone-binding protein 2 [Grapholita molesta] | 221   | 3e-71   | 62            | 3494.34     |
| c9234.  | OBP26     | N           | 170      | ADT78495.1 | pheromone binding protein 1 [Ostrinia nubilalis] | 241   | 2e-79   | 69            | 6152.67     |

(Continued)
compare the OBPs. B. mori was chosen to study the patterns and functions of OBPs, because BmorOBPs were widely recognized and verified. Most PintOBPs had a high similarity to known Pyralidae OBPs, which could possibly be attributed to that both P. interpunctella and Pyralidae belong to Pyraloidea family. Phylogenetic tree showed that the PintPBP2-4 was clustered into the PBP family, and the PintGOBP1 was clustered into the GOBP family. In the PBP family, PintPBP2, PintPBP3 and PintPBP4 were stretched in the same branch with the boot-strap values as high as 62 (Fig 5). Based on the number of conserved cysteines, OBPs can be divided into three subclasses: classic OBPs, Plus-C OBPs and Minus-C OBPs [35]. As for P. interpunctella, PintOBP7, PintOBP10, PintOBP15 and PintOBP19 were clustered into the Minus-C OBP family. Meanwhile PintOBP5 belonged to the Plus-C OBP family. According to multiple amino acid sequence alignments, 16 OBPs (PintOBP1-4, PintOBP6, PintOBP8-9, PintOBP11-17, PintOBP20, PintPBP1-3 and PintGOBP1) totally matched with C1-X25-30-C2-X18-19-C3-X36-42-C4-X8-14-C5-X8-C6 (X stands for any amino acid), and they were identified as classic OBPs (Fig 6) [36].

Base on FPKM measure, the OBPs with an FPKM value greater than 1,000 were defined as high-expression genes [30]. The FPKM analysis revealed that 10 OBP genes (PintOBP8, PintOBP10, PintOBP11, PintOBP15, PintOBP17, PintPBP1-4 and PintGOBP1) were highly abundant in antennae of P. interpunctella (FPKM>1,000) (Table 3). Furthermore, the qRT-PCR expression levels of 29 PintOBP genes indicated that nine OBP genes (PintOBP4, PintOBP6, PintOBP9, PintOBP13, PintOBP17 PintOBP20, PintOBP22 and PintPBP2-3) were significantly expressed in the male antennae (1.8 to 33.5 times compared with females). Eight OBPs (PintOBP5, PintOBP7, PintOBP12, PintOBP15-16, PintOBP18, PintPBP1 and PintGOBP1) were significantly expressed in the female antennae (1.7 to 3.8 times compared with males). The other eight OBP genes (PintOBP1-3, PintOBP8, Pint10-11, PintOBP14 and PintOBP21) showed similar expression levels in the male and female antennae (Fig 7).

### Candidate CSPs in antennae of Plodia interpunctella

In the antennal transcriptomes of P. interpunctella, 15 putative CSPs were identified with lengths ranging from 291 bp to 492 bp. All identified PintCSPs were verified according to the four-cysteines pattern C1-X6-8-C2-X18-19-C3-X2-C4 (Fig 8) [36]. Among the 15 PintCSP genes, eight had intact ORFs with lengths ranging from 318 bp to 492 bp. The BLASTx results indicated that 13 identified PintCSPs shared relatively higher amino acid identities (>50%) with Lepidoptera CSPs in NCBI (Table 4).

A neighbor-joining tree of 78 CSP sequences was constructed based on Lepidoptera species from C. punctiferalis, O. furnacalis, C. suppressalis and B. mori. PintCSPs were distributed on various branches throughout the cladogram (Fig 9). The phylogenetic tree showed that PintCSP14, PintCSP2, PintCSP5 and PintCSP1 were clustered together with OfurCSPs, with relatively higher bootstrapping values.

### Table 3. (Continued)

| Gene ID   | Gene name | Full length | ORF (aa) | Blastx annotation (Reference/Name/Species) | Score | E-value | Identity (%) | FPKM values Female | FPKM values Male |
|-----------|-----------|-------------|----------|----------------------------------------|-------|---------|--------------|-------------------|------------------|
| c16802.  | PBP3      | Y 170       | AAD39447.1 | pheromone binding protein [Ostrinia nubilalis] | 234   | 9e-77   | 67           | 99.62             | 2860.16          |
| c34173.  | PBP4      | Y 124       | AAF06142.1 | pheromone binding protein [Syananthedon exigiosa] | 181   | 1e-56   | 67           | 1390.9            | 2307.02          |
| c34904.  | GOBP1     | Y 190       | AGS36742.1 | GOBP1 [Sesamia inferens]              | 254   | 3e-84   | 75           | 2790.14           | 1853.61          |

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The FPKM analysis revealed that only PintCSP4 was highly abundant in antennal transcriptomes of *P. interpunctella* (FPKM > 1,000) (Table 4). The qRT-PCR results indicated that three PintCSP genes (PintCSP11, PintCSP14 and PintCSP15) were significantly expressed in the male antennae (1.5 to 3.5 times compared with females). Seven PintCSPs (PintCSP1-2, PintCSP5, PintCSP9-10 and PintCSP12-13) were specifically expressed in the female antennae (1.7 to 3.2 times compared with males) (Fig 10).

![Fig 5. Neighbor-joining tree of candidate OBPs from *Plodia interpunctella*, *Conogethes punctiferalis*, *Ostrinia furnacalis*, *Chilo suppressalis* and *Bombyx mori*. The protein names and sequences of OBPs that were used in this analysis are listed in S2 Table.](https://doi.org/10.1371/journal.pone.0189889.g005)

![Fig 6. Sequences alignment of classic PintOBPs.](https://doi.org/10.1371/journal.pone.0189889.g006)
We identified 47 OR genes in the antennal transcriptomes of *P. interpunctella*, in which 36 PintORs had intact ORFs with lengths ranging from 219 bp to 1,422 bp with four to seven transmembrane domains (Table 5).

