



The Mouthparts Enriched Odorant Binding Protein 11 of the Alfalfa Plant Bug *Adelphocoris lineolatus* Displays a Preferential Binding Behavior to Host Plant Secondary Metabolites

Liang Sun^{1,2†}, Yu Wei^{2†}, Dan-Dan Zhang³, Xiao-Yu Ma², Yong Xiao², Ya-Nan Zhang⁴, Xian-Ming Yang², Qiang Xiao¹, Yu-Yuan Guo² and Yong-Jun Zhang^{2*}

¹ Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China, ² State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, ³ Department of Biology, Lund University, Lund, Sweden, ⁴ College of Life Sciences, Huaibei Normal University, Huaibei, China

OPEN ACCESS

Edited by:

Sylvia Anton,
Institut National de la Recherche
Agronomique, France

Reviewed by:

Ewald Grosse-Wilde,
Max Planck Institute for Chemical
Ecology, Germany
William Benjamin Walker,
Swedish University of Agricultural
Sciences, Sweden

*Correspondence:

Yong-Jun Zhang
yjzhang@ippcaas.cn

[†]These authors have contributed
equally to this work.

Specialty section:

This article was submitted to
Invertebrate Physiology,
a section of the journal
Frontiers in Physiology

Received: 01 February 2016

Accepted: 17 May 2016

Published: 01 June 2016

Citation:

Sun L, Wei Y, Zhang D-D, Ma X-Y,
Xiao Y, Zhang Y-N, Yang X-M, Xiao Q,
Guo Y-Y and Zhang Y-J (2016) The
Mouthparts Enriched Odorant Binding
Protein 11 of the Alfalfa Plant Bug
Adelphocoris lineolatus Displays a
Preferential Binding Behavior to Host
Plant Secondary Metabolites.
Front. Physiol. 7:2016.
doi: 10.3389/fphys.2016.00201

Odorant binding proteins (OBPs) are proposed to be directly required for odorant discrimination and represent potential interesting targets for pest control. In the notoriously agricultural pest *Adelphocoris lineolatus*, our previous functional investigation of highly expressed antennal OBPs clearly supported this viewpoint, whereas the findings of the current study by characterizing of AlinOBP11 rather indicated that OBP in hemipterous plant bugs might fulfill a different and tantalizing physiological role. The phylogenetic analysis uncovered that AlinOBP11 together with several homologous bug OBP proteins are potential orthologs, implying they could exhibit a conserved function. Next, the results of expression profiles solidly showed that *AlinOBP11* was predominantly expressed at adult mouthparts, the most important gustatory organ of Hemiptera mirid bug. Finally, a rigorously selective binding profile was observed in the fluorescence competitive binding assay, in which recombinant AlinOBP11 displayed much stronger binding abilities to non-volatile secondary metabolite compounds than the volatile odorants. These results reflect that *AlinOBP11*, even its orthologous proteins across bug species, could be associated with a distinctively conserved physiological role such as a crucial carrier for non-volatiles host secondary metabolites in gustatory system.

Keywords: *Adelphocoris lineolatus*, odorant binding protein, expression profiles, phylogenetic analysis, Fluorescence competitive binding assay

INTRODUCTION

Smell is undoubtedly the most important sensory for insects survival and reproduction (Li and Liberles, 2015; Groot et al., 2016). Olfactory system that can sensitively and selectively detect biologically active odorants attracts great attention from researchers who attempt to explore alternative environment-friendly pest management strategy. In insect olfactory signal transduction pathway, several classes of membrane-bound proteins such as odorant receptor (ORs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs) have been proven to play

central roles in facilitating the conversion of the chemical message to an electrical signal, while the carrier proteins like odorant binding proteins (OBPs) or chemosensory proteins (CSPs) are proposed to bind, deliver and even recognize specific pheromones and odorants to their relevant receptors (Jacquin-Joly and Merlin, 2004; Leal, 2013). For decades, various functional studies toward important olfactory protein families such as OBPs or ORs actually lead to a quick discovering of some high-efficiency pest repellents or attractants (Tanaka et al., 2009; Sun Y. F. et al., 2012; Sun L. et al., 2013). For instance, in the alfalfa plant bug, *A. lineolatus*, behavioral active compounds were successfully screened via ligand binding assay of an antennae highly expressed AlinOBP10 (Sun L. et al., 2013). Synthetic compounds targeting OBP3 or OBP7 which was proven to be responsible for (*E*)- β -farnesene perception elicited significantly behavioral responses in aphids (Sun Y. F. et al., 2012).

Insect OBPs which were first identified in antennal sensillum of silk moth, *Antheraea polyphemus* (Vogt and Riddiford, 1981) belong to the superfamily of small acidic soluble carrier proteins and could be recognized by six highly conserved cysteines (Leal et al., 1999; Sandler et al., 2000; Tegoni et al., 2004; Pelosi et al., 2014). Studies of both immunocytochemical localization and *in situ* hybridization revealed that OBPs were synthesized by non-neuronal auxiliary cells (trichogen and tormogen cells) and secreted into the sensillum lymph with a very high concentration (up to 10 mM) (Steinbrecht et al., 1995; Hekmat-Scafe et al., 1997; Michael, 2000; De Santis et al., 2006; Sun Y. P. et al., 2013; Sun et al., 2014a). So far, various investigations elucidated that antennal sensillum enriched OBPs indeed played essential roles in recognition of physiologically relevant odorants (Jacquin-Joly et al., 2000; Pophof, 2004; Große-Wilde et al., 2006; He et al., 2010). For example, one subclass of OBP families named pheromone binding proteins, PBPs, was highly abundant in long trichoid sensilla and showed significantly specific binding affinities to insect sex pheromones (Vogt and Riddiford, 1981; Krieger et al., 1996; Leal et al., 1999; Klusák et al., 2003; Pophof, 2004; Große-Wilde et al., 2006; De Santis et al., 2006). Sensilla basiconica expressed OBPs were proposed to be involved in terpenoids or other plant volatiles detection (Feng and Prestwich, 1997). Meanwhile, in two Lepidopteran species, the cotton leafworm *Spodoptera littoralis* (Poivet et al., 2012) and the diamondback moth *Plutella xylostella* (Zhu et al., 2016), OBPs were even demonstrated to be associated with the interesting behavior why larvae are attracted by conspecific moth sex pheromone.

However, the functions of insect OBPs may be more complicated and could not be restrict within olfactory cue recognition. In *Drosophila melanogaster*, most OBPs were detected in both gustatory and olfactory sensilla and some numbers were even expressed exclusively in taste organs (Galindo and Smith, 2001). Jeong et al. (2013) proposed that feeding behavior of *D. melanogaster* can be suppressed by a gustatory organ expressed OBP49a responding to bitter compounds. Two OBP genes, *Obp57d* and *Obp57e* in *D. sechellia* have been demonstrated to be involved in the evolution of taste perception and host-plant preference (Matsuo et al., 2007). Particularly, expressions of *Aedes aegypti* OBP22 in antennae

and reproductive organs indicated its multiple functions (Li et al., 2008). Likewise, physiological roles of male reproductive organs expressed orthologous OBP10 in two sibling moth species has been proposed to act as a specific carrier for female oviposition deterrents that could help *Helicoverpa* offspring avoid cannibalism (Sun Y. L. et al., 2012).

The alfalfa plant bug, *A. lineolatus*, a typical polyphagous insect pest outbreaks frequently in cotton field since the transgenic *Bacillus thuringiensis* cotton largely cultivation in China (Lu et al., 2010). Worse still, flight behavior enable it to migrate among different host plants (Lu et al., 2009), and many other important crops like alfalfa (*Medicago sativa* L.), green bean (*Phaseolus vulgaris*), and tea plant (*Camellia sinensis*) suffer from its serious destroy (Lu and Wu, 2008). Evidence suggested that this bug heavily relies on chemical cues for host plant location and migration (Lu and Wu, 2008). Thus, studies aiming at the physiological and molecular basis of insect chemosensation may help explore an alternatively effective pest control method.

