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Odorant-binding proteins display high affinities for behavioral attractants and repellents in the natural predator *Chrysopa pallens*

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ABSTRACT

Chrysopa pallens is an important natural predator of various pests in many different cropping systems. Understanding the sophisticated olfactory system of insect antennae is crucial for studying the physiological bases of olfaction and could also help enhance the effectiveness of *C. pallens* in biological control. However, functional studies of the olfactory genes in *C. pallens* are still lacking. In this study, we cloned five odorant-binding protein (OBP) genes from *C. pallens* (CpalOBPs). Quantitative RT-PCR results indicated that the five CpalOBPs had different tissue expression profiles. Ligand-binding assays showed that farnesol, farnesene, cis-3-hexenyl hexanoate, geranylacetone, beta-ionone, octyl aldehyde, decanal, nerolidol (Ki < 20 μM), and especially 2-pentadecanone (Ki = 1.19 μM) and 2-hexyl-1-decanol (Ki = 0.37 μM) strongly bound to CpalOBP2. CpalOBP15 exhibited high binding affinities for beta-ionone, 2-tridecanone, trans-nerolidol, and dodecyl aldehyde. Behavioral trials using the 14 compounds exhibiting high binding affinities for the CpalOBPs revealed that nine were able to elicit significant behavioral responses from *C. pallens*. Among them, farnesene and its corresponding alcohol, farnesol, elicited remarkable repellent behavioral responses from *C. pallens*. Our study provides several compounds that could be selected to develop slow-release agents that attract/repel *C. pallens* and to improve the search for strategies to eliminate insect pests.

1. Introduction

A sophisticated olfactory system is a key physiological trait in insect behaviors, including host seeking, mating, oviposition, toxin avoidance, and negative taxis (Zhou, 2010). In insects, olfactory genes are expressed in and around olfactory receptor (OR) neurons, which are located at the sensilla in antennae (Schultze et al., 2013, 2014), mouthparts (Syed and Leal, 2007; Wasserman and Itagaki, 2003), and ovipositors (Anderson and Hallberg, 1990). Odorant-binding proteins (OBPs) comprise a family of small, water-soluble, extracellular proteins that are found in abundance within the sensillum lumen, and are thought to affect insect chemoreception by enhancing the solubility of exogenous odorant molecules and delivering them to the chemosensory receptors (Leal, 2012; Vogt, 2003, 2004; Zhou, 2010). Two models have been proposed for the mechanisms of OR activation (Leal, 2012). The first model suggests that a ligand per se activates its OR in moths (Leal et al., 2005) and mosquitoes (Wogulis et al., 2006). In contrast, the second model hypothesizes that a pheromone-binding protein, such as Lush in *Drosophila melanogaster*, forms an OBP/odorant complex that directly activates the OR (Laughlin et al., 2008).

The first OBP was identified as a pheromone-binding protein in the giant moth *Antheraea polyphemus*, and it was specifically bound to a radioactive sex pheromone (Vogt and Riddiford, 1981). Thereafter, a large number of proteins and associated genes were identified in a wide range of insects in the orders of the Neoptera (Pelosi et al., 2005, 2006; Pelosi and Maida, 1995; Zhou, 2010). In addition, many papers have been published on functional and structural studies in Lepidoptera (Liu et al., 2013, 2015), Diptera (Lagarde et al., 2011a; Laughlin et al., 2008), Hymenoptera (Spinelli et al., 2012), and Hemiptera (He and He, 2014; He et al., 2011; Zhou et al., 2014). The typical characteristics of an insect OBP are six highly conserved cysteines, with a specific spacing among them, that form three disulfide bridges crucial for stabilizing the three-dimensional structure (Lagarde et al., 2011b; Tsitsanou et al., 2012). In addition to the classical OBPs (six conserved cysteines), some non-classical OBPs have been identified, including Plus-C OBPs (eight conserved cysteines), Minus-C OBPs (only four conserved cysteines), Dimer OBPs (two six-cysteine signatures), and Atypical OBPs (9–10 cysteines and a long C-terminus) (Hekmat-Scafe et al., 2002; Li et al., 2013a; Zhou et al., 2004).

The lacewing *Chrysopa pallens* (Neuroptera: Chrysopidae) is an important natural predator of various pests, such as aphids, coccids,

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thrips, mites, planthoppers, and the eggs and young larvae of lepidopteran insects (Winterton and de Freitas, 2006), in many different cropping systems including maize, cotton, and rice (Bai et al., 2005; Brooks, 1994). In agreement with the feeding habits of larval and adult lacewings, aphid sex pheromone compounds, enantiomers of nepetalactol and nepetalactone [(1R,4aS,7S,7aR)-nepetalactol and (4aS,7S,7aR)-nepetalactone], were shown to attract lacewings and parasitoids (Boo et al., 1998; Hooper et al., 2002; Koczor et al., 2010).