In the neighbor-joining tree of ORs (Fig 11), the PintOR1 was clustered into the ORco family and four PintORs (PintOR5, PintOR7, PintOR22 and PintOR30) were clustered into the pheromone receptor (PR) family. Two groups of ORs (PintOR14 and PintOR35, PintOR29 and PintOR26) were clustered into the same branch with bootstrapping values of 98 and 87, respectively. All of the other PintORs were distributed on various branches throughout the phylogenetic tree.

PintOR1 (ORco) showed the highest qRT-PCR expression level among the 47 PintORs, with FPKM values of 576.23 and 430.52 in the male and female antennae, respectively. However, the other 46 typical ORs showed a relatively lower expression level (FPKM ranged from 0 to 214). The qRT-PCR results indicated that nine OR genes (PintOR1, PintOR5, PintOR15, PintOR18, PintOR22, PintOR38, PintOR41-42 and PintOR47) were highly expressed in the male antennae. Meanwhile, 16 OR genes (PintOR3, PintOR7, PintOR9-11, PintOR23-25, PintOR28, PintOR31-34, PintOR36, PintOR39, PintOR40, PintOR43-46, PintOR48, PintOR49) were expressed at lower levels in the male antennae.
PintOR28, PintOR30-31, PintOR35, PintOR37, PintOR40 and PintOR45-46) exhibited female antenna-specific expressions (Fig 12).

### Candidate GRs

In the present study, we identified nine candidate PintGR encoding transcripts from antennal transcriptome of *P. interpunctella*. Five PintGR genes had intact ORFs with lengths ranging from 198 bp to 1,461 bp. The BLASTx results indicated that seven identified PintGRs shared relatively higher amino acid identities (>50%) with Lepidoptera GRs in NCBI (Table 6).

In the neighbor-joining tree of GRs (Fig 13), PintGRs were present on various branches throughout the cladogram. PintGR1 and PintGR8 were clustered into the same branch, with a bootstrapping value of 65.

The FPKM analysis showed that all PintGRs had a relatively low expression level (FPKM ranged from 0.27 to 33.37). The qRT-PCR results indicated that PintGR1 and PintGR8 were highly expressed in females, while PintGR3 was highly expressed in males. The FPKM values are as follows:

- **PintGR1**: Female 1.9, Male 0
- **PintGR3**: Female 343.76, Male 276.61
- **PintGR8**: Female 463.43, Male 384.09

### Table 4. The Blastx matches of *Plodia interpunctella* candidate CSP and SNMP genes.

| Gene ID | Gene name | Full length | ORF (aa) | Blastx annotation (Reference/Name/Species) | Score | E-value | Identity (%) | FPKM values |
|---------|-----------|-------------|---------|--------------------------------------------|-------|---------|--------------|-------------|
| c21705. | CSP1      | Y           | 164     | AGE97647.1 | chemosensory protein 8 [*Aphis gossypii*] | 251   | 2e-83   | 78           | Female 1.9, Male 0 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c6549.  | CSP2      | N           | 150     | APB03439.1 | chemosensory protein 3 [*Sitobion avenae*] | 192   | 1e-60   | 91           | Female 0.7, Male 0 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c39316. | CSP3      | Y           | 150     | AGR39578.1 | chemosensory protein 8 [*Agrotis ipsilon*] | 177   | 5e-55   | 63           | Female 343.76, Male 276.61 |
|         | graph_c3  |             |         |                                            |       |         |              |             |
| c31754. | CSP4      | N           | 146     | JAV45874.1 | chemosensory protein 7 [*Mythimna separata*] | 210   | 6e-88   | 80           | Female 1503.31, Male 1403.08 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c33894. | CSP5      | Y           | 141     | BAV56812.1 | chemosensory protein 8 [*Ostrinia furnacalis*] | 194   | 2e-61   | 66           | Female 463.43, Male 384.09 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c50839. | CSP6      | N           | 137     | ALS03837.1 | chemosensory protein 12 [*Ectropis obliqua*] | 195   | 3e-62   | 78           | Female 0.27, Male 0.37 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c25625. | CSP7      | N           | 136     | BAV56814.1 | chemosensory protein 10 [*Ostrinia furnacalis*] | 120   | 1e-32   | 45           | Female 33.2, Male 28.58 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c32629. | CSP8      | Y           | 133     | AFR92093.1 | chemosensory protein 9 [*Helicoverpa armigera*] | 162   | 2e-49   | 61           | Female 4.91, Male 3.65 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c80512. | CSP9      | N           | 130     | APB03440.1 | chemosensory protein 4 [*Sitobion avenae*] | 261   | 3e-88   | 97           | Female 0.38, Male 0.13 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c30384. | CSP10     | Y           | 128     | AEB54579.1 | CSP5 [*Helicoverpa armigera*] | 191   | 2e-60   | 69           | Female 386.2, Male 302.77 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c36418. | CSP11     | Y           | 128     | AIX97839.1 | chemosensory protein [*Cnaphalocrocis medinalis*] | 115   | 7e-31   | 44           | Female 1.57, Male 3.43 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c32937. | CSP12     | Y           | 127     | BAV56806.1 | chemosensory protein 2 [*Ostrinia furnacalis*] | 180   | 2e-65   | 65           | Female 339.32, Male 272.62 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c50413. | CSP13     | N           | 110     | AGE97642.1 | chemosensory protein 2 [*Aphis gossypii*] | 194   | 3e-62   | 89           | Female 1.26, Male 0 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c52233. | CSP14     | Y           | 106     | JAV45868.1 | chemosensory protein 13 [*Mythimna separata*] | 165   | 6e-51   | 75           | Female 0.53, Male 0.54 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c32777. | CSP15     | N           | 97      | AKT26494.1 | chemosensory protein 20 [*Spodoptera exigua*] | 168   | 2e-52   | 85           | Female 2.61, Male 3.45 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c16843. | SNP1      | N           | 510     | AOG12884.1 | sensory neuron membrane protein [*Eoqetia hippophaeolus*] | 863   | 0.0     | 79           | Female 71.33, Male 152.34 |
|         | graph_c0  |             |         |                                            |       |         |              |             |
| c35212. | SNP2      | Y           | 495     | ADQ73889.1 | sensory neuron membrane protein 2 [*Ostrinia nubilalis*] | 762   | 0.0     | 70           | Female 252.42, Male 365.34 |
|         | graph_c1  |             |         |                                            |       |         |              |             |
| c74901. | SNP3      | N           | 121     | KPI91875.1 | Sensory neuron membrane protein 1 [*Papilio xuthus*] | 146   | 3e-38   | 54           | Female 0.53, Male 0.27 |
|         | graph_c0  |             |         |                                            |       |         |              |             |

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expressed in the male antennae (1.9 and 3.7 times compared with females, respectively). Moreover, five GRs (PintGR3, PintGR5-7 and PintGR9) displayed female antenna-specific expressions (Fig 14.).