Previously, 14 OBP transcripts of *A. lineolatus* were identified (Gu et al., 2011a) and functional studies of several antennae highly expressed OBPs such as AlinOBP1, AlinOBP5, AlinOBP10, and AlinOBP13 indicated their potential olfactory roles (Gu et al., 2011b; Sun L. et al., 2013; Wang et al., 2013; Sun et al., 2014a). Subsequently, Hull et al. (2014) identified 33 putative OBP transcripts in the tarnished plant bug, *Lygus lineolaris*, and suggested that several OBP genes included *LylinOBP19* can be expressed in gustatory organs, implying they may be related to taste compound detected. However, whether OBPs could express at taste organs and fulfill potential gustatory functions in *A. lineolatus* remains largely unknown. In the current study, we mainly focus our attention on *AlinOBP11*, a putative orthologous OBP gene of *LylinOBP19* in *A. lineolatus* and our current results of tissue distribution pattern, ligand binding assay, and phylogenetic analysis would provide detail cues for its functional discussion.

MATERIALS AND METHODS

Insect Rearing and Tissue Collection

A. lineolatus adults were collected from alfalfa fields at the Langfang Experimental Station of Chinese Academy of Agricultural Sciences, Hebei Province, China. The laboratory colony was established in plastic containers (20 × 13 × 8 cm), which were maintained at 29 ± 1°C, 60 ± 5% relative humidity, and 14 h light:10 h dark cycle. The adults and newly emerged nymphs were reared on green beans and 10% honey. Different tissues from *A. lineolatus* adults of both sexes including antennae, mouthparts, heads (without antennae and mouthparts), thoraxes, abdomens, legs, and wings were collected for qRT-PCR. Each tissue was collected from three biological pools and all the specimens were immediately stored in -80°C for further process.

RNA Isolation and cDNA Synthesis

Total RNA of each sample was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the first-strand cDNA was synthesized by FastQuant RT-kit with gDNA Eraser (TianGen, Beijing, China) according to the manufacturer's instructions.

qRT-PCR

qRT-PCR assay regarding different developmental stages and tissues were carried out using an ABI 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Two house-keeping genes *Alinβ-actin* (GenBank No.GQ477013) and *AlinElongation factor* (GenBank No.AEY99651) were used as endogenous controls to normalize the target gene expression and correct for sample-to-sample variation. Taqman primers of *Alinβ-actin* and *AlinOBP11* cited Gu et al. (2011a) and primers of *AlinElongation factor* were designed using Primer Express 3.0 (Applied Biosystems) and listed in **Table S1**. For the qRT-PCR reaction, the cDNA was diluted to concentration of 200 ng/μL. Each reaction was performed in a 25 μL mixture of 12.5 μL of Premix Ex Taq (TaKaRa), 1 μL of each primer (10 mM), 0.5 μL probe (10 mM), 0.5 μL of Rox Reference Dye II, 1 μL of sample cDNA (200 ng), and 8.5 μL of sterilized H₂O. Negative controls were non-template reactions (H₂O instead of cDNA). The reaction cycling parameters were as follows: 95°C for 10 s, 40 cycles at 95°C for 20 s, 60°C for 34 s. For the data reproducibility, qRT-PCR reaction for each sample was performed in three technical replicates and three biological replicates. Since our preliminary experiment demonstrated that the amplification efficiency between targeted genes and reference gene was similar (data not shown), the comparative $2^{-\Delta\Delta CT}$ method was used to calculate the relative quantification between tissues (Livak and Schmittgen, 2001).

The comparative analyses of target gene among different tissues and developmental stages were determined using a one-way nested analysis of variance (ANOVA), followed by Tukey's honestly significance difference (HSD) test using the software SPSS Statistics 18.0 (SPSS Inc., Chicago, IL, USA).

Phylogenetic Construction and Selective Pressure Analysis

The 92 OBP sequences of five mirid bug species (GenBank accession numbers and references can be seen in **Table S2**) were used to infer the evolutionary history with the software MEGA 6.0 with a *p*-distance model and a pairwise deletion of gaps (Tamura et al., 2013). The bootstrap support of tree branches was assessed by re-sampling amino acid positions 1000 times. Estimation of the non synonymous (dN) to synonymous (dS) substitution rate (ω) was performed by the maximum likelihood method (Anisimova et al., 2001) using the Codeml program in the PAML 4.6 package (Yang, 1997).

Western Blot Assay

The polyclonal antiserum against the recombinant AlinOBP11 was produced by injecting robust adult rabbits subcutaneously and intramuscularly with the highly purified recombinant protein. Recombinant protein was emulsified with an equal volume of Freund's complete adjuvant (Sigma, St. Louis, MO, USA) for the first time injection (500 μg) and then with incomplete adjuvant for the three additional injections (300 mg each time). The interval between each injection was approximately half a month, and blood was collected 7 days after the last injection and centrifuged at 6000 rpm for 20 min. The serum was purified based on a MAb Trap kit (GE Healthcare)

following the manufacturer's instructions. The rabbits were maintained in large cages at room temperature, and all of the operations were performed according to ethical guidelines to minimize the pain and discomfort of the animals.

Crude extracts from different tissues of female and male adult bugs included the antennae, mouthparts, legs, wings, and bodies (without aforesaid parts) were separated on 15% SDS-PAGE, respectively. Samples were transferred to a polyvinylidene fluoride membrane (PVDF, Millipore, Carrigtwohill, Ireland) at the condition of 200 mA for 50 min, and then membrane was blocked using 5% dry skimmed milk (BD Biosciences, San Jose, CA, USA) in phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBST) for 2 h at room temperature. After washing three times with PBST (10 min each time), the blocked membrane was incubated with purified rabbit anti-AlinOBP11 antiserum (dilution 1:2000) for 1 h. Three times washing with PBST again, the membrane was incubated with anti-rabbit IgG horseradish peroxidase (HRP) conjugate and HRP-streptavidin complex (Promega, Madison, WI, USA) at a dilution of 1:10000 for 1 h. The membrane was then incubated with the western blot substrates of the enhanced chemiluminescence western blot kit (CoWinbiotech, China), and the bands were visualized by exposing to X-OMATBT films (Kodak, New York, USA).

Fluorescence Competitive Binding Assay

The recombinant protein expression and purification was performed according to our previous protocols (Sun L. et al., 2013; Sun et al., 2014a). Briefly, the plasmid containing *AlinOBP11* gene was constructed and transformed into *Escherichia coli* BL21 (DE3) competent cells for recombinant protein expression, and the protein was largely induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37°C for 3–6 h. The purification was performed using two rounds of Ni ion affinity chromatography (GE-Healthcare), and the His-tag was removed with recombinant enterokinase (Novagen). The highly purified proteins were desalted through extensive dialysis, and then the size and purity of the recombinant proteins were verified by 15% SDS-PAGE.