To enhance the effectiveness of *C. pallens* in biological control using the "push–pull" strategy in which combinations of repellent and attractive stimuli are used to regulate the populations of insect pests and natural enemies (Cook et al., 2007), studies on olfactory gene function and olfactory behavior are essential. In our previous study, 14 OBPs were identified from the *C. pallens* transcriptome (Li et al., 2013b). Compared with the many available reports on functional and structural studies of olfactory genes in pests, such data from *C. pallens* are rare. Therefore, we cloned five OBP genes from *C. pallens*. Quantitative PCR (qPCR) results indicated that these five OBPs had different tissue expression profiles, suggesting different functions in chemoreception. The binding properties of the five OBPs with plant volatiles revealed that 14 compounds exhibited high binding affinities for the *C. pallens* OBPs (CpalOBPs), of which nine were able to elicit significant behavioral responses from *C. pallens*. Among them, farnesene and its corresponding alcohol, farnesol, elicited remarkable repellent behavioral responses from *C. pallens*. Our study provides several compounds that could be selected to develop slow-release agents that attract/repel *C. pallens* and to improve the search for strategies to eliminate insect pests.

2. Material and methods

2.1. Insect rearing and collection

The *C. pallens* individuals used in this study were collected from a cotton field at the Institute of Cotton Research (CAAS, Henan, China). The experimental insects were the offspring of a single female and were reared in a laboratory on *Acyrthosiphon pisum*. The rearing conditions were 25 ± 1 °C, 14-h/10-h light/dark cycle, and 65 ± 5% relative humidity. The pupae were kept in separate cages for eclosion, after which the adults were supplied with *A. pisum*. For cDNA cloning and qPCR, antennae, heads, thoraxes, abdomens, legs, and wings were collected from 3-day-old virgin female and male *C. pallens* individuals. The tissues were immediately frozen and stored at −80 °C until RNA isolation.

2.2. cDNA cloning, sequence alignment, and phylogenetic analysis

Total RNA was extracted from the antennae of *C. pallens* using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA templates were synthesized using a Reverse Transcription System (Promega, Madison, WI, USA) according to the manufacturer's instructions. The full-length sequences were confirmed by end-to-end PCR with specific primers designed using Primer Premier 5.0. The primer sequences are listed in Table S1. An amino acid sequence alignment of the corresponding OBPs was created using ClustalX 2.0 (Larkin et al., 2007), and visualized using Jalview 2.4.0 b2 (Waterhouse et al., 2009). Five CpalOBPs were chosen for phylogenetic tree construction along with the OBPs from other insects, which were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/). A phylogenetic tree was constructed based on the amino acid sequences using the neighbor-joining algorithm implemented in MEGA6 (Tamura et al., 2011), with the default settings and 1000 bootstrap replicates. The sequences used in the phylogenetic tree are listed in Table S2.

2.3. Real-time qPCR and data analysis

Total RNA was isolated using an SV Total RNA Isolation System (Promega) according to the manufacturer's instructions. Single-stranded cDNA templates were synthesized using 1 μg of total RNA from various samples using a Reverse Transcription System (Promega) according to the manufacturer's instructions. qPCR was performed in a Mastercycler ep realplex (Eppendorf, Hamburg, Germany) with primers designed based on *C. pallens* nucleotide sequences using Beacon Designer 7.7 (Table S1). The *C. pallens GTP-binding protein* and *ribosomal protein* genes were used as reference genes. The expression levels of the tested mRNAs were determined using GoTaq qPCR Master Mix (Promega) according to the manufacturer's instructions and calculated using the comparative $2^{-\Delta\Delta CT}$ method (Pfaffl, 2001). Each reaction involved three technical replicates performed on three independent biological replicates.

2.4. Escherichia coli expression and purification of recombinant proteins

An *E. coli* expression system was used to express the CpalOBPs. Recombinant pET-32a(+)/CpalOBPs were constructed using an In-Fusion HD Cloning Kit (Clontech, Temecula, CA, USA) according to the manufacturer's instructions, and the recombinant plasmids were transformed into competent *E. coli* BL21 cells. Positive clones were incubated at 37 °C following a previously described protocol (Li et al., 2013a). Recombinant proteins in the supernatants were purified using HisTrap FF affinity columns (GE Healthcare, Piscataway, NJ, USA). The His-tags were cleaved from the proteins using 40 μl of enterokinase (Dingguo, Beijing, China) while loaded on the HisTrap FF columns at 37 °C for 10 h. After completion of the cleavage reaction, the recombinant proteins were eluted with phosphate-buffered saline. Finally, the eluted proteins were desalted using a HiTrap Desalting Kit (GE Healthcare), lyophilized, and stored at −80 °C until use.

2.5. Competitive fluorescence binding assay

Emission fluorescence spectra were measured on an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) in a right-angle configuration with a 1-cm light path quartz cuvette. The proteins were dissolved in 50 mM Tris–HCl buffer (pH 7.4), whereas all ligands used in the binding experiments were added as 1 mM methanol solutions.