Fig 9. Neighbor-joining tree of candidate CSPs from *Plodia interpunctella*, *Conogethes punctilheralis*, *Ostrinia furnacalis*, *Chilo suppressalis* and *Bombyx mori*. The protein names and sequences of CSPs that were used in this analysis are listed in S3 Table.

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Fig 10. *P. interpunctella* CSP transcript levels in different antennae measured by qRT-PCR. MA: male antennae; FA: female antennae. The internal control β-actin was used to normalize transcript levels in each sample. The standard error represented by the error bar, and the asterisk above each bar denote significant differences (p<0.05).

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Table 5. The Blastx matches of *Plodia interpunctella* candidate OR genes.

| Gene ID   | Gene name | Full length | ORF (aa) | Blastx annotation (Reference/Name/Species) | Score | E-value | Identity (%) | FPKM values Female | FPKM values Male |
|-----------|-----------|-------------|----------|---------------------------------------------|-------|---------|--------------|--------------------|------------------|
| c40585.   | OR1       | Y           | 474      | AFQ94048.1 | olfactory receptor 2 [*Chilo suppressalis*] | 928   | 0.0     | 92           | 430.52             | 576.23           |
| c32962.   | OR2       | Y           | 452      | AIT69911.1 | olfactory receptor 71 [*Ctenopseustis herana*] | 608   | 0.0     | 64           | 2.59               | 3.04             |
| c36497.   | OR3       | N           | 449      | ALT31655.1 | odorant receptor 1 [*Cnaphalocrocis medinalis*] | 702   | 0.0     | 76           | 8.04               | 2.88             |
| c28679.   | OR4       | Y           | 430      | ALM26234.1 | odorant receptor 44 [*Athetis dissimilis*] | 712   | 0.0     | 77           | 3.45               | 3.52             |
| c39092.   | OR5       | Y           | 429      | AGI96750.1 | olfactory receptor 13 [*Spodoptera litura*] | 399   | 3e-133  | 44           | 20                 | 189.41           |
| c31116.   | OR6       | Y           | 424      | ANZ03153.1 | olfactory receptor 40 [*Cnaphalocrocis medinalis*] | 525   | 0.0     | 57           | 13.84              | 3.03             |
| c38802.   | OR7       | Y           | 417      | AFP66948.1 | odorant receptor 4 [*Amyelois transitella*] | 565   | 0.0     | 65           | 4.25               | 0.13             |
| c38263.   | OR8       | Y           | 411      | ALM26235.1 | odorant receptor 45 [*Athetis dissimilis*] | 608   | 0.0     | 71           | 4.55               | 5.05             |
| c36791.   | OR9       | Y           | 409      | AOG12913.1 | odorant receptor [*Eogystia hippocophaeacous*] | 258   | 7e-79   | 34           | 6.05               | 2.25             |
| c34023.   | OR10      | N           | 409      | AOG12906.1 | odorant receptor [*Eogystia hippocophaeacous*] | 400   | 1e-134  | 50           | 7.72               | 4.54             |
| c31249.   | OR11      | N           | 408      | CUQ99400.1 | Olfactory receptor 17 [*Manduca sexta*] | 435   | 2e-148  | 52           | 2.41               | 0.0              |
| c39086.   | OR12      | Y           | 406      | AIG51899.1 | odorant receptor [*Helicoverpa armigera*] | 266   | 2e-82   | 38           | 5.66               | 4.67             |
| c36343.   | OR13      | Y           | 404      | ALM26238.1 | odorant receptor 53 [*Athetis dissimilis*] | 441   | 3e-151  | 51           | 7.28               | 4.62             |
| c40271.   | OR14      | Y           | 404      | AOG12941.1 | odorant receptor [*Eogystia hippocophaeacous*] | 468   | 3e-161  | 55           | 5.2                | 5.11             |
| c16387.   | OR15      | Y           | 400      | AOG12915.1 | odorant receptor [*Eogystia hippocophaeacous*] | 508   | 3e-177  | 62           | 3.23               | 3.89             |
| c40164.   | OR16      | Y           | 396      | AQQ73507.1 | olfactory receptor 27 [*Heliconius melpomene rosina*] | 506   | 1e-176  | 62           | 13.69              | 7.6              |
| c37581.   | OR17      | Y           | 393      | AI01084.1  | odorant receptor [*Dendrolimus kikuchii*] | 506   | 9e-177  | 62           | 3.37               | 3.56             |
| c36558.   | OR18      | N           | 391      | ANZ03145.1 | odorant receptor 32 [*Cnaphalocrocis medinalis*] | 439   | 2e-150  | 55           | 6.6                | 10.04            |
| c34205.   | OR19      | Y           | 389      | AIG51856.1 | odorant receptor [*Helicoverpa armigera*] | 402   | 7e-136  | 50           | 18.62              | 11.28            |
| c39368.   | OR20      | Y           | 386      | JAV45828.1 | odorant receptor 37 [*Mythimna separata*] | 522   | 0.0     | 66           | 9.04               | 4.63             |
| c32622.   | OR21      | Y           | 380      | JAI18048.1 | Odorant Receptor [*Epiphyas postvittana*] | 459   | 1e-158  | 59           | 3.65               | 0.71             |
| c37029.   | OR22      | Y           | 377      | AC12370.1  | olfactory receptor 13 [*Helicoverpa armigera*] | 335   | 3e-109  | 48           | 3.62               | 214.15           |
| c37794.   | OR23      | N           | 364      | ALM26250.1 | odorant receptor 85 [*Athetis dissimilis*] | 332   | 1e-108  | 46           | 6.52               | 3.87             |
| c37397.   | OR24      | Y           | 355      | JAI18015.1 | Odorant Receptor [*Epiphyas postvittana*] | 452   | 1e-155  | 60           | 16.24              | 4.61             |
| c29168.   | OR25      | Y           | 345      | ALM26219.1 | odorant receptor 30 [*Athetis dissimilis*] | 211   | 2e-61   | 34           | 25.21              | 0.05             |
| c37849.   | OR26      | Y           | 335      | AQQ73504.1 | olfactory receptor 24 [*Heliconius melpomene rosina*] | 323   | 2e-105  | 48           | 4.79               | 2.77             |
| c27537.   | OR27      | Y           | 328      | AIG51873.1 | odorant receptor [*Helicoverpa armigera*] | 442   | 9e-153  | 68           | 10.2               | 8.72             |