For the ligand binding assays, 45 compounds include 41 volatiles and four non-volatiles were selected based on previously reported isolation from *A. lineolatus* host plants (Meisner et al., 1977; Halloin, 1982; Aldrich, 1988; Loughrin et al., 1995; Röse and Tumlinson, 2004; Millar, 2005). The binding assay was performed on an F-380 fluorescence spectrophotometer (Tianjin, China) at room temperature (25°C) with a 1-cm light path quartz cuvette and 10-nm slits for both excitation and emission. The excitation wavelength was 337 nm, and the emission spectrum was recorded between 390 and 460 nm. Firstly, the constant of AlinOBP11 with the fluorescent probe N-phenyl-1-naphthylamine (1-NPN) was measured, a final concentration of 2 μM protein solution in 50 mM Tris-HCl (pH 7.4) was titrated with aliquots of 1 mM 1-NPN dissolved in methanol to final concentrations ranging from 1 to 16 μM. Then the affinities of other ligands were tested through competitive binding assays using 1-NPN as the fluorescent reporter at a concentration of 2 μM, and the concentration of each competitor ranged from 2 to 30 μM. The fluorescence intensities at the

maximum fluorescence emission between 390 and 460 nm were plotted against the free ligand concentration to determine the binding constants. The bound chemical was evaluated based on its fluorescence intensity with the assumption that the protein was 100% active with a stoichiometry of 1:1 (protein: ligand) saturation. The binding curves were linearized using a Scatchard plot, and the dissociation constants of the competitors were calculated from the corresponding IC_{50} values based on the following equation: $K_i = [IC_{50}] / (1 + [1-NPN]/K_{1-NPN})$, where $[1-NPN]$ is the free concentration of 1-NPN and K_{1-NPN} is the dissociation constant of the complex protein/1-NPN.

RESULTS

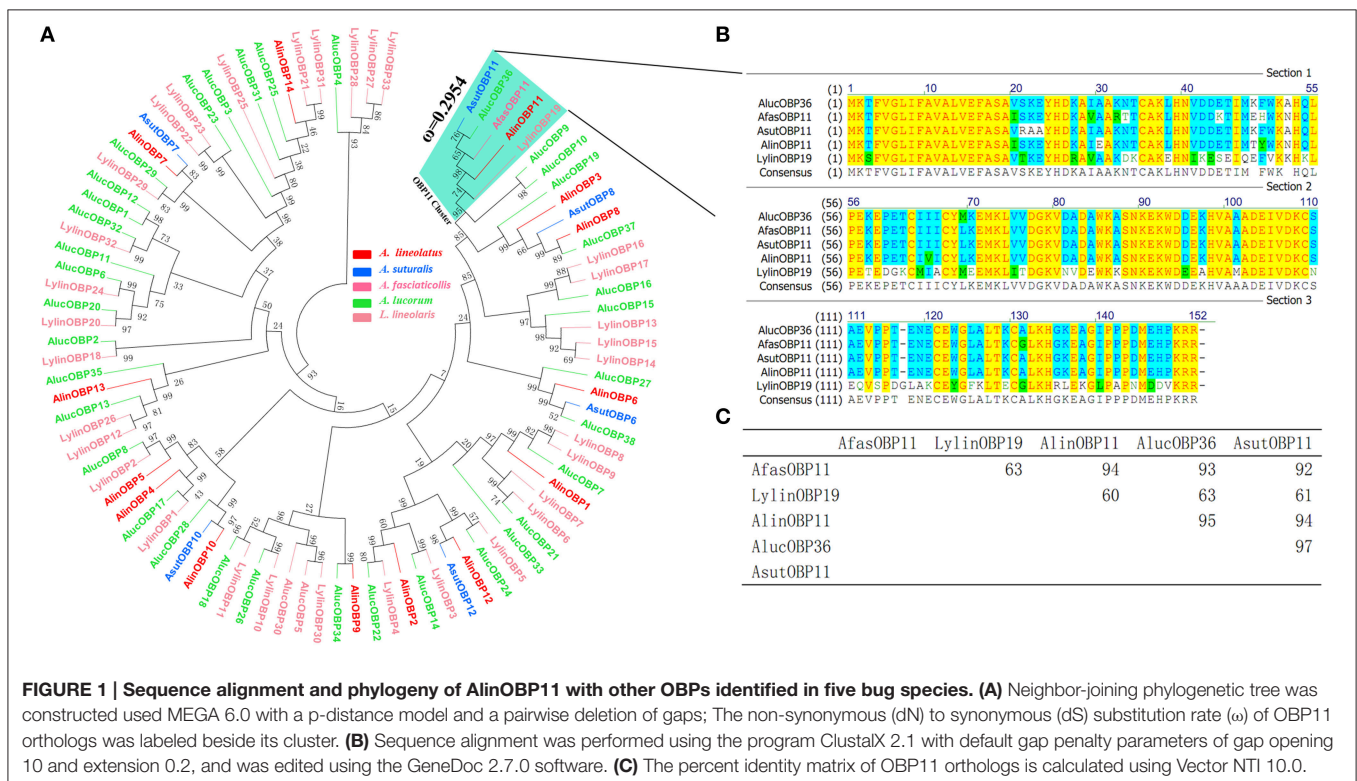
Phylogenetic Tree Construction and Selective Pressure Analysis

A phylogenetic tree of 92 OBPs was constructed using the neighbor-joining method to analyze evolutionary relationships between AlinOBP11 and other OBPs of different mirid species. **Figure 1** revealed a divergent OBP repertoire. AlinOBP11 and four other OBPs i.e., AlucOBP36, AfasOBP11, AsutOBP11, and LylinOBP19 from each bug species clustered into one same clade with bootstrap support value up to 74 (**Figure 1A**). Sequence alignment analysis showed AlinOBP11 has 94, 95, 94, and 60% identity to AsutOBP11, AlucOBP36, AfasOBP11, and LylinOBP19, respectively (**Figures 1B,C**). These results reflect AlinOBP11 and these four OBPs form a clear orthologous group across bug species.

To evaluate potential selective pressure acting on this *OBP11* orthologous cluster, we calculated the ratio of non synonymous to synonymous substitutions (dN/dS or ω) of this cluster with branch models using PAML and compared the log likelihoods ($\ln L$) for the one ratio model M0 (assuming one ω ratio for all branches) and the free ratio model M1 (assuming one ω ratio for each branch) in likelihood ratio tests. The results uncovered that the one ratio model (M0) could not be rejected ($p > 0.01$) and all branches shared a normalized ω ratio of 0.2954 (**Figure 1A**), implying that purifying selection was acting on this cluster and *AlinOBP11* would share a relatively conserved physiological function with its orthologous genes (Qiao et al., 2009; Zhou et al., 2010; Vandermoten et al., 2011).

Specific Tissue and Developmental Expression Profiles of *AlinOBP11*

The results of our western blot assay showed that clear protein bands could be found at mouthparts, legs, antennae as well as other tissues, which seems that *AlinOBP11* can be ubiquitously expressed at adult tissues of both sexes (**Figure 2**). To compare the expression levels of *AlinOBP11* among different tissues, we then conducted the qRT-PCR assay. Interestingly, unlike previously reported uniformly antennae predominant expressed *AlinOBPs* (Gu et al., 2011a; Sun L. et al., 2013; Sun et al., 2014a), our current results revealed that *AlinOBP11* was strongly expressed at mouthparts, and slightly expressed at legs, antennae, and other tissues (**Figure 3**). Meanwhile, *AlinOBP11* transcript abundance varied among different developmental instars and