Aliquots of 2 μM OBP solutions were titrated with 1 mM 1-N-phenylnaphthylamine (1-NPN) to a final concentration of 20 μM to measure the OBP affinities for the fluorescent probe. The 1-NPN fluorescent probe was excited at 337 nm, and emission spectra were recorded from 360 to 500 nm. OBP dissociation constants for 1-NPN were calculated from Scatchard plots of the binding data. The comparative binding of other ligands was measured using 2 μM 1-NPN, and the corresponding fluorescence intensities were recorded. Dissociation constants (Ki) of the OBPs for each ligand were calculated from the corresponding concentrations of ligands halving the initial fluorescence value of 1-NPN (IC_{50} values) based on the binding curves, using the following equation: $Ki = IC_{50} / (1 + C_{1\text{-NPN}} / K_{1\text{-NPN}})$. In this equation, $C_{1\text{-NPN}}$ is the free concentration of 1-NPN, and $K_{1\text{-NPN}}$ is the dissociation constant of the complex protein/1-NPN (Wei et al., 2008). Each reaction was repeated in triplicate.

2.6. Behavioral trials

The behavioral responses of *C. pallens* adults (5 days after emergence) to the 14 putative ligands for recombinant CpalOBP5, CpalOBP7, and/or CpalOBP10 were examined using a glass Y-tube olfactometer. The central tube was 25 cm (length) × 30 m (diameter), with arms of 20 cm (length) × 30 mm (diameter), and an angle of 75° between the arms. Two filter paper strips were placed in the arms of the olfactometer; one held a control and the other held the test chemical.

A constant airflow (0.2 l/min), which was filtered through activated-charcoal and deionized water, was introduced into each arm of the olfactometer.

C. pallens individuals received 5 min to choose between the two arms of the Y-tube olfactometer, and the insect was then discarded. A response was identified when *C. pallens* walked into one arm of the olfactometer beyond 5 cm. After testing one insect, the arms were exchanged to avoid an asymmetric bias effect and the test compound was replaced with a new one (10 μl on filter paper; 10 mg/ml). To minimize the visual distraction for *C. pallens*, the Y-tube olfactometer was placed in darkness. At least 40 insects were used. Before behavioral measurements, the insects were starved for ~12 h. All tests were conducted at 24 ± 2 °C and 60 ± 5% relative humidity. All olfactometers used were washed with ethanol and dried at 80 °C for 5 min. The chi-squared test was used to analyze the behavioral assay data.

3. Results

3.1. Gene cloning and sequence analysis of CpalOBPs

Five OBP genes were cloned and designated *CpalOBP1*, *CpalOBP2*, *CpalOBP5*, *CpalOBP13*, and *CpalOBP15* (GenBank accession numbers KP082905, KP082903, KP403738, KP403734, and KP403736, respectively). The predicted open reading frames of *CpalOBP1*, *CpalOBP2*, *CpalOBP5*, *CpalOBP13*, and *CpalOBP15* had lengths of 450, 417, 408, 633, and 771 bp, respectively, which encoded 149, 138, 135, 210, and 256 amino acids, respectively. The initial 19, 19, 21, 20, and 16 amino acids of the respective sequences were predicted signal peptides (Fig. 1). The predicted protein molecular weights and isoelectric points of *CpalOBP1*, *CpalOBP2*, *CpalOBP5*, *CpalOBP13*, and *CpalOBP15* were 16.47 kDa and 5.36, 15.72 kDa and 4.6, 15.12 kDa and 8.49, 23.76 kDa and 5.29, and 29.63 kDa and 5.45, respectively. The phylogenetic tree of the five CpalOBPs and 35 OBPs from other insects indicated four possible protein subfamilies (Fig. 2). The results showed that CpalOBP1 and CpalOBP2 belonged to the Classic OBPs, CpalOBP5 to the Minus-C OBPs, CpalOBP13 to the Plus-C OBPs, and CpalOBP15 to the Atypical OBPs.

3.2. Tissue expression profiles of the CpalOBPs

Tissue expression patterns can often provide functional information. The expression levels of the *CpalOBPs* in different adult tissues were detected by qPCR (Fig. 3). The *CpalOBPs* in our study displayed various tissue expression profiles. *CpalOBP1*, *CpalOBP2*, and *CpalOBP13* were highly expressed in the antennae of both sexes. Among these genes, *CpalOBP2* expression was restricted to the antennae, while *CpalOBP1* and *CpalOBP13* had low levels of expression in the other tissues examined. *CpalOBP5* was highly expressed in the wings. *CpalOBP15* was ubiquitously expressed in most of the tissues examined at relatively high levels.

3.3. In vitro expression and purification of the five CpalOBPs

The five CpalOBPs were successfully expressed as soluble proteins using a bacterial system. A His-tag affinity column was used to purify the proteins, followed by treatment with an enterokinase to remove the His-tag. More than 10 mg of each purified CpalOBP was obtained from each 1.0 L culture. The purified proteins were identified by SDS-PAGE (Fig. 4).