(Continued)
Candidate IRs

In the present study, we identified 14 candidate PintIR genes encoding transcripts from antennal transcriptome of P. interpunctella (Table 6). Nine PintIRs had intact ORFs with lengths ranging from 384 bp to 2,706 bp. In the neighbor-joining tree of IRs (Fig 15), PintIR1 and PintIR2 were phylogenetically clustered into the highly conserved IR8a and IR21a sub-families, respectively. The FPKM analysis revealed that all PintIRs showed a low expression level (FPKM value ranged from 0.36 to 113.52). The qRT-PCR results indicated that PintIR1, PintIR3-5, PintIR10, and PintIR13-14 were highly expressed in the female antennae (1.2 to 5.3 times compared with males) (Fig 14).
Discussion

In recent years, RNA-Seq transcriptome sequencing technology has been widely used due to the development of high-throughput sequencing technology, resulting in great progress in non-model organisms [11, 37–39]. In the present study, we used NGS technology to analyze...
the antennal transcriptome of *P. interpunctella*. Sequence analysis and assembly results demonstrated that Illumina sequencing technology could effectively and rapidly captured a large portion of the transcriptome, providing molecular foundations for rapid characterization of functional genes and better reference of target genes [40].

Table 6. The Blastx matches of *Plodia interpunctella* candidate GR and IR genes.

| Gene ID       | Gene name | Full length | ORF (aa) | Blastx annotation (Reference/Name/Species) | Score | E-value | Identity (%) | FPKM values |
|---------------|-----------|-------------|---------|--------------------------------------------|-------|---------|--------------|-------------|
| c28105.       | GR1       | N           | 509     | gustatory receptor *Helicoverpa armigera*   | 721   | 0.0     | 78           | 2.14        |
| c36972.       | GR2       | Y           | 487     | Gustatory Receptor *Epiphyas postvittana*  | 456   | 5e-155  | 55           | 11.23       |
| c19072.       | GR3       | N           | 410     | gustatory receptor *Helicoverpa armigera*   | 796   | 0.0     | 92           | 0.57        |
| c37959.       | GR4       | Y           | 401     | gustatory receptor 46 *Bombyx mori*        | 180   | 3e-49   | 32           | 33.37       |
| c35051.       | GR5       | Y           | 381     | gustatory Receptor *Helicoverpa armigera*  | 183   | 4e-52   | 51           | 2.54        |
| c37548.       | GR6       | Y           | 297     | gustatory receptor 3 *Ectropis obliqua*    | 474   | 4e-165  | 76           | 7.56        |
| c27749.       | GR7       | N           | 254     | gustatory receptor 4 *Helicoverpa armigera*| 81.6  | 4e-14   | 38           | 0.79        |
| c69579.       | GR8       | N           | 80      | gustatory receptor 2 *Helicoverpa assulta* | 151   | 2e-43   | 97           | 0.27        |
| c35211.       | GR9       | Y           | 66      | gustatory receptor 4 *Helicoverpa armigera*| 70.5  | 1e-12   | 57           | 18.78       |
| c38794.       | IR1       | Y           | 902     | ionotrop ic receptor *Ostrinia furnacalis* | 1439  | 0.0     | 77           | 137.3       |
| c37198.       | IR2       | Y           | 863     | ionotrop ic receptor *Ostrinia furnacalis* | 1256  | 0.0     | 70           | 12.95       |
| c38992.       | IR3       | Y           | 703     | ionotrop ic receptor *Eogystia hippophaecolus* | 1071  | 0.0     | 75           | 8.91        |
| c38347.       | IR4       | Y           | 646     | ionotrop ic receptor *Ostrinia furnacalis* | 808   | 0.0     | 66           | 8.16        |
| c36292.       | IR5       | N           | 581     | ionotrop ic receptor *Ostrinia furnacalis* | 909   | 0.0     | 76           | 4.24        |
| c40504.       | IR6       | Y           | 550     | ionotrop ic receptor *Ostrinia furnacalis* | 803   | 0.0     | 70           | 22.37       |
| c39043.       | IR7       | N           | 518     | ionotrop ic receptor 75q.2 *Helicoverpa assulta* | 525   | 0.0     | 67           | 16.37       |
| c38844.       | IR8       | Y           | 498     | ionotrop ic receptor *Eogystia hippophaecolus* | 473   | 7e-159  | 61           | 18.83       |
| c37486.       | IR9       | Y           | 456     | ionotrop ic receptor *Ostrinia furnacalis* | 509   | 1e-173  | 62           | 5.43        |
| c37941.       | IR10      | N           | 436     | ionotrop ic receptor *Opencophthera brunatala* | 459   | 4e-154  | 50           | 6.8         |
| c39660.       | IR11      | Y           | 351     | ionotrop ic Receptor 7 *Mythimna separata* | 364   | 2e-118  | 52           | 16.63       |
| c35003.       | IR12      | N           | 283     | ionotrop ic receptor *Helicoverpa armigera* | 501   | 3e-177  | 82           | 1.32        |
| c20608.       | IR13      | N           | 218     | ionotrop ic receptor *Epiphyas postvittana* | 213   | 7e-62   | 50           | 0.36        |
| c36292.       | IR14      | Y           | 128     | ionotrop ic receptor *Ostrinia furnacalis* | 229   | 3e-68   | 80           | 4.07        |