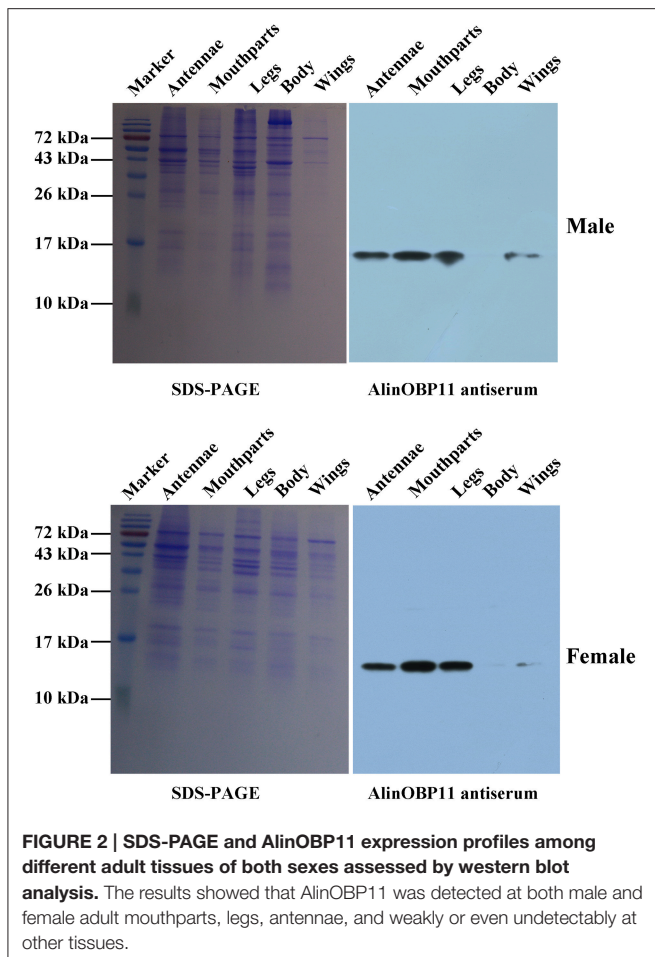


FIGURE 2 | SDS-PAGE and AlinOBP11 expression profiles among different adult tissues of both sexes assessed by western blot analysis. The results showed that AlinOBP11 was detected at both male and female adult mouthparts, legs, antennae, and weakly or even undetectably at other tissues.

significantly higher expression level was observed in adult bugs (Figure 3).

In vitro Expression and Purification of AlinOBP11

The recombinant AlinOBP11 was successfully expressed using a bacterial system. Induced targeted recombinant appeared at both supernatant and insoluble inclusion bodies and the former was selected to be purified using two rounds of Ni ion affinity chromatography (GE Healthcare, Little Chalfont, UK). The finally purified AlinOBP11 recombinant protein on the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis displayed a single band (Figure S1).

Ligand-Binding of Recombinant AlinOBP11

Before the ligand-binding analysis, we measured the binding affinities of fluorescence probe 1-NPN with purified AlinOBP11. The results showed AlinOBP11 could solidly bind to 1-NPN with binding affinity of $5.86 \pm 0.47 \mu\text{M}$ (Figure 4A). Consequently, the binding properties of AlinOBP11 to compounds with different functional groups were analyzed and the results suggested it had a relatively narrow binding profile. Notably, all the tested non-volatile compounds showed strong binding

abilities to AlinOBP11, and quercetin was the best ligand ($K_i = 2.63 \pm 0.23 \mu\text{M}$), followed by gossypol ($K_i = 3.43 \pm 0.32 \mu\text{M}$), rutin hydrate ($K_i = 7.78 \pm 1.23 \mu\text{M}$), and (-)-catechin ($K_i = 15.26 \pm 0.70 \mu\text{M}$). Additionally, the tested host volatiles such as aliphatic alcohols, aldehydes, ketones, esters, aromatics could hardly bind to recombinant AlinOBP11, except of three terpenoids α -phellandrene, nerolidol, and *trans, trans*-farnesol, which can bind to AlinOBP11 and their binding constant K_i was 20.07 ± 0.41 , 20.76 ± 0.55 , and $19.26 \pm 1.78 \mu\text{M}$, respectively (Figures 4B,C; Table 1).

DISCUSSION

Insect OBPs may serve as important molecular target for designing and screening new effectively behavioral blocking agents used in the application of eco-friendly pest management strategies as they are considered to be strongly expressed in antennal sensillum lymph and are involved in olfactory cues discrimination, binding and transduction (Qiao et al., 2009; He et al., 2011; Sun Y. F. et al., 2012; Pelosi et al., 2013, 2014; Sun L. et al., 2013; Sun et al., 2014a). However, a plenty of studies suggested that OBPs' expression patterns are not restricted in olfactory organs and thus their physiological functions would be more complex and diversified (Park et al., 2000; Foret and Maleszka, 2006; Li et al., 2008; Sun Y. F. et al., 2012; Yuan et al., 2015). To confirm whether OBPs in the Hemiptera mirid bug species could fulfill putative gustatory function, in the present study we especially focus on a putative non-olfactory organ biased OBP gene, the *OBP11* in *A. lineolatus*.

Previously, Gu et al. identified 14 putative OBP genes from the antennal cDNA library of *A. lineolatus* and suggested *AlinOBP11* was strongly expressed at adult legs of both sexes (Gu et al., 2011a). Subsequently, a large number of potential OBP genes were identified in the tarnished plant bug, *L. lineolaris* and the green plant bug, *Apolygus lucorum* via transcriptome strategy, and more OBP transcripts were found to be expressed at gustatory organs such as legs and mouthparts (Hull et al., 2014; Yuan et al., 2015). Therefore, we firstly re-confirmed the tissue expression profiles of *AlinOBP11* after taking mouthparts into account, the most important gustatory organs of Hemiptera species. The results of our western blot analysis revealed that clear single bands could be seen at mouthparts, legs as well as antennae of both male and female adult bugs (Figure 2). Interestingly, we found that relative mRNA level of *AlinOBP11* was extraordinarily higher at adult mouthparts of both sexes than that of previous reported legs and other tissues (Figure 3). In addition, higher expression level was also observed in adult bugs than different instars of nymph (Figure 3). If we considered the tissue distribution patterns of all the 14 identified OBP genes, according to the inference that mRNA expression is indicative of physiological function of its encoded protein, a putative functional subdivision of different OBP genes in the same species of *A. lineolatus* would occur and the mouthparts-biased *AlinOBP11* could be separated from other OBPs such as *AlinOBP1*, *10*, *13* which have been demonstrated strongly