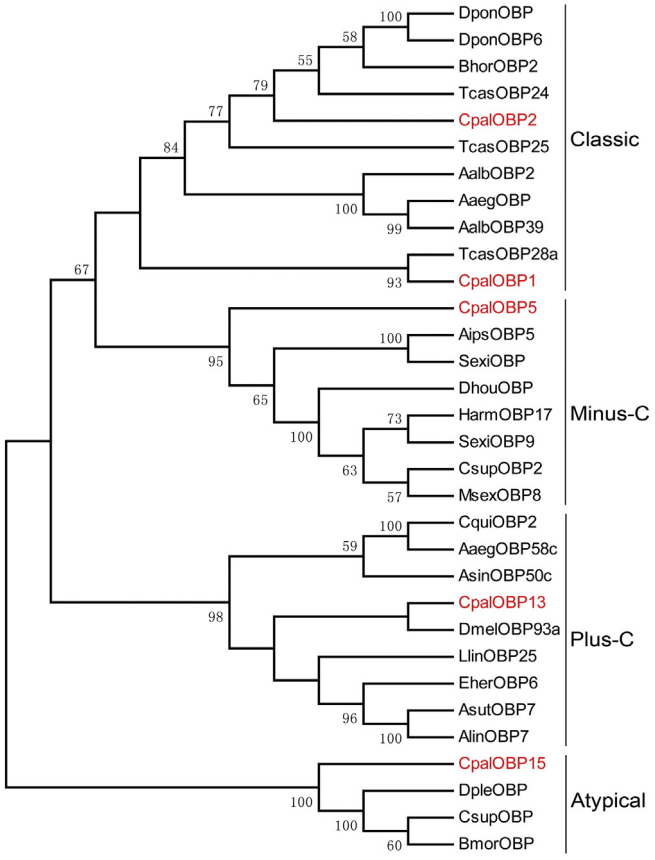

Fig. 2. Phylogenetic tree of candidate OBPs from *C. pallens* and other species. Tcas: *Tribolium castaneum*; Dpon: *Dendroctonus ponderosae*; Bhor: *Batocera horsfieldi*; Aaeg: *Aedes aegypti*; Aalb: *Aedes albopictus*; Ham: *Helicoverpa armigera*; Sexi: *Spodoptera exigua*; Csup: *Chilo suppressalis*; Aips: *Agrotis ipsilon*; Dhou: *Dendrolimus houi*; Msex: *Manduca sexta*; Cqui: *Culex quinquefasciatus*; Asin: *Anopheles sinensis*; Llin: *Lygus lineolaris*; Dmel: *Drosophila melanogaster*; Asut: *Adelphocoris suturalis*; Alin: *Adelphocoris lineolatus*; Eher: *Euschistus heros*; Bmor: *Bombyx mori*; and Dple: *Danaus plexippus*. Values at nodes indicate the bootstrap percentages based on 1000 replicates, and branches with bootstrap values above 50% are marked. OBPs from *C. pallens* are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Ligand-binding assays of the five CpalOBPs

To investigate and compare the binding specificities of the five CpalOBPs with 87 ligands, fluorescence levels were measured as reported previously (Li et al., 2013a). The dissociation constants for CpalOBP1, CpalOBP2, CpalOBP5, CpalOBP13, and CpalOBP15 combined with the fluorescent probe 1-NPN were 15.26, 4.08, 32.34, 12.72, and 6.70, respectively, based on the binding curves and Scatchard plots (Fig. 5A).

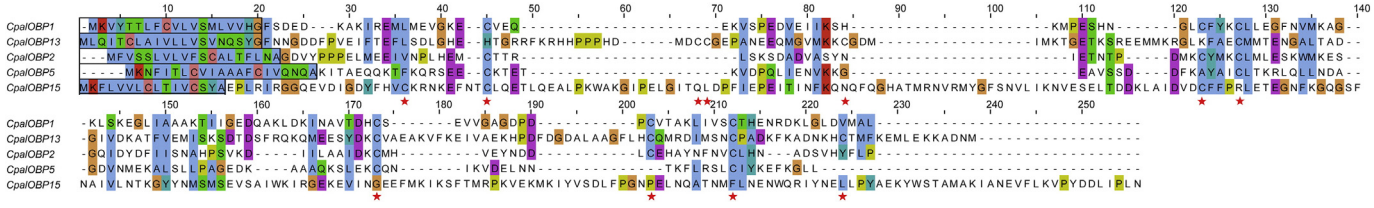

Fig. 1. Alignment of CpalOBP amino acid sequences. Predicted signal peptides are boxed, and conserved cysteines are labeled with red pentagrams. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