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The unigene annotation showed that 55,379 unigenes (63.44%) were unmapped in those databases, which could be attributed to the short sequence reads generated by the sequencing technology. It also suggested that the unmapped sequences could represent unannotated or new genes. In fact, fewer than 5% of unmapped unigenes are likely to represent new genes. Generally, the 5' ends of sequences show less conservation than the body. Therefore, partial transcripts (unigenes representing the 5' CDS, but not the body) may not be found matches in the databases. For GO analysis, the antennal unigenes were annotated into different functional groups [16], which were similar to those in the antennal transcriptomes of *Conogethes punctiferalis* [13], *Spodoptera littoralis* [41] and *Helicoverpa armigera* [11]. Therefore, we inferred that the success rates of functional annotation of genes highly depended on the sequence length of the splicing unigene: the shorter the length of the sequence, the less possibility of the annotation. Others reasons might also result in partial information failure, such as the
incompleteness of *P. interpunctella* gene transcription group information, and/or the insufficiency of the sequence of partial RNA-Seq sequencing data in public database.

Olfactory-related genes might be used as potential targets for management programs of *P. interpunctella*. As the first step of odor detection [6], OBPs have attracted wide interests of researchers [13, 17, 42]. In the present study, we identified 29 PintOBP genes from antennal transcriptome of *P. interpunctella*. The number of identified PintOBPs was equivalent to that from *H. armigera* (26) [11], *Dendrolimus kikuchii* (27) [17] and *Agrotis ipsilon* (33) [21], and it was significantly greater than that from *Cnaphalocrocis medinalis* (12) [12], *C. punctiferalis* (14) [13], *Manduca sexta* (18) [43] and *S. exigua* (11) [44]. The small number of OBPs in above species could be attributed to that the actual number of OBPs was less in *P. interpunctella*, or there should be more OBPs that were not caught by the sequencing. Therefore, we speculated that the transcriptomic sequencing might not be strong enough to detect all the OBPs, especially for some OBPs with low expression levels in the antennae [45].

The OBP trees from five Lepidopteran species indicated that after a long history evolution, the Lepidopteran OBPs differentiated into several branches (Fig 5), which was consistent with previous reports [46]. In the evolutionary tree for GOBPs and PBPs, these two sub-families were clustered respectively, indicating that these genes might have the same ancestor gene and differentiate along sex isolation and speciation. The qRT-PCR results indicated that nine PintOBP genes (PintOBP4, PintOBP6, PintOBP9, PintOBP13, PintOBP17 PintOBP20, PintOBP22 and PintPBP2-3) were significantly expressed in the male antennae, suggesting that these OBPs played essential roles in the detection of sex pheromones. Eight PintOBPs (PintOBP5, PintOBP7, PintOBP12, PintOBP15-16, PintOBP18, PintPBP1 and PintGOBP1) were significantly expressed in the female antennae, revealing that these OBPs played important roles in the detection of general odorants, such as host plant volatiles [21].

CSPs represent a newly-discovered class of soluble carrier proteins with similar functions to OBPs in insect chemoreception [47]. CSPs have been found in insect chemosensory tissues and non-chemosensory organs, such as antennae [11], legs [48], labial palps [49], tarsi [50], brain [51], proboscis [52], pheromone gland [53–54] and wings [55]. We identified 15 putative CSP encoding transcripts, and found that six PintCSP genes were significantly expressed in the female antennae. These PintCSPs might play important roles in the detection of general odorants, such as host plant volatiles.
OR proteins are key players in insect olfaction [56]. We identified 47 PintOR genes in antennal transcriptome of *P. interpunctella*. The number of PintORs identified in this study was less than that identified from the antennal transcriptomes of *Bombyx mori* (72) [57], *C. punctiferalis* (62) [58] and *Ostrinia furnacalis* (56) [59]. However, the difference in identified OR gene numbers might be caused by sequencing methods and depth, or sample preparation. In the neighbor-joining tree of ORs, four PintORs (PintOR5, PintOR7, PintOR22 and PintOR30) were clustered into the PR family, indicating that parts or all of them contributed to sex pheromone detection. The qRT-PCR results indicated that PintOR5 and PintOR22 were highly expressed in the male antennae, suggesting they are highly related to sex pheromone. PintOR7 and PintOR30 specifically expressed in the female antennae. The expression profiles of these sequences showed that not all of them were male-specific [60]. Recent studies also showed that some PR genes are expressed in both sexes [54]. The OR tree showed that the PintORco (PintOR1) was highly conserved.
In recent years, 12 HarmIRs in *H. armigera* [11], 17 SlitIRs in *S. littoralis* [61] and 15 Cpo-mlRs in *C. pomonella* [22] have been identified. In this study, we identified 14 PintIRs, including highly conserved IR co-receptors PintIR1 and PintIR2 (IR8a and IR21a) from antennal transcriptome of *P. interpunctella*. Therefore, we speculated that IRs were relatively highly conserved sequences, implying that IRs had conservative features.

Several recent reports simultaneously tested the qRT-PCR expression of olfactory-related genes in various tissues of insect, including bodies, heads, legs or abdomens [13–15, 20–21, 54]. In present study, we only focused on the qRT-PCR analysis of *P. interpunctella* antennae. To the best of our knowledge, *P. interpunctella* moths do not eat anything, suggesting they have no food demands, so location of mate partners and oviposition sites should be the main function of olfactory. While most olfactory genes related to recognition of pheromone and host volatiles distribute in insect antennae, therefore, we only compared the expression between female and male antennae of *P. interpunctella*, to verify the olfactory-related genes.

**Conclusion**

In this study, we identified a few olfactory gene families in antennal transcriptome of *P. interpunctella*, including 29 PintOBPs, 15 PintCSPs, three PintSNMPs, 47 PintORs, nine PintGRs and 14 PintIRs. The identification of antennal olfactory-related proteins in *P. interpunctella* reinforced our knowledge on the molecular and cellular basis of insect chemoreception. More importantly, our data suggested that new methods could be developed to control this pest by interfering their olfactory perception.

