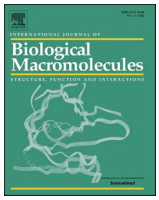
International Journal of Biological Macromolecules 289 (2025) 138475

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



# The morphology and molecular mechanisms of enhanced olfaction in the grain aphid *Sitobion miscanthi*

Jia Fan [a,*,1], Xin Jiang [a,1], Qian Li [a], Miaomiao Yu [a], Siyu Zhang [a], Wenxin Xue [a], Frédéric Francis [b], Changqing Su [c,d], Gudbjorg Inga Aradottir [e], Yanxia Liu [a], Yong Zhang [a,*], Julian Chen [a]

[a] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China
[b] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux B-5030, Belgium
[c] Center for Wetland Conservation and Research, Hengshui University, Hengshui 053000, Hebei Province, People's Republic of China
[d] Collaborative Innovation Center for Wetland Conservation and Green Development of Hebei Province, Hengshui 053000, People's Republic of China
[e] Mamore Research and Innovation Limited, Harpenden, Hertfordshire, UK

ARTICLE INFO

*Keywords:*
Aphid
Olfactory sensillum
Odorant binding protein (OBP)
Parallel reaction monitoring (PRM)
Wing phenotype

ABSTRACT

Winged aphids develop more sensitive olfaction than the wingless phenotype to identify potential habitat from afar. Two types of olfactory sensilla, primary rhinarium (PRh) and secondary rhinarium (SRh) are responsible for aphid olfactory perception, of which, SRh is involved in the perception of both *E*-β-farnesene (EBF) and plant volatiles. Odorant binding proteins (OBPs) play a vital role in the response of insect olfactory nerves located in the rhinarium to external odor stimuli. However, the role of SRhs in winged aphids have received little attention to date and the underlying mechanisms of how OBPs work for the olfactory enhancement in winged aphids are unclear. Here, we compared and analyzed the number of SRhs on the antennae of winged and wingless aphids in the grain aphid *Sitobion miscanthi*, then highly expressed OBPs in antennae of winged aphids were screened based on both transcriptomic and proteomic data and identified by parallel reaction monitoring parallel reaction monitoring (PRM). Furthermore, the affinity of highly expressed OBPs was tested. The results showed that winged aphids have more SRhs, and the three highly expressed OBPs, SmisOBP6/7/10, in winged antennae shared broad and overlapped ligands spectra with plant volatiles and pheromones. Furthermore, OBP9, as an OBP with high expression in antennae, not only binds EBF, but also shows extensive affinity to various ligand types. Our results highlight the importance of the higher number of SRhs as the morphological basis of enhanced olfactory perception in winged aphid and further indicates the involvement of OBP6/7/10 as the molecular support of this process.

## 1. Introduction

A sophisticated olfaction system is vital to insects to detect external odorant stimuli including plant volatiles and pheromones that guide their behavioral responses, such as mating, foraging, host location, and oviposition. Two key pheromones influencing aphid behaviour are the aphid sex and alarm pheromones, for which (1R,4aS,7S,7aR)-nepetalactol and (4aS,7S,7aR)-nepetalactone are the two commonly shared functional components of the aphid sex pheromone [1–3], and (*E*)-β-farnesene (EBF) is the key component of the alarm pheromone that repel aphids in most aphid species [4,5]. Odorant binding proteins (OBPs) carry out a mass-selection from the wide range of volatile chemicals in the insect habitat, and are mainly expressed at the antennae. The affinity spectrum of OBPs, while often broad, is not entirely inclusive. They are responsible for detecting and delivering candidate active compounds or their analogues to olfactory receptors [6,7]. Only matching, specific odorant can activate olfaction receptor and therefore trigger the electrical impulses to the brain, which determines how the insect responds.

Since insect OBPs were first identified in insect antennae [8], their

* Corresponding authors.
*E-mail addresses:* jfan@ippcaas.cn (J. Fan), zhangyong02@caas.cn (Y. Zhang).
[1] Equal contribution.

https://doi.org/10.1016/j.ijbiomac.2024.138475
Received 27 February 2024; Received in revised form 12 August 2024; Accepted 4 December 2024
Available online 16 December 2024

olfactory function has received much interest and been well studied. Although OBPs are generally diverse in insects, aphid OBPs are highly conserved among species. The number of OBP families in aphids is between 9 and 12 (with complete coding sequence, *Acyrthosiphon pisum* [9–12]. Aphid OBP3 [13], OBP7 (*A. pisum, Myzus persicae, Rhopalosiphum padi,* and *S. miscanthi,* [14–18]) and OBP9 (*A. pisum* and *M. persicae*, [16,19]) were successively reported for their affinity with *E*-β-farnesene, a commonly functional component of aphid alarm pheromone. Most of the other reported aphid OBPs show broad binding spectrum to binding plant volatiles [13,16,19].

The grain aphid *S. miscanthi* is a dominant wheat pest in China, and was also widely misidentified and reported as *Sitobion avenae* [20,21]. Depending on environmental cues, through parthenogenetic reproduction, the offspring that has identical genetic background as their mother can develop into either winged or wingless phenotype [22,23]. This adaptive phenotypic plasticity makes aphids an ideal tool for the study of epigenetics. At the initial phase, aphid populations expand very fast with a high proportion of wingless aphids. When the aphid population density increases or the plant quality reduces, a higher proportion of aphids will develop into winged morph and fly away or migrate to search for suitable habitats and start their colony expansion again. Hence, the highly developed chemosensory system of winged aphid is critical for host selection during long-distance migration [24,25].

Generally, there are 5 types of antennal sensilla in aphids, the primary rhinaria (PRh), secondary rhinaria (SRh), sensilla trichoid (type I and type II, STr), sensilla campaniform (SCa) and sensilla coeloconica (SCo). Three of those are mainly involved in chemo-sensory function, namely PRh [24,26,27], SRh [26] and STr type II [28,29], and the first two types are responsible for olfactory perception. The 2 PRhs are separately located on the last segment of the antenna and the segment before it (5th and 6th segments). The four sensilla trichoid type II are located on the top of the last segment. However, the number and location of SRh vary in species and even among phenotypes within the species, developing and appearing only when entering the adult stage. Previous ecological studies showed that the secondary sensory rhinarium play roles in the perception of both plant volatile [26] as well as EBF [25]. Observations of the 4 or 6 groups of neurons in the sensilla of the SRh by transmission electron microscopy also support such a function on multiple odor detection [24].

To provide a detailed insight into how SRhs play a role in aphid olfaction at the molecular level, we chose to study both winged and wingless *S. miscanthi* from the same colony with identical genetic background to compare their antennal sensillum and further analyze the differential expression of antennal OBPs at both mRNA and protein level and identifying it further using the parallel reaction monitoring (PRM) technique. The binding affinities of four OBPs were also tested.

## 2. Materials and methods

### 2.1. Insects

Langfang-1 [21], a grain aphid (*S. miscanthi*) isolate that was originally collected from wheat in Langfang, Hebei province, China was used for morphological observation and sequencing. An isogenic colony was started from a single parthenogenetic female and was maintained on wheat (*Triticum aestivum*) at 21–25 °C on a 16 h: 8 h light/dark cycle. Both winged and wingless aphids of 48–96 h from emergence were used for experiments throughout the study.