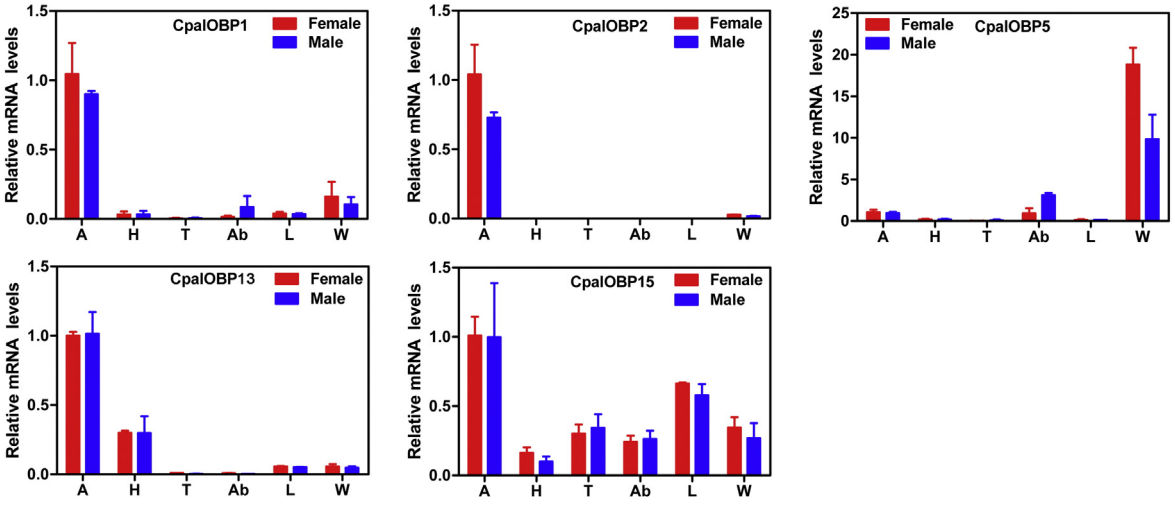

Fig. 3. Relative mRNA expression levels of the five *CpalOBPs* in different adult tissues. The relative expression levels were normalized using GTP-binding protein and ribosomal protein. Error bars represent standard errors. H, heads; T, thoraxes; Ab, abdomens; L, legs; W, wings; A, antennae.

In a subsequent experiment, 1-NPN was used as the fluorescent reporter to determine the binding activities of the five CpalOBPs to different odorants. The IC_{50} and Ki values for all 87 compounds tested were calculated based on the binding curves (Fig. 5B and C). In general, CpalOBP1, CpalOBP5, and CpalOBP13 displayed a weak binding (Ki > 20 μM) to most of the 87 ligands tested. However, there were 10 ligands that showed relatively higher binding affinities (Ki < 20 μM) for CpalOBP2 and four ligands for CpalOBP15 (Table 1). CpalOBP15 exhibited high binding affinities for beta-ionone, 2-tridecanone, trans-nerolidol, and dodecyl aldehyde. Farnesol, farnesene, cis-3-hexenyl hexanoate, geranylacetone, beta-ionone, octyl aldehyde, decanal, nerolidol (Ki < 20 μM), and especially 2-pentadecanone (Ki = 1.19 μM) and 2-hexyl-1-decanol (Ki = 0.37 μM) bound strongly to CpalOBP2. Meanwhile, CpalOBP1 only showed a binding affinity for phlorizin dehydrate (Ki = 15.93 μM).

3.5. Behavioral trials

The behavioral responses to the 14 compounds that exhibited high binding affinities (Ki < 20 μM) for the five CpalOBPs were examined in a Y-tube olfactometer. Nine of the 14 compounds were able to elicit behavioral responses in *C. pallens* (Fig. 6). *C. pallens* showed a significant attraction to cis-3-hexenyl hexanoate ($\chi^2 = 6.26$, $P < 0.05$), decanal ($\chi^2 = 8.26$, $P < 0.01$), and 2-hexyl-1-decanol ($\chi^2 = 13.02$, $P < 0.01$). In contrast, *C. pallens* showed a significant aversion to 2-pentadecanone ($\chi^2 = 6.04$, $P < 0.05$), 2-tridecanone ($\chi^2 = 12.12$, $P < 0.01$), geranylacetone ($\chi^2 = 11.28$, $P < 0.01$), octyl aldehyde ($\chi^2 = 6.04$ $P < 0.05$), farnesol ($\chi^2 = 4.32$, $P < 0.05$), and farnesene ($\chi^2 = 8.26$, $P < 0.01$).

4. Discussion

C. pallens is an important natural enemy of pests in different agro-ecosystems (Bai et al., 2005; Brooks, 1994; Winterton and de Freitas, 2006). Understanding the olfactory behavior of *C. pallens*, including hunting, mating, and oviposition, can improve biological control in sustainable agriculture. However, most studies have focused on pest olfaction (Gu et al., 2013; Sparks et al., 2014; Vogt et al., 2009) and studies on natural enemy insects are scarce. Consequently, five CpalOBPs were cloned and functionally characterized in this study. The present findings could increase the understanding of the olfactory processes of *C. pallens* during hunting.