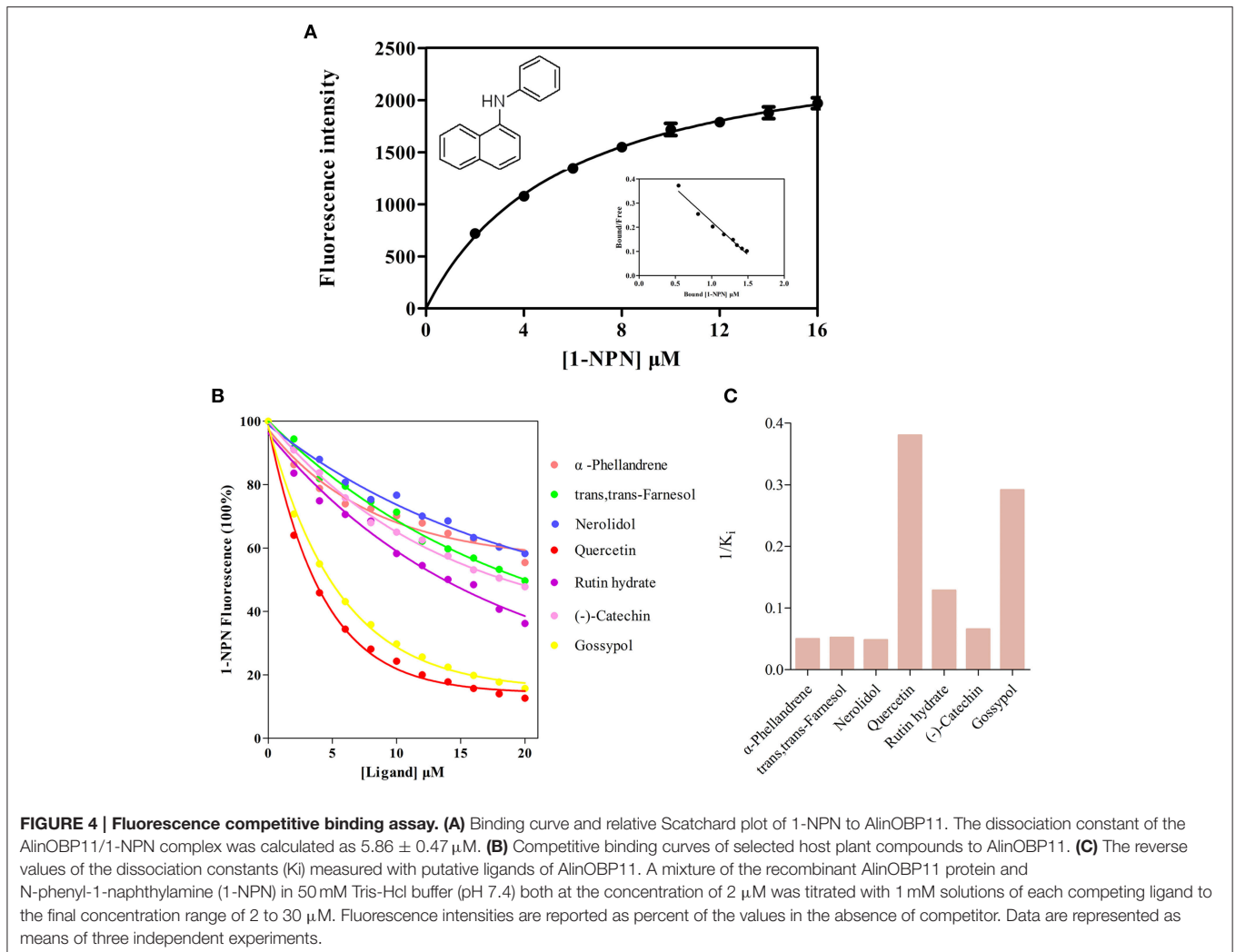
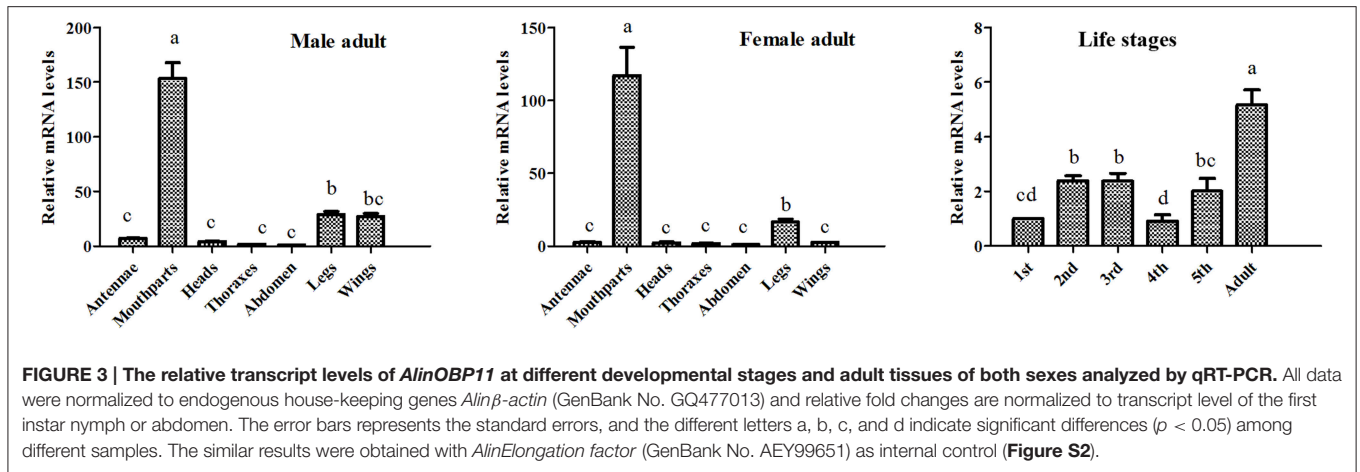


TABLE 1 | Binding affinities of all of the selected compounds to the recombinant AlinOBP11 protein.

Ligand	CAS Number	AlinOBP11	
		IC ₅₀ (μM)	K _i (μM)
GENERAL ODORANTS			
2-Hexanol	626-93-7	u.d.	u.d.
Pentanol	71-41-0	u.d.	u.d.
Valeraldehyde	110-62-3	u.d.	u.d.
Hexanal	66-25-1	u.d.	u.d.
Heptanal	111-71-7	u.d.	u.d.
Octanal	124-13-0	u.d.	u.d.
Nonanal	124-19-6	u.d.	u.d.
2-Hexanone	591-78-6	u.d.	u.d.
2-Heptanone	110-43-0	u.d.	u.d.
2-Octanone	111-13-7	u.d.	u.d.
3-Hexanone	589-38-8	u.d.	u.d.
6-Methyl-5-hepten-2-one	110-93-0	u.d.	u.d.
Amyl acetate	628-637-7	u.d.	u.d.
Nonyl acetate	1143-13-5	u.d.	u.d.
Undecane	1120-21-4	u.d.	u.d.
Indole	120-72-9	u.d.	u.d.
Benzaldehyde	100-52-7	u.d.	u.d.
3,4-Dimethyl-benzaldehyde	5973-71-7	u.d.	u.d.
Acetophenone	98-86-2	u.d.	u.d.
Methyl salicylate	119-36-8	u.d.	u.d.
GREEN LEAF VOLATILES			
1-Hexanol	111-27-3	u.d.	u.d.
<i>cis</i> -3-Hexen-1-ol	928-96-1	u.d.	u.d.
<i>trans</i> -2-Hexenal	6278-26-3	u.d.	u.d.
<i>cis</i> -3-hexenyl acetate	3681-71-8	u.d.	u.d.
TERPENOIDS			
<i>E</i> -β-Ocimene	3016-19-1	u.d.	u.d.
Limonene	5989-27-5	u.d.	u.d.
α-Phellandrene	99-83-2	26.16 ± 0.52	20.07 ± 0.41
β-Pinene	18172-67-3	u.d.	u.d.
(+)-α-Pinene	7785-70-8	u.d.	u.d.
β-Ionone	79-77-6	u.d.	u.d.
Myrcene	123-35-3	u.d.	u.d.
Nerolidol	7212-44-4	27.23 ± 0.71	20.76 ± 0.55
β-Caryophyllene	87-44-5	u.d.	u.d.
α-Humulene	6753-98-6	u.d.	u.d.
<i>trans</i> -β-Farnesene	18794-84-8	u.d.	u.d.
<i>trans,trans</i> -Farnesol	106-28-5	25.21 ± 2.36	19.26 ± 1.78
PUTATIVE SEX PHEROMONES			
Hexyl butyrate	2639-63-6	u.d.	u.d.
Hexyl hexanoate	6378-65-0	u.d.	u.d.
Butyl butyrate	109-21-7	u.d.	u.d.
Ethyl butyrate	105-54-4	u.d.	u.d.
<i>trans</i> -2-hexenyl butyrate	53398-83-7	u.d.	u.d.
HOST PLANT SECONDARY METABOLITES			
(-)-Catechin	18829-704	19.26 ± 0.93	15.26 ± 0.70
Rutin hydrate	207671-50-9	9.89 ± 1.61	7.78 ± 1.23
Quercetin	117-39-5	3.36 ± 0.30	2.63 ± 0.23
Gossypol	303-45-7	4.45 ± 0.34	3.43 ± 0.32

U.d. means that the IC₅₀ value exceeds 30 μM and thus that the binding affinities (K_i) of the candidate competitive ligand were not calculated in this study.

expressed at antennae sensillum and fulfilled vital roles in bug olfactory cue perception (Gu et al., 2011b; Sun L. et al., 2013; Sun et al., 2014a). Indeed, unlike the antennae which are equipped with various olfactory sensilla (Chinta et al., 1997; Sun et al., 2014b), mouthparts of Hemiptera bug species consist of piercing-sucking stylets and labium, the former is used to eject saliva for food ingestion and is considered to be directly related to oviposition behavior (Romani et al., 2005), while the latter has 11–12 uniporous gustatory sensilla which are responsible for assessing the suitability of food substrates (Ave et al., 1978; Hatfield and Frazier, 1980). Our current results of tissue expression pattern merely confirmed that *AlinOBP11* was preferentially expressed at *A. lineolatus* adult mouthparts. Although it is not known whether *AlinOBP11* was expressed in stylets or the gustatory sensillum of labium, we can conceivably speculate that this provocatively specific expression profile would benefit the alfalfa plant bugs, to a great extent, in many importantly behavioral performances such as egg laying, host plants selection, and even of toxic substances avoidance.