**Supporting information**

**S1 Table.** Primers used for RT-qPCR.

(DOCX)

**S2 Table.** Amino acid sequences of PintOBPs used in phylogenetic analyses.

(DOC)

**S3 Table.** Amino acid sequences of PintCSPs used in phylogenetic analyses.

(DOC)

**S4 Table.** Amino acid sequences of PintORs used in phylogenetic analyses.

(DOC)

**S5 Table.** Amino acid sequences of PintGRs used in phylogenetic analyses.

(DOC)

**S6 Table.** Amino acid sequences of PintIRs used in phylogenetic analyses.

(DOC)

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References
1. Mohandass S, Arthur FH, Zhu KY, Throne JE. Biology and management of Plodia interpunctella (Lepidoptera: Pyralidae) in stored products. J Stored Prod Res. 2007; 43(3): 302–311. https://doi.org/10.1016/j.jspr.2006.08.002
2. Razazzian S, Hassani MR, Imani S, Shoja M. Life table parameters of Plodia interpunctella (Lepidoptera: Pyralidae) on four commercial pistachio cultivars. J Asia Pac Entomol. 2015; 19(1): 55–59. https://doi.org/10.1016/j.aspen.2014.12.002
3. Olsson POC, Anderbrant O, Löfstedt C, Borg-Karlson AK, Libikas I. Electrophysiological and behavioral responses to chocolate volatiles in both sexes of the pyralid moths Ephestia cautella and Plodia interpunctella. J Chem Ecol. 2005; 31(12): 2947–2961. https://doi.org/10.1007/s10886-005-8406-z PMID: 16365716
4. Leal WS. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol. 2013; 58: 373–379. https://doi.org/10.1146/annurev-ento-120811-153635 PMID: 23020622
5. Liu Z, Smagge G, Lei ZR, Wang JJ. Identification of male- and female-specific olfaction genes in antennae of the oriental fruit fly (Bactrocera dorsalis). PloS ONE. 2016; 11(2): e0147783. https://doi.org/10.1371/journal.pone.0147783 PMID: 26845547
6. Pelosi P, Zhou JJ, Ban LP, Calvello M. Soluble proteins in insect chemical communication. Cell Mol Life Sci. 2006; 63(14): 1658–1676. https://doi.org/10.1007/s00018-005-5607-0 PMID: 16786224
7. Sato K, Touhara K. Insect olfaction: receptors, signal transduction, and behavior. Springer Berlin Heidelberg, 2008. pp. 203–220
8. Vogt RG, Riddiford LM. Pheromone binding and inactivation by moth antennae. Nature. 1981; 293: 161–163. PMID: 18074618
9. Leal WS. Pheromone reception. Top Curr Chem. 2005; 240:1–36. https://doi.org/10.1007/b98314
10. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011; 29: 644–652. https://doi.org/10.1038/nbt.1883 PMID: 21572440
11. Liu Y, Gu SH, Zhang YJ, Guo YY, Wang GR. Candidate olfaction genes identified within the Helicoverpa armigera antennal transcriptome. PloS ONE. 2012; 7(10): e48260. https://doi.org/10.1371/journal.pone.0048260 PMID: 23110222
12. Liu S, Wang WL, Zhang YX, Zhang BX, Rao XJ, Liu XM, et al. Transcriptome sequencing reveals abundant olfactory genes in the antennae of the rice leaffolder, Cnaphalocrocis medinalis (Lepidoptera: Pyralidae). Entomol Sci. 2017; 20(1): 177–188. https://doi.org/10.1111/ens.12253
13. Jia XJ, Wang HX, Yan ZG, Zhang MZ, Wei CH, Qin XC, et al. Antennal transcriptome and differential expression of olfactory genes in the yellow peach moth, Conogethes punctiferalis (Lepidoptera: Crambidae). Sci Rep. 2016; 6: 29067. https://doi.org/10.1038/srep29067 PMID: 27364081
14. Zhang J, Wang B, Dong SL, Cao DP, Dong JF, Walker WB, et al. Antennal transcriptome analysis and comparison of chemosensory gene families in two closely related noctuidae moths, Helicoverpa armigera and H. assulta. PloS ONE. 2015; 10(2), e0117054. https://doi.org/10.1371/journal.pone.0117054 PMID: 25659090
15. Cao DP, Liu Y, Wei JJ, Liao XY, Walker WB, Li JH, et al. Identification of candidate olfactory genes in *Chilo suppressalis* by antennal transcriptome analysis. Int J Biol Sci. 2014; 10(8), 846. https://doi.org/10.7150/ijbs.9297 PMID: 25078661

16. Chang XQ, Nie XP, Zhang Z, Zeng FF, Lv L, Zhang S, et al. De novo analysis of the oriental armyworm *Mythimna separata* antennal transcriptome and expression patterns of odorant-binding proteins. Comp Biochem Phys D: Genomics and Proteomics. 2017; 22, 120–130. https://doi.org/10.1016/j.cbd.2017.03.001 PMID: 28395238

17. Zhang SF, Zhang Z, Wang HB, Kong XB. Antennal transcriptome analysis and comparison of olfactory genes in two sympatric defoliators, *Dendrolimus houi* and *Dendrolimus kikuchii* (Lepidoptera: Lasiocampidae). Insect Biochem Molec. 2014; 52, 69–81. https://doi.org/10.1016/j.ibmb.2014.06.006 PMID: 24998398

18. Zhang LW, Kang K, Jiang SC, Zhang YN, Wang TT, Zhang J, et al. Analysis of the antennal transcriptome and insights into olfactory genes in *Hyphantria cunea* (Drury). PloS ONE. 2016; 11(10), e0164729. https://doi.org/10.1371/journal.pone.0164729 PMID: 27741288

19. Zhang TT, Coates BS, Ge X, Bai SX, He KL, Wang ZY. Male- and female-biased gene expression of olfactory-related genes in the antennae of Asian corn borer, *Ostrinia furnacalis* (Gueneé)(Lepidoptera: Crambidae). PloS ONE. 2015; 10(6), e0128550. https://doi.org/10.1371/journal.pone.0128550 PMID: 26062030