### 2.2. Scanning electron microscopy

Isolated aphid heads were kept in 70 % ethanol for 2 days, and put through 80 %, 90 %, 95 %, 100 % ethanol, 5 min for each concentration. After being $CO_2$-dried, using critical point drying technique, specimens were mounted on aluminum stubs, and then gold-coated with a sputter coater (SC502, Polaron, Quorum Technologies, United Kingdom). The antennae were observed with a SEM (Hitachi S-4800 FESEM, Japan) and the sensilla on the antennae were classified according to their surface morphology. The morphology, number and distribution of olfactory sensilla as well as other functional sensilla were observed and recorded from 20 winged and 10 wingless antennae. The number and distribution were recorded across the antennae, mainly from pedicel and the first, third and fourth sub-segments of the flagellum.

### 2.3. Differential expression analysis of transcriptome

The differential expression analysis focusing on 13 OBPs were performed based on the RNA-seq data as in our previous reported work [10]. For details, the FPKM method as described in Mortazavi [30] was employed for the calculation of gene expression. Genes with a fold change $\geq$1.5 or fold change $\leq -1.5$, and $P < 0.05$ were denoted as differentially expressed genes.

### 2.4. TMT quantitative proteomic analysis

#### 2.4.1. Protein extraction

Winged and wingless aphids 48–96 h after emergence were collected separately. 600 pairs of antennae for one replicate were collected into 1.5 mL centrifuge tube and snap frozen by liquid nitrogen, then stored at −80 °C. Three replications were carried out for each phenotype. Antennae were ground into powder in liquid nitrogen, and then 4 times volume of lysis buffer (8 M urea, 1 % Triton-100, 10 mM dithiothreitol, 1 % Protease Inhibitor Cocktail, 3 μM TSA, 50 mM NAM, and 2 mM EDTA) were added. Lysed proteins were isolated through centrifugation at 20,000 ×*g* for 10 min at 4 °C, and precipitated in pre-cold 20 % TCA at 4 °C for 2 h. After washing the protein precipitation by cold acetone for 3 times, it was dissolved into 8 M urea. The concentration of total protein was measured by the BCA protein assay kit (Bio-Rad, Hercules, CA, USA).

#### 2.4.2. Trypsin digestion

Trypsin digestion mainly followed the FASP method [31]. For details, we used 10 mM dithiothreitol to reduce the protein solution at 60 °C for 60 min and 50 mM iodoacetamide to alkylate at room temperature in darkness for 40 min to digestion. After that, we added 300 mM TEAB to dilute the protein sample. Lastly, we added trypsin at a mass ratio of trypsin to the protein of 1: 50 to perform the first digestion at 37 °C, 12 h.

#### 2.4.3. TMT labeling

After trypsin digestion, the peptide was desalted using the Strata X C18 SPE column (Phenomenex, Torrance, CA, USA) and vacuum dried. The peptide was reconstituted in 0.5 M TEAB and treated with TMT reagent (Thermo Fisher Scientific, Carlsbad, CA, USA). Concisely, one unit of TMT reagent was thawed and reconstituted in acetonitrile. The peptide mixtures were then incubated at room temperature for 2 h and pooled, desalted, and dried by vacuum centrifugation.

#### 2.4.4. HPLC fractionation

The trypsin-digested peptides were fractionated by high pH reverse-phase HPLC using Agilent Zorbax Extend C18 column (5 μm particles, 4.2 mm ID, 150 mm length, Agilent, Santa Clara, CA, USA). Firstly, peptides were separated into 75 fractions in a gradient of 8 % to 98 % acetonitrile (pH 9.0) over 60 min. After that, the peptides were combined into 15 fractions and dried by vacuum centrifuging.

#### 2.4.5. LC-MS/MS analysis

The tryptic peptides were dissolved in solvent A (0.1 % formic acid in 2 % acetonitrile) and then loaded directly onto a reversed-phase analytical column of 15 cm in length and 75 μm in diameter (Thermo Fisher Scientific, Carlsbad, CA, USA). The gradient including an increase from 6 % to 24 % solvent B (0.1 % formic acid in 90 % acetonitrile) over

26 min, 24 % to 36 % in 8 min, and climbing to 80 % in 3 min then holding at 80 % for the last 3 min, all performed on an EASY-nLC 1000 UPLC system (Thermo Fisher Scientific, Carlsbad, CA, USA) at a constant flow rate of 350 nL/min. The peptides were processed by NSI source and then analyzed by tandem mass spectrometry (MS/MS) in Orbitrap Fusion (Thermo Fisher Scientific, Carlsbad, CA, USA) coupled to UPLC online. The applied electrospray voltage was 2.0 kV. The *m/z* scan range was 350 to 1550 for a full scan, and intact peptides were detected in the Orbitrap at a resolution of 60,000. Peptides for MS/MS were then selected using the NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 15,000. The data acquisition mode uses a dependent scanning (DDA) program, that is, after the first scan, the first 20 peptides with the highest signal intensity are chosen to enter the peptide precursor and enter the HCD collision cell in turn, using 35 % of the fragmentation energy. Secondary mass spectrometry was performed in sequence. To improve the effective utilization, the automatic gain control was set (AGC) to 5E4, the signal threshold to 5000 ions/s, the maximum injection time to 200 ms, and the dynamic exclusion time of the tandem mass spectrometry scan to 30 s to prevent the replication of the parent ion scanning.

#### 2.4.6. Database search

We used Proteome Discoverer 2.3 (Thermo, USA) to process the resulting MS/MS data. Tandem mass spectra were searched against the OBP queries as well as an integrated local protein database LM2019-0351. The minimum length of the peptide was set to 7 amino acid residues; the maximum number of peptide modifications was 5. In the first search, the mass tolerance for precursor ions was set as 20 ppm and 5 ppm in the main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification and oxidation on Met was specified as variable modifications. The quantitative method was set to TMT-6 plex, and the false discovery rate (FDR) for protein identification and peptide-spectrum match (PSM) identification was set to lower than 1 %. Differentially abundant proteins (DAPs) were chosen to meet the criteria (fold change $\geq$1.4 or fold change $\leq -1.4$, and $P < 0.05$).

### 2.5. Comparison of transcriptomic and proteomic data

According to the DE analysis results of both transcriptome and proteome, Venn diagrams of the differentially expressed antennal OBPs in group 1 (transcripts of winged vs. wingless, UP), group 2 (transcripts of winged vs. wingless, DOWN), group 3 (transcripts of winged vs. wingless, no difference (ND)), group 4 (proteins of winged vs. wingless_UP), group 5 (proteins of winged vs. wingless ND) were constructed using Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/).

### 2.6. Targeted proteomics quantification (PRM)

The PRM approach was used to confirm the changes in DAPs identified in the TMT-based quantitative proteomic analysis. In this experiment, four independent samples, namely complete antennae of winged and wingless aphids, and the antennae after removal of PRhs (5th and 6th segments) of both phenotypes with three biological replicates were characterized by an acquired MS/MS spectrum.