To date, four OBP subfamilies have been found in the insect, namely the Classic, Minus-C, Plus-C, and Atypical OBP subfamilies (Zhou, 2010). A phylogenetic tree of the five CpalOBPs, together with 27 OBPs from other insects, showed that the CpalOBPs were well clustered into the four distinct subfamilies, with CpalOBP1 and CpalOBP2 belonging to the Classic OBPs, CpalOBP5 to the Minus-C OBPs, CpalOBP13 to

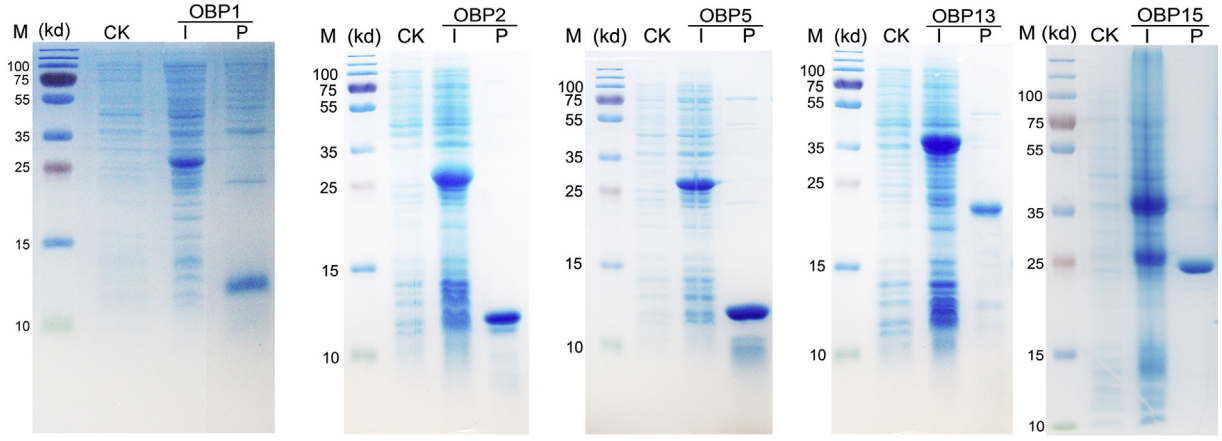

Fig. 4. SDS-PAGE analyses showing the expression and purification of the recombinant CpalOBPs. M: molecular markers; CK and I: bacterial cells before and after induction by IPTG, respectively; P: purified protein after cleavage by thrombin.