20. Li GW, Du J, Li YP, Wu JX. Identification of putative olfactory genes from the oriental fruit moth *Grapholita molesta* via an antennal transcriptome analysis. PloS ONE. 2015; 10(11), e0142193. https://doi.org/10.1371/journal.pone.0142193 PMID: 26540284

21. Gu SH, Sun L, Yang RN, Wu KM, Guo YY, Li XC, et al. Molecular characterization and differential expression of odorant receptor genes in the antennae of the black cutworm moth *Agrotis ipsilon*. PloS ONE. 2014; 9(8), e103420. https://doi.org/10.1371/journal.pone.0103420 PMID: 25837076

22. Bengtsson JM, Trona F, Montagne N, Anfora G, Ignell R, Witzgall P, et al. Putative chemosensory receptors of the coding moth, *Cydia pomonella*, identified by antennal transcriptome analysis. PloS ONE. 2012; 7(2), e31620. https://doi.org/10.1371/journal.pone.0031620 PMID: 22363688

23. Harrison PW, Manik JE, Wedel N. Incomplete sex chromosome dosage compensation in the Indian meal moth, *Plodia interpunctella*, based on de novo transcriptome assembly. Genome Biol Evol. 2012; 4(11), 1118–1126. https://doi.org/10.1093/gbe/evs086 PMID: 23034217

24. McTaggart SJ, Hannah T, Bridgett S, Garbutt JS, Kaur G, Boots M. Novel insights into the insect transcriptome response to a natural DNA virus. BMC Genomics. 2015; 16; 310. https://doi.org/10.1186/s12864-015-1499-z PMID: 26062030

25. Tang PA, Wu HJ, Xue H, Ju XR, Song W, Zhang QL, et al. Characterization of transcriptome in the Indian meal moth *Plodia interpunctella* (Lepidoptera: Pyralidae) and gene expression analysis during development stages. Gene. 2017; 622: 29–41. https://doi.org/10.1016/j.gene.2017.04.018 PMID: 28412460

26. Mitchell RF, Hughes DT, Luetje CW, Millar JG, Soriano-Agaton F, Hanks LM, et al. Sequencing and characterization of odorant receptors of the cerambycide beetle Megacyllene caryae. Insect Biochem Mol Biol. 2012; 42(7): 499–505. https://doi.org/10.1016/j.ibmb.2012.03.007 PMID: 22504490

27. Conesa A, Gött S, García-Gómez JM, Terol J, Talón M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 2005; 21(18): 3674–3676. https://doi.org/10.1093/bioinformatics/bti610 PMID: 16381474

28. Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. 2000; 28(1): 33–36. https://doi.org/10.1093/nar/28.1.33 PMID: 10592175

29. Kanelhisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Res. 2008; 36: D480–D484. https://doi.org/10.1093/nar/gkm882 PMID: 18077471

30. Huang OY, Sun PD, Zhou XG, Lei CL. Characterization of head transcriptome and analysis of gene expression involved in caste differentiation and aggression in *Odontotermes formosanus* (Shiraki). PloS ONE. 2012; 7(11): e50383. https://doi.org/10.1371/journal.pone.0050383 PMID: 23209730

31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011; 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121 PMID: 21546353

32. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008; 5: 621–628. https://doi.org/10.1038/nmeth.1226 PMID: 18516045

33. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001; 25(4): 402–408. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609
34. Feng B, Guo QS, Zheng KD, Qin YX, Du YJ. Antennal transcriptome analysis of the piercing moth *Orae sia emarginata* (Lepidoptera: Noctuidae). PloS ONE. 2017; 12(6): e0179433. https://doi.org/10.1371/journal.pone.0179433 PMID: 28614384

35. Hekmat-Scafe DS, Scafe CR, Mckinney AJ, Tanouye MA. Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. Genome Res. 2002; 12(9): 1357–1369. https://doi.org/10.1101/gr.239402 PMID: 12213773

36. Xu YL, He P, Zhang L, Fang SQ, Dong SL, Zhang YJ, et al. Large-scale identification of odorant-binding proteins and chemosensory proteins from expressed sequence tags in insects. BMC Genomics. 2009; 10: 632. https://doi.org/10.1186/1471-2164-10-632 PMID: 20034407

37. Pitts RJ, Rinker DC, Jones PL, Rokas A, Zwiebel LJ. Transcriptome profiling of chemosensory appendages in the malaria vector *Anopheles gambiae* reveals tissue-and sex-specific signatures of odor coding. BMC Genomics. 2011; 12: 271. https://doi.org/10.1186/1471-2164-12-271 PMID: 21619637

38. Mamidala P, Wijeratne AJ, Wijeratne S, Poland T, Qazi SS, Cusson M, et al. Identification of odor-processing genes in the emerald ash borer, *Agrilus planipennis*. PloS ONE. 2013; 8(2): e56555. https://doi.org/10.1371/journal.pone.0056555 PMID: 23424668

39. Gu SH, Wu KM, Guo YY, Field LM, Pickett JA, Field LM, et al. Identification and expression profiling of odorant binding proteins and chemosensory proteins between two wingless morphs and a winged morph of the cotton aphid *Aphis gossypii* Glover. PloS ONE. 2013; 8(9): e73524. https://doi.org/10.1371/journal.pone.0073524 PMID: 24073197

40. Leitch O, Papanicolaou A, Lennard C, Kirkbride KP, Anderson A. Chemosensory genes identified in the antennal transcriptome of the blow fly *Calliphora stygia*. BMC Genomics. 2015; 16: 255. https://doi.org/10.1186/s12864-015-1466-8 PMID: 25880816

41. Jacquin-Joly E, Legaë F, Montagne N, Monsempes C, Francois MC, Poulain J, et al. Candidate chemosensory genes in female antennae of the noctuid moth *Spodoptera littoralis*. Int J Biol Sci. 2012; 8(7): 1036–1050. https://doi.org/10.7150/ijbfs.4469 PMID: 22904672

42. Gu SH, Zhou JJ, Gao S, Wang DH, Li XC, Guo YY, et al. Identification and comparative expression analysis of odorant binding protein genes in the tobacco cutworm *Spodoptera littoralis*. Sci Rep. 2015; 5: 13800. https://doi.org/10.1038/srep13800 PMID: 26346731

43. Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wiché D, Hansson BS. Antennal transcriptome of *Manduca sexta*. Proc Natl Acad Sci. 2011; 108(18): 7449–7454. https://doi.org/10.1073/pnas.1017963108 PMID: 21498690

44. Zhu JY, Zhang LF, Ze SZ, Wang DW, Yang B. Identification and tissue distribution of odorant binding protein genes in the beet armyworm, *Spodoptera exigua*. J Insect Physiol. 2013; 59(7): 722–728. https://doi.org/10.1016/j.jinsphys.2013.02.011 PMID: 23499610

45. Wanner KW, Isman MB, Feng Q, Plettner E, Theilmann DA. Developmental expression patterns of four chemosensory protein genes from the Eastern spruce budworm, *Chrostionera fumiferana*. Insect Mol Biol. 2005; 14(3): 289–300. https://doi.org/10.1111/j.1365-2583.2005.00559.x PMID: 15926898

46. Liu NY, Liu CC, Dong SL. Functional differentiation of pheromone-binding proteins in the common cutworm *Spodoptera litura*. Comp Biochem Physiol A Mol Integr Physiol. 2013; 165(2): 254–262. https://doi.org/10.1016/j.cbpa.2013.03.016 PMID: 23507568

47. Wanner KW, Willis LG, Theilmann DA, Isman MB, Feng QL, Plettner E. Analysis of the insect os-d-like gene family. J Chem Ecol. 2004; 30: 889–911. https://doi.org/10.1023/B:JOEC.0000028457.51147.d4 PMID: 15274438

48. Picimon B-FJ, Dietrich K, Krieger J, Breier H. Identity and expression pattern of chemosensory proteins in *Heliothis virescens* (Lepidoptera, Noctuidae). Insect Biochem Mol Biol. 2001; 31(12): 1173–1181. https://doi.org/10.1016/S0965-1748(01)00063-7 PMID: 11583930

49. Maleszka R, Stange G. Molecular cloning, by a novel approach, of a cDNA encoding a putative olfactory protein in the labial palps of the moth *Cactoblastis cactorum*. Gene. 1997; 202(1–2): 39–43. https://doi.org/10.1016/S0378-1119(97)00448-4 PMID: 9427543

50. Angell S, Ceron F, Scailor A, Monti M, Monteforti G, Min-nocci A, et al. Purification, structural characterization, cloning and immunocytochemical localization of chemoreception proteins from *Schistocerca gregaria*. The FEBS Journal. 1999; 262(3): 745–754. https://doi.org/10.1046/j.1432-1327.1999.00438.x

51. Whittfield CW, Band MR, Bonaldo MF, Kumar CG, Liu L, Pardinas J, et al. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behaviours in the honey bee. Genome Res. 2002; 12:555–566. https://doi.org/10.1101/gr.5302 PMID: 11932240

52. Nagman-Le Meillour P, Cain AH, Jacquin-Joly E, Francois MC, Ramachandran S, Maida R, et al. Chemosensory proteins from the proboscis of *Mamestra brassicae*. Chem Senses. 2000; 25(5):541–553. https://doi.org/10.1093/chemse/25.5.541 PMID: 11015326
53. Dani FR, Michelucci E, Francese S, Mastrobuoni G, Cap-pellozza S, La Marca Get al. Odorant-binding proteins and chemosensory proteins in pheromone detection and release in the silkmoth Bombyx mori. Chem Senses. 2011; 36(4): 335–344. https://doi.org/10.1093/chemse/bjq137 PMID: 21220518

54. Wei HS, Li KB, Zhang S, Cao YZ, Yin J. Identification of candidate chemosensory genes by transcriptome analysis in Loxostege sticticalis Linnaeus. PloS ONE. 2017; 12(4): e0174036. https://doi.org/10.1371/journal.pone.0174036 PMID: 28423037

55. Ban L, Scaloni A, Brandazza A, Angeli S, Zhang L, Yan Y, et al. Chemosensory proteins of Locusta migratoria. Insect Mol Biol. 2003; 12(2): 125–134. https://doi.org/10.1046/j.1365-2583.2003.00394.x PMID: 12653934

56. Slone JD, Pask GM, Ferguson ST, Millar JG, Berger SL, Reinberg D, et al. Functional characterization of odorant receptors in the ponerine ant, Harpegnathos saltator. P Natl Acad Sci. 2017; 114(2): 8586–8591. https://doi.org/10.1073/pnas.1704647114 PMID: 28696298

57. Gong DP, Zhang HJ, Zhao P, Xia QY, Xiang ZH. The odorant binding protein gene family from the genome of silkworm, Bombyx mori. BMC Genomics. 2009; 10: 332. https://doi.org/10.1186/1471-2164-10-332 PMID: 19624863

58. Ge X, Zhang TT, Wang ZY, He KL, Bai SX. Identification of putative chemosensory receptor genes from yellow peach moth Conogethes punctiferalis (Gueneé) antennae transcriptome. Sci Rep. 2016; 6: 32636. https://doi.org/10.1038/srep32636 PMID: 27659493

59. Yang B, Ozaki K, Ishikawa Y, Matsuo T. Identification of candidate odorant receptors in asian corn borer Ostrinia furnacalis. PloS ONE. 2015; 10(3): e0121261. https://doi.org/10.1371/journal.pone.0121261 PMID: 25803580

60. Koenig C, Hirsh A, Bucks S, Klinner C, Vogel H, Shukla A, et al. A reference gene set for chemosensory receptor genes of Manduca sexta. Insect Biochem Mol Biol. 2015; 66: 51–63. https://doi.org/10.1016/j.ibmb.2015.09.007 PMID: 26365739

61. Poivet E, Gallot A, Montagne N, Glaser N, Legael F, Jacquin-Joly E. A Comparison of the olfactory gene repertoires of adults and larvae in the Noctuid moth Spodoptera littoralis. PloS ONE. 2013; 8(3): e60263. https://doi.org/10.1371/journal.pone.0060263 PMID: 23956215