### 2.7. Total protein extraction

Total protein extraction sample was ground individually in liquid nitrogen and lysed in buffer containing 100 mM $NH_4HCO_3$ (pH 8), 6 M Urea and 0.2 % SDS, followed by 5 min of ultrasonication on ice. The lysate was centrifuged (12,000 *g* 15 min, 4 °C)and the supernatant was transferred to a clean tube. Extracts were reduced with 10 mM DTT for 1 h at 56 °C, and subsequently alkylated with iodoacetamide for 1 h at room temperature in the dark. Then samples were mixed with 4 times volume of acetone via vortexing and incubated at −20 °C for 2 h. Then centrifuged and the precipitation was collected and dissolved by dissolution buffer, which contained 0.1 M triethylammonium bicarbonate (TEAB, pH 8.5) and 6 M urea.

#### 2.7.1. Trypsin treatment

Trypsin (3 μL of 1 μg/μL) and TEAB buffer (500 μL of 50 mM) were added into 100 μL lysis buffer with 120 μg of each protein sample lysed into, mixed and digested at 37 °C overnight. Equal volume of 1 % formic acid was then mixed with digested sample and centrifuged (12,000 *g*) for 5 min at room temperature. The supernatant was loaded to the C18 desalting column, washed 3 times with 1 mL of solution (0.1 % formic acid, 4 % acetonitrile), then eluted twice by 0.4 mL of elution buffer (0.1 % formic acid, 75 % acetonitrile). The eluents were combined and lyophilized.

#### 2.7.2. LC-MS/MS analysis pre-experiment

Each sample was mixed with the same amounts of peptides. 1 μg of the mixture was analyzed with "Label-free" method using EASY-nLCTM 1200 UHPLC system (Thermo Fisher, CA, USA) coupled with a Q Exactive series mass spectrometer (Thermo Fisher, CA, USA). The sample was injected into a home-made C18 Nano-Trap column (2 cm × 75 μm, 3 μm) and peptides were separated on a home-made analytical column (15 cm × 150 μm, 1.9 μm). The separated peptides were analyzed by Q Exactive series mass spectrometer (Thermo Fisher, CA, USA), with ion source of Nanospray Flex™(ESI), spray voltage of 2.4 kV and ion transport capillary temperature of 320 °C. Full scan ranges from *m/z* 350 to 1500 with resolution of 60,000 (at m/z 200), an automatic gain control (AGC) target value is 3 × 106 and a maximum ion injection time is 20 ms. The top 20(40) precursors of the highest abundant in the full scan were selected and fragmented by higher energy collisional dissociation (HCD) and analyzed in MS/MS, where resolution is 15,000 (at m/z 200), the automatic gain control (AGC) target value is 5 × 104, the maximum ion injection time is 45 ms, a normalized collision energy of 27 %, an intensity threshold of 2.2 × 104, and the dynamic exclusion parameter of 20 s, the raw data of MS detection is named ".raw". The offline data was searched by PD2.2 software. The "missed cleavage" was set as 0, and 1–3 unique peptides were selected for each protein. After selecting the peptides, the information of the target peptide such as *m/z*, charge number and charge type, were input into the "inclusion list". The mixed peptides described above were analyzed by "full scan" followed by "PRM" pattern. The chromatographic separation and full scan condition are the same as above. The PRM was set as resolution of 30,000 (at 200 m/z) with an AGC target value of 5 × 104, a maximum ion injection time of 80 ms, a normalized collision energy of 27 %. The off-line data was analyzed by Skyline software to determine whether the selected peptides were usable based on reproducibility and stability.

#### 2.7.3. LC-MS/MS Analysis of formal experiment

Same amount of trypsin treated-peptide of each sample was taken, and spiked with an equal amount of the labeled peptide DSPSAPVNVTVR (red bold V for heavy isotope labeling) as an internal standard. Samples were analyzed by "full scan" followed by "PRM" pattern as described above. The off-line data was analyzed by Skyline software, and the peak area was corrected using the internal standard peptide. The final quantity of target proteins in winged and wingless sample groups was normalized by wingless OBP7 using Gq-α protein as the reference.

### 2.8. Binding affinity test

#### 2.8.1. Protein (SmisOBP6/7/9/10) expression and purification

The mature SmisOBP6/7/9/10 (SmisOBP10 with His tags) were obtained within expression vector pET-30a, which mainly following previous work as described by Prestwich [32] and Wang [16] with some modifications. Namely, SmisOBP6/7/9 expression was induced by exposure to 0.8 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at

28 °C for 8 h, while SmisOBP10 was induced by exposure to IPTG at 37 °C for 8 h.

Purification of SmisOBP6/7/9/10 was accomplished with serial chromatographic steps on anion-exchange resins HiTrap Q/SP HP columns (GE Healthcare Biosciences, Uppsala, Sweden). Protein samples were analyzed by SDS-PAGE after every purification step, the concentration of purified protein was conducted by Protein Assay kit (Qubit™ Protein Assay kit, Q33211, Invitorgen), and the purified SmisOBPs were analyzed by liquid chromatography (LC–MS).

### 2.8.2. Fluorescence ligand-binding assay

Fluorescence competitive ligand-binding assays were performed primarily as described by Fan [15]. Fluorescence was measured in a right angle configuration on a Lengguang 970 CRT spectrofluorimeter (Shanghai Jingmi Science, Chengdu, China) at room temperature using a 1-cm light path fluoremeter quartz cuvette. Samples were excited at 337 nm, and emission spectra were recorded between 370 and 500 nm. SmisOBP6/7/9/10 were diluted to a concentration of 2 μM in 16 mM tris buffer with different pH (supplementary Table 2). The affinities of 1-*N*-phenylnaphthylamine (1-NPN) with SmisOBP6/7/9/10 were measured