Our fluorescence competition assay provides further insight into understanding of physiological roles of *AlinOBP11*. The results clearly showed that recombinant *AlinOBP11* protein displayed preferential binding abilities to tested non-volatile host plant secondary metabolites than all the volatile compounds (Figures 4B,C; Table 1). These results correspond well to its specific tissue distribution within mouthparts which could give a functional implication that *AlinOBP11* could function as carrier in gustatory system for non-volatile compounds detection when plant bugs begin to search suitable food substrates by using the mouthparts to rub or tap on plant surfaces or insert plant tissues. Additionally, plant secondary compounds play key roles in the long-term evolution of plant-herbivore interactions (Elsayed, 2011; Mithöfer and Boland, 2012), and the content level variation of quercetin, gossypol, and rutin hydrate, three *AlinOBP11* best ligands, over the course of host plant maturation have been demonstrated to be involved in herbivore defense. In particular, gossypol and rutin hydrate were proposed to increase the resistance of cotton plants in response to mirid bug feeding, while the content of quercetin in cotton tended to perform a negatively correlation between their interactions (Lin et al., 2011). Thus, *A. lineolatus* might employ the mouthparts-biased expressed *AlinOBP11* to perceive and discriminate these functional different non-volatile secondary metabolites; however, this speculation still needs to be supported by more evidences.

Gene duplication was pointed to be the main mechanism underlying the fast expansion and functional evolution of chemosensory genes (Zhou et al., 2010; Zhang and Löfstedt, 2013); nevertheless, physiological functions of putative orthologs also attract great interest. In aphid species, the distribution of orthologous *OBP* genes may reflect their life styles and host relationships. As an example, homologous *OBP3* proteins of different aphid species were proved to be associated with recognition of alarm pheromone (*E*)-β-farnesene (Qiao et al., 2009; Vandermoten et al., 2011; Sun Y. F. et al., 2012). We reconstructed the phylogenetic trees used reported OBPs of several bug species (Figure 1) and the results clearly suggest *AlinOBP11* and *AsutoBP11*, *AlucOBP36*, *AfasOBP11*, and *LylinOBP19* fall

into the same clade and support they are potential orthologs across bug species which was consistent with Hull's assumption (Hull et al., 2014). Selective pressure assess by calculation of dN/dS or $\omega = 0.295$ (Figure 1) also indicates that genes in this cluster are under purifying selection and would perform conserved functions (Qiao et al., 2009; Zhou et al., 2010; Vandermoten et al., 2011). Meanwhile, we found the *AlinOBP11* was predominately expressed at mouthparts similar to tissue expression profiles of previous reported *LylinOBP19* (Hull et al., 2014) and our further studies of *AfasOBP11* and *AsutOBP11* (data not shown here). However, Hua et al. (2012) suggested that *AlucOBP36* (named as *AlucOBP3* in their study) was antennae-biased expressed. This could be explained by different genetic relationships and evolutionary processes of these bugs. *A. lineolatus*, *A. suturalis*, and *A. fasciaticollis* belong to the same genus *Adelphocoris*, while *A. lucorum* belongs to the other genus *Apolygus*. Notably, the *in vitro* functional studies of antennae expressed *AlucOBP36* resembled our results of *AlinOBP11*, which also showed better binding abilities to non-volatile host plant secondary compounds of rutin hydrate, but not to quercetin and gossypol (Hua et al., 2012), and this could be attributed to the mutations of several amino acids in these two proteins' binding pockets.

In conclusion, this study characterizes a mouthparts enriched *OBP11* protein in *A. lineolatus* which preferentially binds to non-volatile plant secondary compounds; to our current knowledge, *AlinOBP11* represents the first physiological function of mouthparts highly expressed OBP in Hemiptera species. As putative orthologous genes probably exhibited conserved physiological function, orthologous *OBP11* could be involved in mirid bug feeding behaviors and serve as potential molecular targets for the development of eco-friendly pest management strategies against mirid bugs' outbreaks.

REFERENCES

- Aldrich, J. R. (1988). Chemical ecology of the Heteroptera. *Annu. Rev. Entomol.* 33, 211–238. doi: 10.1146/annurev.en.33.010188.001235
- Anisimova, M., Bielawski, J. P., and Yang, Z. H. (2001). Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution. *Mol. Biol. Evol.* 18, 1585–1592. doi: 10.1093/oxfordjournals.molbev.a003945
- Ave, D., Frazier, J. L., and Hatfield, L. D. (1978). Contact chemoreception in the tarnished plant bug *Lygus lineolaris*. *Entomol. Exp. Appl.* 24, 217–227. doi: 10.1111/j.1570-7458.1978.tb02776.x
- Chinta, S., Dickens, J. C., and Baker, G. T. (1997). Morphology and distribution of antennal sensilla of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae). *Inter. J. Insect Morphol. Embryol.* 26, 21–26. doi: 10.1016/S0020-7322(96)00022-0
- De Santis, F., Francois, M.-C., Merlin, C., Pelletier, J., Maibeche-Coisne, M., Conti, E., et al. (2006). Molecular cloning and *in situ* expression patterns of two new pheromone-binding proteins from the corn stemborer *Sesamia nonagrioides*. *J. Chem. Ecol.* 32, 1703–1717. doi: 10.1007/s10886-006-9103-2
- Elsayed, G. (2011). Plant secondary substances and insects behaviour. *Arch. Phytopathol. Plant Prot.* 44, 1534–1549. doi: 10.1080/03235408.2010.507957
- Feng, L., and Prestwich, G. D. (1997). Expression and characterization of a Lepidopteran general odorant binding protein. *Insect Biochem. Mol. Biol.* 27, 405–412. doi: 10.1016/S0965-1748(97)00012-X

AUTHOR CONTRIBUTIONS

LS and YZ conceived and designed the experimental plan. LS, XM, and YX performed the experiments. LS, YW, DZ, YZ, XY, QX, and YG analyzed the data. LS and DZ drafted the manuscript.

ACKNOWLEDGMENTS

This work was supported by China National “973” Basic Research Program (2012CB114104), the National Natural Science Foundation of China (31272048, 31321004, 31471778, and 31501652) and Research Foundation of State Key Laboratory for Biology of Plant Diseases and Insect Pests (SKLOF201514).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fphys.2016.00201>

Figure S1 | SDS-PAGE analyses of *AlinOBP11* expression and purification. Protein markers are shown in the left side; –, crude bacterial extract before induction with IPTG; + crude bacterial extracts after induction with IPTG; Sup, supernatant of disrupted PET/*AlinOBP11*; Pel, inclusion body of disrupted PET/*AlinOBP11*; P/His-tag, purified *AlinOBP11* protein with His-tag; P, finally purified *AlinOBP11* protein obtained after two rounds of purification.

Figure S2 | The relative transcript levels of *AlinOBP11* at different developmental stages and adult tissues of both sexes evaluated by qRT-PCR with *AlinElongation factor* (GenBank No. AEY99651) as internal control. The results clearly showed *AlinOBP11* was strongly expressed at adult mouthparts.

Table S1 | The primers used in this article.

Table S2 | The protein names, GenBank accession numbers, and references of OBPs used in the phylogenetic analysis.