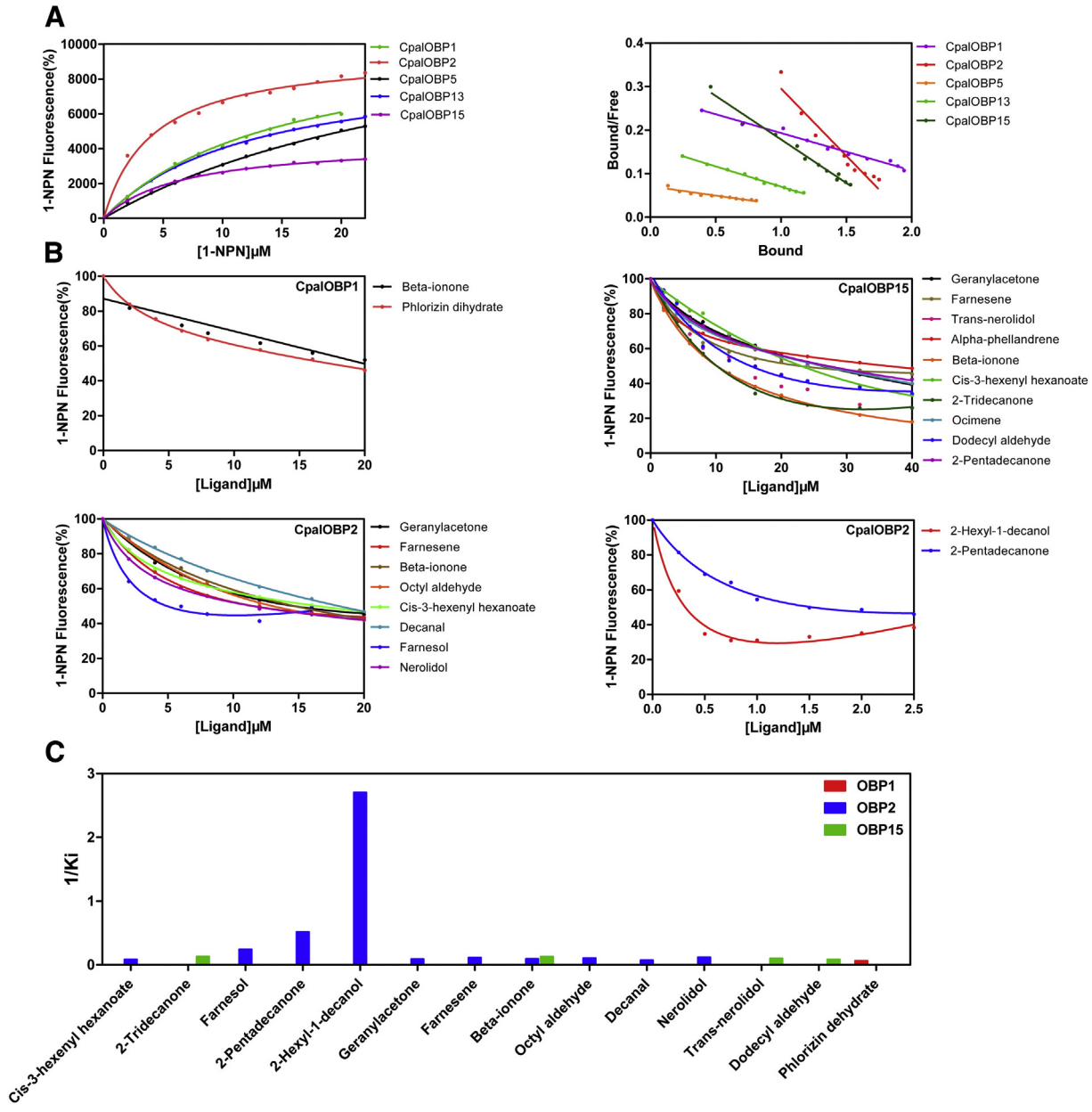

Fig. 5. Ligand-binding assays of the CpalOBPs. (A) Binding curve for 1-NPN and Scatchard plot of CpalOBPs. (B) Binding curves of selected ligands. (C) Comparisons of binding affinities (indicated by 1/Ki) of CpalOBPs with 14 components.

the Plus-C OBPs, and CpalOBP15 to the Atypical OBPs. These findings suggested that the five CpalOBPs play different roles in odorant recognition, and that rapid evolution has occurred through gene duplication.

To understand the physiological functions of the five CpalOBPs, we examined their tissue expression profiles in *C. pallens* adults. The qPCR data revealed that *CpalOBP1*, *CpalOBP2*, and *CpalOBP13* were highly expressed in the antennae of both sexes. Antennae are the major chemosensory organs of insects. Therefore, the antennae-enriched genes may be involved in insect olfaction. Consistent with previous studies, *CpalOBP5* was highly expressed in the wings (Gu et al., 2011; Hua et al., 2012, 2013), whereas *CpalOBP15* was ubiquitously expressed in most of the tissues examined. Olfactory sensilla have been found on the wings and legs of some insects (Leal, 2012), but no such sensilla or chemosensory functions of the wings and legs have been described in *C. pallens*. The high expression of *CpalOBP5* in the wing and ubiquitous expression of *CpalOBP15* deserve further study.

A fluorescence competitive binding assay was used to determine the binding activities of the five CpalOBPs for 87 volatile compounds, including 37 from cotton (Yu et al., 2007), 10 from maize (Carroll et al., 2006; De Moraes et al., 1998; Itoh et al., 2002), seven from tobacco (De Moraes et al., 1998; Yan et al., 2005), 14 from faba bean (Webster et al., 2008), and the remainder from other plants (Obata et al., 1983). The results showed that CpalOBP2 had high binding capacities for 10 plant volatiles, suggesting that it plays a role in the transportation of plant volatiles. In particular, seven ligands, 2-hexyl-1-decanol, 2-pentadecanone, octyl aldehyde, nerolidol, volatiles from cotton and rice, farnesene and its corresponding alcohol, farnesol, showed a good affinity (Ki < 10 μM) for CpalOBP2 only. These data indicate that CpalOBP2 is involved in detecting and recognizing the alarm pheromone in aphid species. CpalOBP15 exhibited high binding affinities for 2-Tridecanone, released by cauliflower leaf (Seenivasagan and Paul, 2011), and beta-ionone, a fragrant component of flowers and other tissues of many plants (Gora and Brud, 1983; Werkhoff et al.,

Table 1
Binding data for the recombinant CpalOBPs with different plant volatiles.

Ligands	OBP1		OBP2		OBP15	
	IC_{50}	Ki	IC_{50}	Ki	IC_{50}	Ki
Cis-3-hexenyl hexanoate	>20	>20	16.63	11.90	>20	>20
2-Tridecanone	>20	>20	>20	>20	9.82	7.76
Farnesol	>20	>20	5.80	4.15	>20	>20
2-Pentadecanone	>20	>20	1.67	1.19	>20	>20
2-Hexyl-1-decanol	>20	>20	0.52	0.37	>20	>20
Geranylacetone	>20	>20	15.24	10.90	>20	>20
Farnesene	>20	>20	12.41	8.87	>20	>20
Beta-ionone	>20	>20	15.03	10.74	9.92	7.85
Octyl aldehyde	>20	>20	13.59	9.72	>20	>20
Decanal	>20	>20	19.80	14.16	>20	>20
Nerolidol	>20	>20	11.66	8.34	>20	>20
Trans-nerolidol	>20	>20	>20	>20	12.74	10.08
Fodecyl aldehyde	>20	>20	>20	>20	15.07	11.92
Phlorizin dehydrate	17.90	15.93	>20	>20	>20	>20