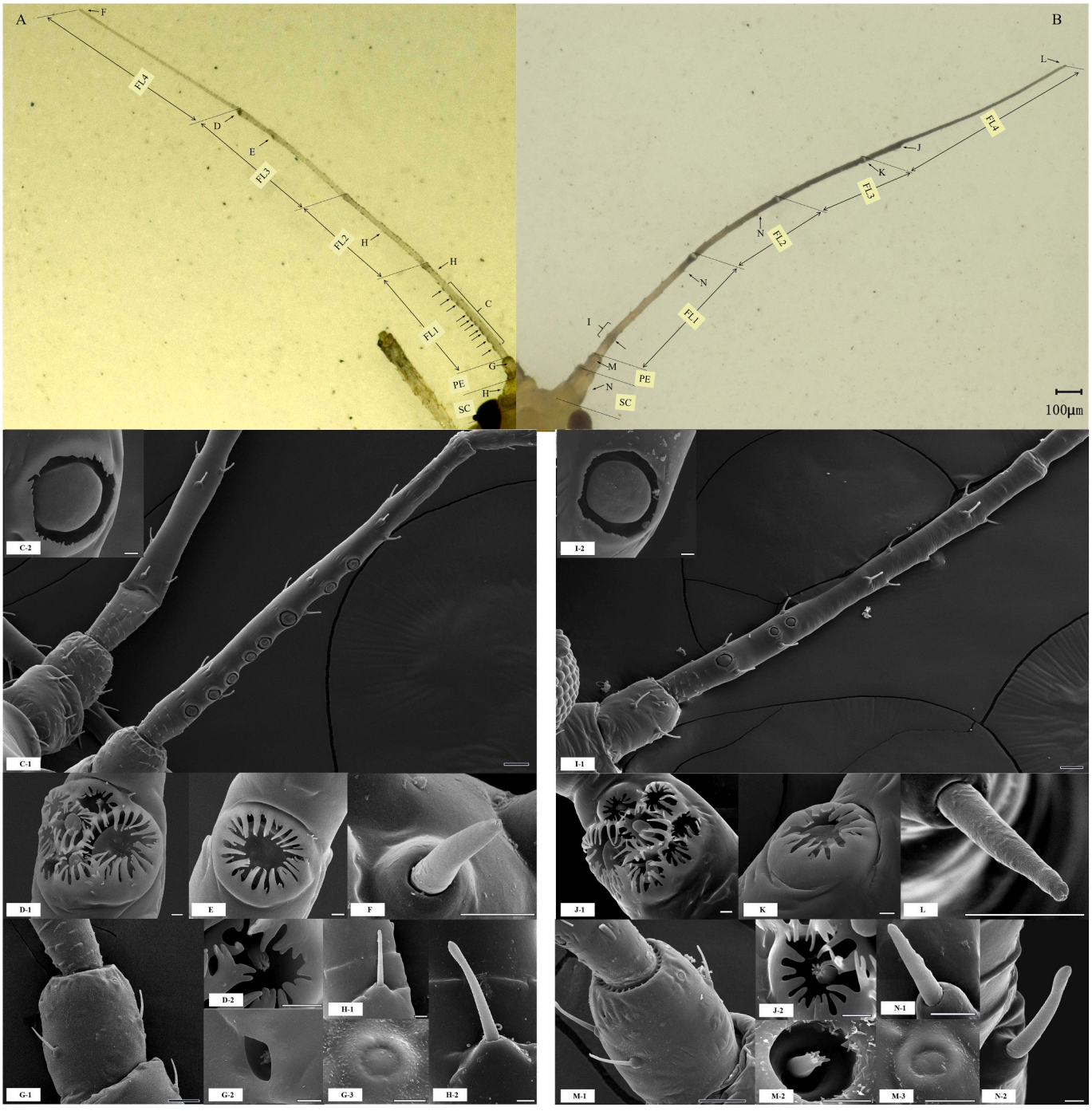



**Fig. 1.** Gross antennal morphology (A/B) and the close-up view (C—N) of sensillum from both winged and wingless parthenogenetic aphids. The antenna consists of 6 segments, numbered from the base and include a SC (scape), a PE (pedicel) and 4 FLs (flagellum). C/I, PRhs on FL3 composed of multiple coeloconic pegs. D-1/J-1, SRhs). E/K, PRhs on FL4. F/L, STr type II. D-2/G-2/J-2/M-2, SCo. G-3/M-3, SCa. H1/N1, STr type I. Scale bar 2 μm for (A), 2 μm for (B–E), 0.05 μm for (F), and 10 μm for (G).

with samples of 1-NPN at a final concentration of 0–16 μM. The dissociation constants of ligands (Ki) were calculated as Ki = [$IC_{50}$]/(1 + [1-NPN]/$K_{1\text{-NPN}}$), with $IC_{50}$ being the concentration of one competitive ligand that caused a 50 % reduction in fluorescence intensity, [1-NPN] being the free 1-NPN concentration and $K_{1\text{-NPN}}$ the association constant of OBP/1-NPN [33]. The curves were linearized using Scatchard plots, and the association constant of 1-NPN ($K_{1\text{-NPN}}$) was estimated on a direct plot through nonlinear regression with an equation corresponding to a single binding site using GraphPad Prism version 7.00 (GraphPad Software, San Diego California, USA).

To investigate the ligand-binding abilities of SmisOBPs, three kinds of competitive ligands were used: (i) aphid alarm pheromone components, including *E*-β-farnesene(EBF), (−)-α-pinene, (−)-β-pinene and (+)-limonene which are released by other aphids following natural enemy predation or physical damage [5,34], (ii) components of the aphid sexual pheromone: (4aSR,7SR,7aRS)-nepetalactone (NEPtone) and (1RS,4aSR,7SR,7aRS)-nepetalactol (NEPtol); (iii) green leaf volatiles: (*Z*)-3-hexen-1-ol, methyl salicylate (MeSA, an insect-induced plant volatile), and (Z)-3-Hexenyl acetate.

## 3. Results

### 3.1. Antennal sensilla of both winged and wingless aphids under SEM

There are 6 segments on the adult antennae of *Sitobion miscanthi* (Fig. 1A/B). They were grouped into scape (SC, 1st segment), pedicel (PE, 2nd segment) and flagellum (FL1-FL4, 3-6th segment) respectively. Firstly, the sensillum type and occurrence position between winged and wingless were identical. Further, there was no statistical difference in the number of each type of sensillum except the SRh (Table 1). There were 2 types of olfactory sensillum, namely PRhs (FL3 and FL4, Fig. 1D/E/J/K) and SRh (FL1, Fig. 1C/I) and they were all located on the flagellum. Meanwhile, STr type II, with gustatory function occurred on the flagellum as well (the end of FL4, Fig. 1F/L). SCo (Fig. 1D-2/G-1/G-2/J-2/M-1/M-2) and SCa (Fig. 1G-1/G-3/M-1/M-3) were present on the pedicel, and STr type I (Fig. 1H/N) were present all over the antennae. In summary, the only difference between antennae of winged and wingless aphids was that winged aphids had significantly more SRhs than wingless aphids (Fig. 1C-1/I-1, Table 1). On average 7 (6–11) and 2 SRhs (1–4) were present on FL1 of winged and wingless aphids respectively (Table 2).

### 3.2. Differential expression of SmisOBPs between winged and wingless antennal transcriptomes

To characterize the phenotype associated SmisOBP expression patterns between winged and wingless aphids, we performed a differential expression analysis based on the antennal transcriptome data.

A total of 147,665 distinct transcripts (mean length = 652 bp) were assembled, 133,331 of which were screened as unigenes (mean length = 594 bp). Gene expression analysis showed that, compared with wingless aphids, there were 1517 genes with significantly differential expression (1117 up-regulated and 410 down-regulated, Supplementary Table 3) in winged antennae. Of 13 SmisOBPs, SmisOBP6/7/10 were up-regulated in winged aphid. On the contrary, SmisOBP1 and SmisOBP5 were up-regulated in wingless aphids (Fig. 2A). the expression of the other SmisOBPs, SmisOBP2/3/4/8/9/13/14 and SmisOBP15 were not statistically different at the mRNA level. The GO classification results on differentially expressed SmisOBPs showed that the genes were mainly enriched in 75 biological processes, 1 cellular component and processes involved in binding functions (Supplementary Table 4). No SmisOBPs were enriched to any known pathway, although 151 pathways enriched 1636 genes (Supplementary Table 5) from the transcriptome. We also noticed that *Gqα,* a previously identified olfactory related gene [35] showed no significant difference between antennae from winged and wingless aphids (Supplementary Table 6) in RNA-seq, which was further selected as a reference protein when processing the PRM data analysis.