- Foret, S., and Maleszka, R. (2006). Function and evolution of a gene family encoding odorant binding-like proteins in a social insect, the honey bee (*Apis mellifera*). *Genome Res.* 16, 1404–1413. doi: 10.1101/gr.5075706
- Galindo, K., and Smith, D. P. (2001). A large family of divergent drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla. *Genetics* 159, 1059–1072.
- Groot, A. T., Dekker, T., and Heckel, D. G. (2016). The genetic basis of pheromone evolution in moths. *Annu. Rev. Entomol.* 61, 99–117. doi: 10.1146/annurev-ento-010715-023638
- Große-Wilde, E., Svatoš, A., and Krieger, J. (2006). A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor *in vitro*. *Chem. Senses* 31, 547–555. doi: 10.1093/chemse/bjj059
- Gu, S. H., Wang, S. P., Zhang, X. Y., Wu, K. M., Guo, Y. Y., Zhou, J. J., et al. (2011a). Identification and tissue distribution of odorant binding protein genes in the lucerne plant bug *Adelphocoris lineolatus* (Goeze). *Insect Biochem. Mol. Biol.* 41, 254–263. doi: 10.1016/j.ibmb.2011.01.002
- Gu, S.-H., Wang, W.-X., Wang, G.-R., Zhang, X.-Y., Guo, Y.-Y., Zhang, Z. D., et al. (2011b). Functional characterization and immunolocalization of odorant binding protein 1 in the lucerne plant bug, *Adelphocoris lineolatus* (Goeze). *Arch. Insect Biochem. Physiol.* 77, 81–99. doi: 10.1002/arch.20427
- Halloin, J. M. (1982). Localization and changes in catechin and tannins during development and ripening of cottonseed. *New Phytol.* 90, 651–657. doi: 10.1111/j.1469-8137.1982.tb03274.x

- Hatfield, L. D., and Frazier, J. L. (1980). Ultrastructure of the labial tip sensilla of the tarnished plant bug, *Lygus lineolaris* (P. de Beauvois) (Hemiptera: Miridae). *Inter. J. Insect Morphol. Embryol.* 9, 59–66. doi: 10.1016/0020-7322(80)90036-7
- He, P., Zhang, J., Liu, N. Y., Zhang, Y. N., Yang, K., and Dong, S. L. (2011). Distinct expression profiles and different functions of odorant binding proteins in *Nilaparvata lugens* Stål. *PLoS ONE* 6:e28921. doi: 10.1371/journal.pone.0028921
- He, X., Tzotzos, G., Woodcock, C., Pickett, J. A., Hooper, T., Field, L. M., et al. (2010). Binding of the general odorant binding protein of *Bombyx mori* BmorGOBP2 to the moth sex pheromone components. *J. Chem. Ecol.* 36, 1293–1305. doi: 10.1007/s10886-010-9870-7
- Hekmat-Scafe, D. S., Steinbrecht, R. A., and Carlson, J. R. (1997). Coexpression of two odorant-binding protein homologs in *Drosophila*: implications for olfactory coding. *J. Neurosci.* 17, 1616–1624.
- Hua, J. F., Zhang, S., Cui, J. J., Wang, D. J., Wang, C. Y., Luo, J. Y., et al. (2012). Identification and binding characterization of three odorant binding proteins and one chemosensory protein from *Apolygus lucorum* (Meyer-Dür). *J. Chem. Ecol.* 38, 1163–1170. doi: 10.1007/s10886-012-0178-7
- Hull, J. J., Perera, O. P., and Snodgrass, G. L. (2014). Cloning and expression profiling of odorant-binding proteins in the tarnished plant bug, *Lygus lineolaris*. *Insect Mol. Biol.* 23, 78–97. doi: 10.1111/imb.12064
- Jacquin-Joly, E., Bohbot, J., Francois, M. C., Cain, A. H., and Nagnan-Le Meillour, P. (2000). Characterization of the general odorant-binding protein 2 in the molecular coding of odorants in *Mamestra brassicae*. *Eur. J. Biochem.* 267, 6708–6714. doi: 10.1046/j.1432-1327.2000.01772.x
- Jacquin-Joly, E., and Merlin, C. (2004). Insect olfactory receptors: contributions of molecular biology to chemical ecology. *J. Chem. Ecol.* 30, 2359–2397. doi: 10.1007/s10886-004-7941-3
- Jeong, Y. T., Shim, J., Oh, S. R., Yoon, H. I., Kim, C. H., Moon, S. J., et al. (2013). An odorant-binding protein required for suppression of sweet taste by bitter chemicals. *Neuron* 79, 725–737. doi: 10.1016/j.neuron.2013.06.025
- Klusák, V., Havlas, Z., Ruliššek, L. R., Vondrášek, J., and Svatoš, A. (2003). Sexual attraction in the silkworm moth: nature of binding of bombykol in pheromone binding protein—an ab initio study. *Chem. Biol.* 10, 331–340. doi: 10.1016/S1074-5521(03)00074-7
- Krieger, J., Von Nickisch-Rosenegk, E., Mameli, M., Pelosi, P., and Breer, H. (1996). Binding proteins from the antennae of *Bombyx mori*. *Insect Biochem. Mol. Biol.* 26, 297–307. doi: 10.1016/0965-1748(95)00096-8
- Leal, W. S. (2013). Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annu. Rev. Entomol.* 58, 373–391. doi: 10.1146/annurev-ento-120811-153635
- Leal, W. S., Nikonova, L., and Peng, G. (1999). Disulfide structure of the pheromone binding protein from the silkworm moth, *Bombyx mori*. *FEBS Lett.* 464, 85–90. doi: 10.1016/S0014-5793(99)01683-X
- Li, Q., and Liberles, S. D. (2015). Aversion and attraction through olfaction. *Curr. Biol.* 25, R120–R129. doi: 10.1016/j.cub.2014.11.044
- Li, S., Picimbon, J. F., Ji, S., Kan, Y., Chuanling, Q., Zhou, J. J., et al. (2008). Multiple functions of an odorant-binding protein in the mosquito *Aedes aegypti*. *Biochem. Biophys. Res. Commun.* 372, 464–468. doi: 10.1016/j.bbrc.2008.05.064
- Lin, F., Wu, D., Lu, Y., Zhang, Y., Wang, M., and Wu, K. (2011). The relationship between the main secondary metabolites and the resistance of cotton to *Apolygus lucorum*. *Acta Phytopyth.* 38, 202–208.
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262
- Loughrin, J., Manukian, A., Heath, R., and Tumlinson, J. (1995). Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21, 1217–1227. doi: 10.1007/BF02228321
- Lu, Y. H., and Wu, K. M. (2008). *Biology and Control of Cotton Mirids*. Beijing: Golden Shield Press.
- Lu, Y. H., Wu, K. M., Jiang, Y. Y., Xia, B., Li, P., Feng, H. Q., et al. (2010). Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* 328, 1151–1154. doi: 10.1126/science.1187881
- Lu, Y., Wu, K., Wyckhuys, K., and Guo, Y. (2009). Comparative flight performance of three important pest *Adelphocoris* species of Bt cotton in China. *B. Entomol. Res.* 99, 543–550. doi: 10.1017/S000748530800655X
- Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T., and Fuyama, Y. (2007). Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. *PLoS Biol.* 5:e118. doi: 10.1371/journal.pbio.0050118
- Meisner, J., Zur, M., Kabonci, E., and Ascher, K. (1977). Influence of gossypol content of leaves of different cotton strains on the development of *Spodoptera littoralis* larvae. *J. Econom. Entomol.* 70, 714–716. doi: 10.1093/jee/70.6.714
- Michael, L. (2000). Immunolocalization of general odorant-binding protein in antennal sensilla of moth caterpillars. *Arthropod Struct. Dev.* 29, 57–73. doi: 10.1016/S1467-8039(00)00013-X
- Millar, J. G. (2005). Pheromones of true bugs. *Top. Curr. Chem.* 240, 37–84. doi: 10.1007/b98315
- Mithöfer, A., and Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annu. Rev. Plant Biol.* 63, 431–450. doi: 10.1146/annurev-arplant-042110-103854
- Park, S. K., Shanhag, S. R., Wang, Q., Hasan, G., Steinbrecht, R. A., and Pikielny, C. W. (2000). Expression patterns of two putative odorant-binding proteins in the olfactory organs of *Drosophila melanogaster* have different implications for their functions. *Cell Tissue Res.* 300, 181–192. doi: 10.1007/s004410050059
- Pelosi, P., Iovinella, I., Felicioli, A., and Dani, F. R. (2014). Soluble proteins of chemical communication: an overview across arthropods. *Front. Physiol.* 5:320. doi: 10.3389/fphys.2014.00320
- Pelosi, P., Mastrogiacomo, R., Iovinella, I., Tuccori, E., and Persaud, K. (2013). Structure and biotechnological applications of odorant-binding proteins. *Appl. Microbiol. Biotechnol.* 98, 61–70. doi: 10.1007/s00253-013-5383-y
- Poivet, E., Rharrabe, K., Monsempe, C., Glaser, N., Rochat, D., Renou, M., et al. (2012). The use of the sex pheromone as an evolutionary solution to food source selection in caterpillars. *Nat. Commun.* 3, 1047. doi: 10.1038/ncomms2050
- Pophof, B. (2004). Pheromone-binding proteins contribute to the activation of olfactory receptor neurons in the silkworms *Antheraea polyphemus* and *Bombyx mori*. *Chem. Senses* 29, 117–125. doi: 10.1093/chemse/bjh012
- Qiao, H., Tuccori, E., He, X., Gazzano, A., Field, L., Zhou, J. J., et al. (2009). Discrimination of alarm pheromone (*E*)- β -farnesene by aphid odorant-binding proteins. *Insect Biochem. Molecul. Biol.* 39, 414–419. doi: 10.1016/j.ibmb.2009.03.004
- Romani, R., Salerno, G., Frati, F., Conti, E., Isidoro, N., and Bin, F. (2005). Oviposition behaviour in *Lygus rugulipennis*: a morpho-functional study. *Entomol. Experiment. Appl.* 115, 17–25. doi: 10.1111/j.1570-7458.2005.00268.x
- Röse, U. R., and Tumlinson, J. (2004). Volatiles released from cotton plants in response to *Helicoverpa zea* feeding damage on cotton flower buds. *Planta* 218, 824–832. doi: 10.1007/s00425-003-1162-9
- Sandler, B. H., Nikonova, L., Leal, W. S., and Clardy, J. (2000). Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. *Chem. Biol.* 7, 143–151. doi: 10.1016/S1074-5521(00)00078-8
- Steinbrecht, R., Laue, M., and Ziegelberger, G. (1995). Immunolocalization of pheromone-binding protein and general odorant-binding protein in olfactory sensilla of the silk moths *Antheraea* and *Bombyx*. *Cell Tissue Res.* 282, 203–217. doi: 10.1007/BF00319112
- Sun, L., Gu, S. H., Xiao, H. J., Zhou, J. J., Guo, Y. Y., Liu, Z. W., et al. (2013). The preferential binding of a sensory organ specific odorant binding protein of the alfalfa plant bug *Adelphocoris lineolatus* AlinOBP10 to biologically active host plant volatiles. *J. Chem. Ecol.* 39, 1221–1231. doi: 10.1007/s10886-013-0333-9
- Sun, L., Xiao, H. J., Gu, S. H., Guo, Y. Y., Liu, Z. W., and Zhang, Y. J. (2014b). Perception of potential sex pheromones and host-associated volatiles in the cotton plant bug, *Adelphocoris fasciaticollis* (Hemiptera: Miridae): morphology and electrophysiology. *Appl. Entomol. Zool.* 49, 43–57. doi: 10.1007/s13355-013-0223-1
- Sun, L., Xiao, H. J., Gu, S. H., Zhou, J. J., Guo, Y. Y., Liu, Z. W., et al. (2014a). The antenna-specific odorant-binding protein AlinOBP13 of the alfalfa plant bug *Adelphocoris lineolatus* is expressed specifically in basiconic sensilla and has high binding affinity to terpenoids. *Insect Mol. Biol.* 23, 417–434. doi: 10.1111/imb.12089
- Sun, Y. F., De Biasio, F., Qiao, H. L., Iovinella, I., Yang, S. X., Ling, Y., et al. (2012). Two odorant-binding proteins mediate the behavioural response of aphids to the alarm pheromone *E*- β -farnesene and structural analogues. *PLoS ONE* 7:e32759. doi: 10.1371/journal.pone.0032759
- Sun, Y. L., Huang, L. Q., Pelosi, P., and Wang, C. Z. (2012). Expression in antennae and reproductive organs suggests a dual role of an odorant-binding protein in two sibling *Helicoverpa* species. *PLoS ONE* 7:e30040. doi: 10.1371/journal.pone.0030040