Notes: IC_{50} values of <20 μM were obtained based on the binding curves. Other tested compounds (not listed) that have high Ki values (>20 μM) for all of the OBPs are beta-caryophyllene, alpha-phellandrene, (+/−)-alpha-pinene, (+)-beta-pinene, ocimene, (−)trans-caryophyllene, ethylbenzene, indole, naphthalene, cumene, tridecane, tetradecane, undecane, dodecane, tetradecane, octadecane, heptadecane, tridecane, benzyl alcohol, 1-hexanol, cis-3-hexen-1-ol, cis-2-hexen-1-ol, geraniol, (+/−)-linalool, eucalyptol, alpha-ionol, 2-heptanol, (+)-cedrol, linalool, trans-3-hexen-1, methyl anthranilate-ol, butyl formate, caproyl acetate, pentyl acetate, ethyl propionate, ethyl benzoate, octyl acetate, cis-3-hexenyl acetate, trans-2-hexenyl acetate, phenylacetaldehyde, beta-cyclocitral, (+)-carvone, damascenone, 6-methyl-5-hepten-2-one, 2-heptanone, (±)-camphor, acetophenone, hexyl butyrate, (+)-limonene oxide, (E3)-hexen-1-ol, camphene, (R)-(+)-limonene, phenethyl alcohol, 3-hexanol, cis-3-hexenol, benzyl acetate, phenethyl acetate, geranyl acetate, cis-3-hexenyl acetate, ethyl butyrate, trans-2-hexenyl butyrate, heptyl acetate, methyl salicylate, butyl acetate, nonyl acetate, isoamyl acetate, cis-3-hexenyl butyrate, benzaldehyde, hexanal, trans-2-hexenal, cis-3-hexenal, 3-hexanone, 2-hexanone, and undecanal.

1992). Meanwhile, CpalOBP1, CpalOBP5, and CpalOBP13 did not show strong binding affinities for the ligands tested, based on the Ki values (Ki > 20 μM). These differences in the binding properties are probably caused by different roles in chemoreception among the CpalOBPs.

Of the 14 compounds that exhibited high binding affinities (Ki < 20 μM) for the CpalOBPs, six elicited significant attractant behavioral responses and three elicited remarkable repellent behavioral responses from *C. pallens* in the behavioral trials. Among these nine volatiles, *C. pallens* showed a significant aversion behavior to farnesene and its corresponding alcohol, farnesol. Since beta farnesene is the aphid alarm pheromone (Bowers et al., 1972), the aphid will leave an area containing farnesene, making it unnecessary for *C. pallens* to search the area. Thus, *C. pallens* might recognize and use the aphid alarm pheromone to improve its hunting efficiency for aphids. The compounds that elicited significant behavioral responses could be selected to develop slow-release agents to attract/repel *C. pallens* and to improve the search for strategies, such as the "push–pull" strategy, in which combinations of repellent and attractive stimuli are used to alter the populations of insect pests and natural enemies to eliminate insect pests (Cook et al., 2007). For future studies, RNAi or knockdowns of CpalOBPs are needed to confirm the roles of these compounds and their influence on the behavior of *C. pallens*, as are efficacy evaluations of the screened potential attractants/repellents in the field.

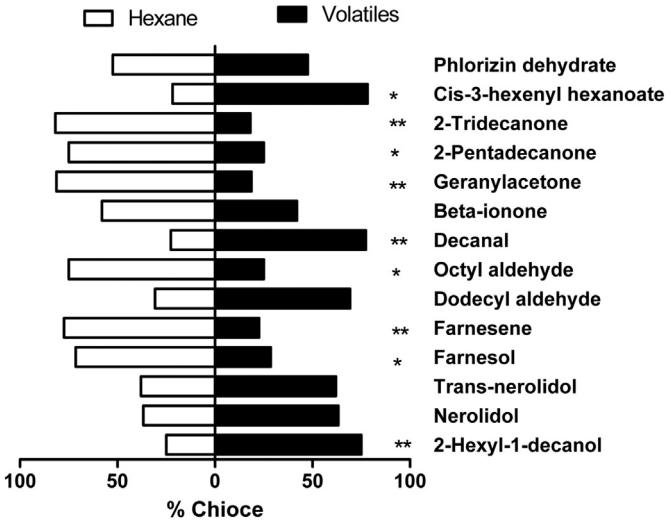

Fig. 6. Behavioral responses of *C. pallens* in a Y-tube olfactometer bioassay when given a choice between different odorant chemicals and hexane (control). The chi-squared test was used to determine significant differences in the numbers of insects choosing a particular odor, with the following levels of significance: $^{*}P < 0.05$ and $^{**}P < 0.01$.

In this study, we cloned five CpalOBP genes from *C. pallens*. qPCR showed that the five genes had diverse expression profiles. Ligand-binding assays and behavioral trials showed that 14 compounds exhibited high binding affinities for the CpalOBPs, of which nine elicited significant behavioral responses from *C. pallens*. The nine compounds that elicited significant behavioral responses could be selected to develop slow-release agents to attract/repel *C. pallens* and to improve the search for strategies to eliminate insect pests.

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