### 3.3. Differential expression of SmisOBPs between winged and wingless antennal proteomes

To complement the transcriptomic analysis on the expression pattern of SmisOBPs, we performed TMT (Tandem Mass Tags)- based proteomic analyses on the same tissues.

Of the 4477 proteins quantified in 2 sample groups (3 replications for each sample, Supplementary Table 7), 239 were differentially expressed (68 were up-regulated and 171 were down-regulated) ($P \leq 0.05$) between the antennae of winged and wingless aphids. Abundant value range of quantified proteins was from 10.9 to 189.0, and the expression of all successfully quantified SmisOBPs, namely, SmisOBP2/3/5/6/7/9/10 were higher than 100 (Fig. 2B). Three SmisOBPs, namely SmisOBP6/7/9/10 were expressed significantly higher in the winged phenotype. And SmisOBP2/3/5 did not show significantly different expression pattern between the two phenotypes. No SmisOBP was found to be down-regulated in winged type (Supplementary Table 7) at a protein level. The GO classification results on SmisOBPs showed that SmisOBP2/6/9 were enriched in the same biological process, 2 cellular component and annotated as odorant binding functions (Supplementary Table 8). It was not able to classify SmisOBP3/5/10 under GO enrichment. In addition, there was no SmisOBP enriched to any known pathway either, although up to 235 proteins were enriched in 108 pathways (Supplementary Table 8) from proteome. SmisOBP13 was also detected but it was considered a unreliable results due to extremely low abundance values, furthermore SmisOBP1, SmisOBP4, SmisOBP8, SmisOBP14, SmisOBP15 identified based on antennal transcriptome were not detected at the protein level. Consistent with the differential transcriptome, *Gqα* did not show a significant difference between winged and wingless antennae (Supplementary Table 6) in TMT.

### 3.4. Comparison of transcriptomic and proteomic data

The expression specificity of most SmisOBPs between the process of transcription and translation was consistent, except for SmisOBP5 and SmisOBP9. Three coding SmisOBPs, SmisOBP6/7/10, were significantly higher expressed in antennae of winged aphid at both mRNA and protein level. Besides, the differential comparison results of SmisOBP2/3 remained no statistical difference between phenotypes under both mRNA and protein levels. SmisOBP5 showed comparative expressions between winged and wingless aphids at the protein level, while, at the mRNA level, the expression was significantly higher in wingless aphids. SmisOBP9 was significantly higher in winged antennae at the protein level, but showed no difference at the mRNA level in winged phenotypes. The other 6 SmisOBPs could not be quantified after the secondary

**Table 1**
The Number and distribution of antennal sensilla in winged and wingless of *S. miscanthi.*

| Antennal segments | Sensilla types | Location | |
|---|---|---|---|
| | | Winged aphid | Wingless aphid |
| Scape | STr typeII | ao | ao |
| Pedicel | SCo | 1 | 1 |
| | SCa | 1 | 1 |
| | STr typeII | ao | ao |
| Flagellum 1 | SRh | 7.4 ± 1.1 | 2.3 ± 0.8 |
| | STr typeII | ao | ao |
| Flagellum 2 | STr typeII | ao | ao |
| Flagellum 3 | PRh | 1 | 1 |
| | STr typeII | ao | ao |
| Flagellum 4 | PRh | 1 | 1 |
| | STr typeI | 4 | 4 |
| | STr typeII | ao | ao |

"ao", all over.

**Table 2**
Primers list for *SmisOBP6/7/9/10*.

| Prime name | Melting temperature | Sequence(5′ → 3′) |
|---|---|---|
| *SmisOBP6*-F | 60 °C | CCCATATG GCTGGGTACGATAGAACATGGAT |
| *SmisOBP6*-R | | GCGAATTC TTAAATTAATTTAGGTGGCGATT |
| *SmisOBP7*-F | 60 °C | CCCATATGTACTTGAGTGAAGCGGCCATTAAAA |
| *SmisOBP7*-R | | GCGAATTCCTATAGTGGTAGATACTCTAAACTTCTGGG |
| *SmisOBP9*-F | 62 °C | CCCATATGGCTGATGATGCAGATGCAGGGGAT |
| *SmisOBP9*-R | | GCGAATTCTTATTTCGATTTTGGTTTCATCTTCACC |
| *SmisOBP10*-F | 60 °C | CCGCTCGAGTCACAGTGGCAGTAGTTCAACA |
| *SmisOBP10*-R | | ATTTGCGGCCGCTCACAGTGGCAGTAGTTCAACA |

F: forward primer. R: reverse primer. Underlined showed the *Nde* I (CATATG), *EcoR* I (GAATTC), *Xho* I (GCTCGA), *Not* I (GCGGCCGC) restriction enzymes sites in the forward and reverse primer.

mass spectrum, most likely due to their low abundance in whole tissue homogenates (Supplementary Table 7).

### 3.5. Targeted proteomics quantification (PRM)

In order to confirm the target SmisOBPs expression, we performed PRM analysis that generates full MS/MS data with high resolution and high mass accuracy and is widely used for the quantification of targeted proteins/peptides [36]. The unique peptide of 5 OBPs, including 3 differentially abundant proteins (DAPs, SmisOBP6/7/10) as well as the other two reported aphid EBF binding proteins (SmisOBP3 and SmisOBP9) in the antennae (Fig. 2D/E), were selected for PRM verification. Much larger amounts of SmisOBP6/7/10 were detected in the winged antennae than wingless antennae, confirming the trend in the abundances of these target proteins quantified by TMT. However, SmisOBP9 did not show difference in abundance, which was identical with results of differential expression analysis of transcriptome.

Furthermore, we successfully identified and quantified each OBP in the antennae when the fifth and sixth segments with the 2 PRhs were removed (the sampled antennae were cut from the middle of the fourth segment). The result demonstrated that the abundance of SmisOBP6 and SmisOBP7 in the antennae after the PRh were removed kept consistent in the whole antennae. Besides, when PRhs were removed, the abundance of SmisOBP2 in winged and wingless aphid changed from no difference before to significantly high expression in winged aphid. While, SmisOBP10 changed from significantly high expression in winged aphid to no statistical difference between winged and wingless phenotypes (Fig. 2D).

### 3.6. Broad binding affinities of SmisOBP6/7/9/10

To better understand how winged phenotype enriched SmisOBP6/7/10 contribute to the more developed olfaction of winged aphids, they were expressed and purified for competitive fluorescence ligand binding test to various ecologically significant volatile compounds for aphids (Table 4). Considering that OBP9 has been reported as an EBF binding protein in aphids [16,19] but has not been identified in *S. miscanthi* yet, SmisOBP9 was also included in this experiment. Like most of reported insect SmisOBPs, SmisOBP6/7/9/10 were found expressed in the inclusion bodies, and after a dissolve and refolding treatment, the refolded SmisOBPs were purified resulting in yields of 0.67 mg/mL, 0.58 mg/mL, 0.57 mg/mL and 0.31 mg/mL respectively. The theoretical molecular weight values for each SmisOBP was very close to their measured values (SmisOBP6, 22.83 kDa; SmisOBP7, 14.28 kDa; SmisOBP9, 15.85 kDa; SmisOBP10, 20.25 kDa, Fig. 3). All purified protein samples were further identified by LC-MS/MS (Q-TOF).