- Sun, Y. P., Zhao, L. J., Sun, L., Zhang, S. G., and Ban, L. P. (2013). Immunolocalization of odorant-binding proteins on antennal chemosensilla of the peach aphid *Myzus persicae* (Sulzer). *Chem. Senses* 38, 129–136. doi: 10.1093/chemse/bjs093
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi: 10.1093/molbev/mst197
- Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R., et al. (2009). Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. *Curr. Biol.* 19, 881–890. doi: 10.1016/j.cub.2009.04.035
- Tegoni, M., Campanacci, V., and Cambillau, C. (2004). Structural aspects of sexual attraction and chemical communication in insects. *Trends Biochem. Sci.* 29, 257–264. doi: 10.1016/j.tibs.2004.03.003
- Vandermoten, S., Francis, F., Haubruge, E., and Leal, W. S. (2011). Conserved odorant-binding proteins from aphids and eavesdropping predators. *PLoS ONE* 6:e23608. doi: 10.1371/journal.pone.0023608
- Vogt, R. G., and Riddiford, L. M. (1981). Pheromone binding and inactivation by moth antennae. *Nature* 293, 161–163. doi: 10.1038/293161a0
- Wang, S. Y., Gu, S. H., Han, L., Guo, Y. Y., Zhou, J. J., and Zhang, Y. J. (2013). Specific involvement of two amino acid residues in *cis*-nerolidol binding to odorant-binding protein 5 AlinOBP5 in the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze). *Insect Mol. Biol.* 22, 172–182. doi: 10.1111/imb.12012
- Yang, Z. H. (1997). PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* 13, 555–556. doi: 10.1093/bioinformatics/13.5.555
- Yuan, H. B., Ding, Y. X., Gu, S. H., Sun, L., Zhu, X. Q., Liu, H. W., et al. (2015). Molecular characterization and expression profiling of odorant-binding proteins in *Apolygus lucorum*. *PLoS ONE* 10:e140562. doi: 10.1371/journal.pone.0140562
- Zhang, D. D., and Löfstedt, C. (2013). Functional evolution of a multigene family: orthologous and paralogous pheromone receptor genes in the turnip moth, *Agrotis segetum*. *PLoS ONE* 8:e77345. doi: 10.1371/journal.pone.0077345
- Zhou, J. J., Vieira, F. G., He, X. L., Smadja, C., Liu, R., Rozas, J., et al. (2010). Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid *Acyrtosiphon pisum*. *Insect Mol. Biol.* 19(Suppl. 2), 113–122. doi: 10.1111/j.1365-2583.2009.00919.x
- Zhu, J., Ban, L., Song, L. M., Liu, Y., Pelosi, P., and Wang, G. (2016). General odorant-binding proteins and sex pheromone guide larvae of *Plutella xylostella* to better food. *Insect Biochem. Mol. Biol.* 72, 10–19. doi: 10.1016/j.ibmb.2016.03.005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Sun, Wei, Zhang, Ma, Xiao, Zhang, Yang, Xiao, Guo and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.