The fluorescent competitive binding assays were conducted using the fluorescence probe N-pheny-1-naphthylamine (1-NPN). The dissociation constants of the SmisOBP(6/7/9/10)/1-NPN complex were 2.35 ± 0.41, 0.27 ± 0.05, 0.27 ± 0.01 and 10.56 ± 2.73 μM respectively (Fig. 3, Table 3).

In the ligand binding assays, SmisOBP6 showed a wide affinity spectrum to diverse levels to plant VOCs, aphid alarm pheromone and aphid sex pheromone. In details, the affinity to (−)-β-pinene (Ki = 9.30 ± 0.19), (+)-Limonene (9.63 ± 0.53) and (Z)-3-hexenol (10.87 ± 0.95) which are three typical component of green leave volatiles, as well as NEPtol (10.67 ± 1.47, one of the two components of aphid sex pheromone) were comparatively stronger than to other ligands, while the affinity to MeSA was the weakest (Ki = 29.09 ± 1.43, lower Ki value means higher binding affinity). For the other ligands including EBF, NEPtone and some plant VOCs, SmisOBP6 showed modest binding properties (Fig. 3, Table 3).

The affinity of SmisOBP7 to EBF, the common active component of aphid alarm pheromone, was higher (Ki =0.24 ± 0.01) than to all the other ligands. We found that SmisOBP7 also had a wide binding spectrum with a range of affinity values much larger (from 0.24 to 11.66) than SmisOBP6. Apart from EBF, it also showed stronger affinities with NEPtol and NEPtone (Ki = 2.43 ± 0.25, sex pheromone) as well as (−)-β-pinene (Ki = 1.93 ± 0.29) and (+)-Limonene (Ki = 2.57 ± 0.11) which are two green leaf volatiles (Fig. 3, Table 4). Among all ligands, affinity of SmisOBP10 to NEPtone was the strongest (Ki =9.71 ± 0.52) and to (−)-α-pinene was the weakest (Ki = 13.82 ± 1.24). Moreover, SmisOBP10 lacked affinity to EBF, (−)-β-pinene and (+)-limonene (Fig. 3, Table 4). The affinity of SmisOBP9 to EBF was stronger (Ki = 0.27 ± 0.01) than to the other ligands. More than that, SmisOBP9 showed broad affinities with strong binding properties to all testing ligands (Ki = 0.27–2.97, Fig. 3, Table 4) including plant VOCs and sex pheromones (NEPtone and NEPtol).

## 4. Conclusion and discussion

The significant differences in the morphological structure of antennae between winged and wingless aphid is that the winged aphid has more SRhs. Three OBPs were identified to be highly expressed in the antennae of winged aphids, their binding characteristics showing a complementary and overall extensive pattern. In addition, OBP9 was shown to be a highly expressed and stable OBP in the antennae of wheat aphid, not only binding strongly to EBF, but also has the property of widely binding various ligand types, which may play an important role in the functional maintenance of the olfactory system of aphids.

### 4.1. A higher number of SRhs is the morphological basis of enhanced olfaction in winged aphids

Although there is only one olfactory sensillum type, namely sensilla placoid, that is responsible for aphid olfaction, they are further grouped into two types, PRhs and SRhs, according to their occurrence and timing of development. The PRh is a type sensillum that the aphid nymph is born with and has the same number and location on the antennae of different developmental stages and phenotypes. On the contrary, SRh does not occur until the aphids develop into the adult stage and vary in quantity, contributing to the dimorphism in the antennae between the winged and wingless morphs. In the present study, the wingless *S. miscanthi* has an average 2 SRhs versus much higher number of 7 SRhs

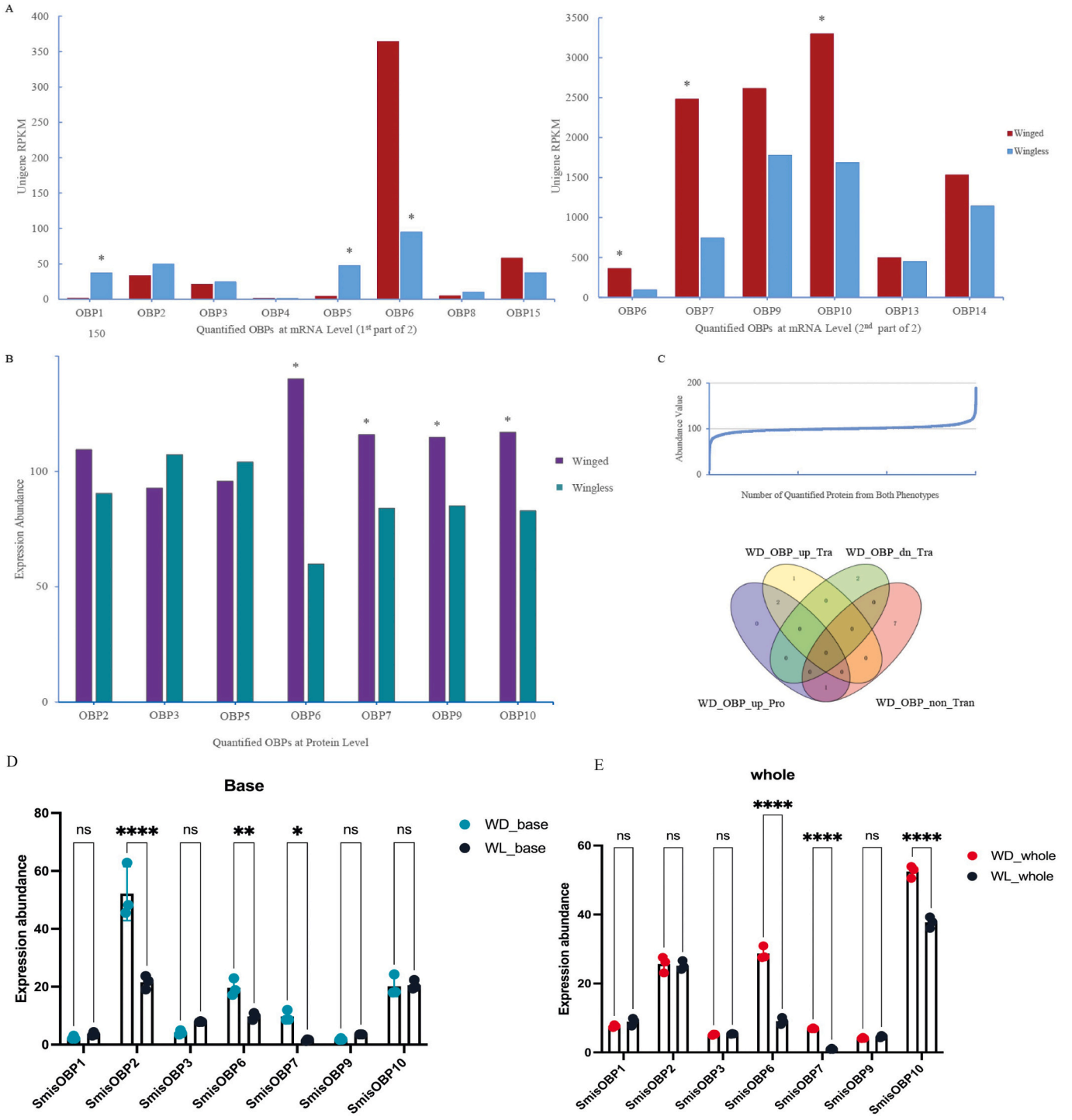



**Fig. 2.** The expression levels of differentially expressed OBPs in antennae. A. The comparative expressions of OBPs at transcript level (2nd generation RNA-seq). B. The comparative expression level of OBPs at protein level (TMT). C. combined analysis. D/E. Targeted proteomics quantification (parallel reaction monitoring, PRM).

in the winged phenotype, which is the main difference in both quantity and type of sensilla between winged and wingless antennae. We therefore conclude that the extra SRhs is the morphological basis of the more developed olfaction in winged *S. miscanthi*. SRhs are not developed at all in certain phenotypes of some aphid species, such as the wingless phenotype of the peach aphid *Myzus percicae* [26,37] and the soybean aphid *Aphis glycines* [25]. The SRhs in winged *A. glycines* can independently detect EBF without PRh. While, when PRhs of the wingless soybean aphid phenotype were removed, as it congenitally has no SRh, its ability to detect EBF was eliminated [27]. In the same way, the removal of PRhs made wingless pea aphid incapable in responding EBF also [37]. As one of the two olfactory sensilla types, the SRh is involved in the perception of plant volatiles [26,38] and EBF [25,37]. A more developed olfaction of winged virginoparae is critical for the aphid as the particular biological function of winged genotype is migration or flying away and depending on the chemical clues, targeting new habitats for achieving colonial expansion at a larger scale.

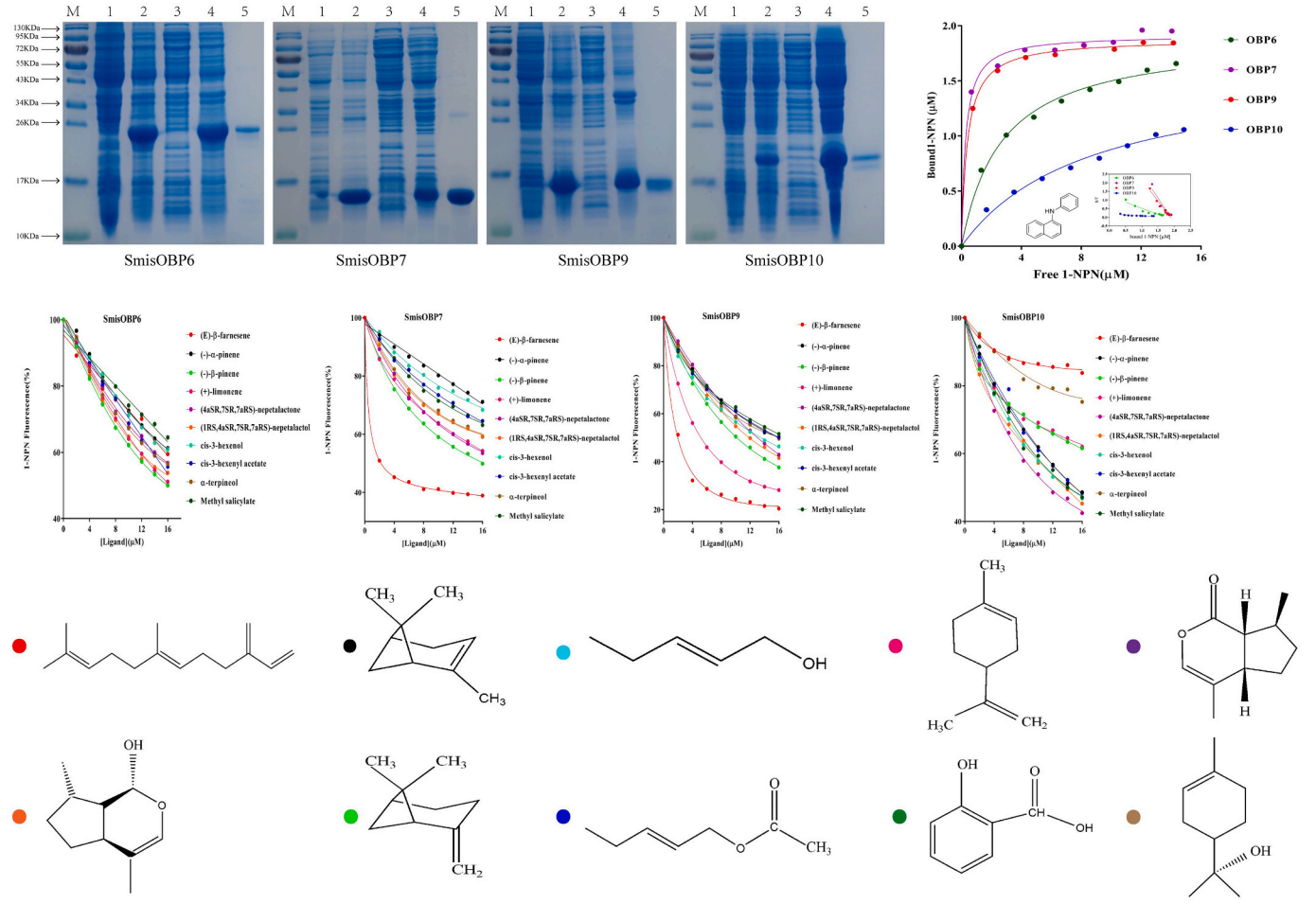



**Fig. 3.** Competitive binding curves of SmisOBP6/7/9/10 to components of aphid alarm pheromones((*E*)-β-Farnesene, (−)-α-pinene, (−)-β-pinene, (+)-limonene), aphid sexual pheromones ((4aSR 7SR 7aRS)-nepetalactone, (1RS 4aSR 7SR 7aRS)-nepetalactol) and the volatiles of wheat green leaf ((Z)-3-Hexen-1-ol, (Z)-3-Hexenyl acetate, Methyl salicylate, α-terpineol). A mixture of the recombinant protein and *N*-phenyl-1-naphthylamine (1-NPN) in 50 mM Tris-HCl buffer (pH 7.4) both at the concentration of 2 μM was titrated with 1 mM solutions of each competing ligand to the final concentration range of 2 μM to 16 μM. Fluorescence values were presented as percent of the values in the absence of competitor. Dates are means of three independent experiments.

**Table 3**
Calculated association constants of OBPs and 1-NPN (*N*-phenyl-1-naphthylamine) probe complexes.

| Protein name | SmisOBP6 | SmisOBP7 | SmisOBP9 | SmisOBP10 |
|---|---|---|---|---|
| Kd(μM) | 2.352 ± 0.45 | 0.27 ± 0.05 | 0.39 ± 0.03 | 10.56 ± 2.73 |

### 4.2. *Highly expressed OBP6/7/10 in antennae of winged aphid*

The highly enriched SmisOBP6/7/10 and their combined binding spectrum revealed the molecular support of an all-round enhanced olfaction in winged aphids.

There has been a comprehensive progress on odorant binding affinities of aphid OBPs in vitro since 2009 [13]. The sub-cellular location of several OBPs have also been uncovered [14,39,40] but only on either on winged or wingless aphids, none have been confirmed on both phenotypes to date. Around 10 OBPs are expressed in only 2 types of olfactory rhinaria, which makes it quite difficult to clarify and get a global view of expression pattern in aphids and to further exploit the mechanism of the enhanced olfaction in winged phenotype.

In the present study, we reported, for the first time, an integrated transcriptomic and proteomic analysis on aphid OBPs of both winged and wingless phenotypes. All 3 DAP SmisOBPs, SmisOBP6/7/10, showed higher expression in winged antennae at transcriptomic (illumina) and proteomic (TMT) level, which were further identified by PRM. Most OBPs are secretory proteins. Which can help to understand why the expression abundance of some OBP in complete antennae is inconsistent with that after removal of PRhs. OBP10, which was originally highly expressed in antennae (complete ones) of winged aphid, showed no difference between winged and wingless aphids after PRh removal. This may be due to the incision caused by the removal of PRhs, resulting in the loss of a large amount of OBP10 along with lymph, resulting in no difference being established.

We further performed a competitive ligand binding test for OBPs. Of the candidate ligands, α-pinene, β-pinene and limonene are generally recognized as plant VOCs. However, their function as aphid alarm pheromone was also reported in the vetch aphid *Megoura viciae* [34]. Therefore, our results can also be summarized as follows. SmisOBP6 showed affinity mainly to plant VOCs as well as the aphid sex pheromone. SmisOBP7 has stronger binding property to pheromones including the alarm pheromone and sex pheromone. And SmisOBP10 displayed high affinity with the sex pheromone. Prior work has shown that OBP7 responds by upregulating its expression to EBF induction in *Sitobion miscanthi*, with up-regulation of OBP7 in winged phenotype being particularly strong [41]. Further, higher expression of OBP6/7/10 in the antennae of the winged aphids and their broad binding spectra to volatile molecules demonstrate well how the extra secondary SRhs enhance the olfaction of winged aphids.

To our knowledge, NEPtone or NEPtol could evoke both positive

**Table 4**
Binding affinities of SmisOBPs for candidate ligands, evaluated in displacement binding assays using the fluorescent probe, 1-NPN.

| Code | OBP6 | | OBP7 | | OBP9 | | OBP10 | |
|---|---|---|---|---|---|---|---|---|
| | LC50 | Ki(μM) | LC50 | Ki(μM) | LC50 | Ki(μM) | LC50 | Ki(μM) |
| (*E*)-β-farnesene | 25.89 ± 1.81 | 13.99 ± 0.97 | 2.04 ± 0.11 | 0.24 ± 0.01 | 1.66 ± 0.08 | 0.27 ± 0.01 | – | – |
| | 36.15.57 | | 36.15.57 | | 6.59 | | – | |
| | 54.81 | | 54.81 | | 65.57 | | 55.03 | |
| | 65.57 | | 65.57 | | 70.32 | | 34.57 | |
| | 56.75 | | 56.75 | | 61.57 | | 56.32 | |
| | 41.78 | | 41.78 | | 44.19 | | 30.89 | |
| | 55.60 | | 55.60 | | 65.01 | | 53.20 | |
| | 36.33 | | 36.33 | | 57.27 | | 26.29 | |
| | – | | – | | 54.39 | | 66.25 | |
| | – | | – | | 29.02 | | 59.56 | |
| | – | | – | | 53.23 | | 62.19 | |
| | – | | – | | 33.06 | | 53.01 | |
| | – | | – | | – | | – | |
| | – | | – | | – | | – | |
| | – | | – | | 66.66 | | – | |
| | – | | – | | 84.74 | | – | |
| | – | | – | | – | | – | |
| | – | | – | | – | | – | |
| | – | | – | | 59.51 | | – | |
| | – | | – | | 40.68 | | – | |
| (4aSR,7SR,7aRS)-Nepetalactone | 25.02 ± 0.90 | 13.52 ± 0.48 | 20.42 ± 2.08 | 2.43 ± 0.25 | 13.13 ± 1.46 | 2.16 ± 0.24 | 11.55 ± 0.61 | 9.71 ± 0.52 |
| (1RS, 4aSR, 7SR,7aRS)-Nepetalactol | 19.73 ± 2.72 | 10.67 ± 1.47 | 27.14 ± 0.87 | 3.23 ± 0.10 | 10.81 ± 1.53 | 1.78 ± 0.25 | 13.68 ± 3.27 | 11.67 ± 2.75 |
| Methyl salicylate | – | 29.09 ± 1.43 | 43.93 ± 3.56 | 4.90 ± 0.42 | 20.26 ± 3.82 | 3.32 ± 0.63 | 14.57 ± 2.27 | 12.25 ± 1.91 |
| α-pinere | | | | | | | | |
| (+)-Limonene | 18.44 ± 0.11 | 9.63 ± 0.53 | 21.64 ± 0.93 | 2.57 ± 0.11 | 5.39 ± 0.47 | 0.89 ± 0.08 | – | – |
| (Z)-3-hexenol | 20.12 ± 1.76 | 10.87 ± 0.95 | – | 7.49 ± 0.90 | 16.04 ± 4.39 | 2.64 ± 0.72 | 14.86 ± 2.31 | 12.49 ± 2.75 |
| (Z)-3-hexenyl acetate | 24.73 ± 5.44 | 13.36 ± 2.94 | 48.32 ± 2.70 | 5.75 ± 0.32 | 16.88 ± 0.68 | 2.77 ± 0.11 | 15.97 ± 0.20 | 13.77 ± 0.72 |
| (−)-α-pinene | 32.96 ± 5.47 | 17.81 ± 2.95 | – | 11.66 ± 2.00 | 16.47 ± 0.91 | 3.07 ± 0.81 | 16.44 ± 1.47 | 13.82 ± 1.24 |
| (−)-β-pinene | 17.22 ± 0.34 | 9.30 ± 0.19 | 16.23 ± 2.44 | 1.93 ± 0.29 | 10.06 ± 0.32 | 1.63 ± 0.05 | – | – |
| α-terpineol | 23.98 ± 1.85 | 13.76 ± 1.00 | 32.03 ± 2.60 | 3.81 ± 0.31 | 18.67 ± 4.91 | 3.07 ± 0.81 | – | – |

"–", not detectable.

electro-physiological and behavioral responses of asexual aphids [42,43]. However, it remains unclear how virginoparae detect sex pheromone. As suggested in *M. persicae* [44], an explanation could be that as the sexual and asexual aphids use the same set of genes, the active response is an evolutionary relic in virginoparae. Otherwise, the function of NEP other than sexual attraction has long been demonstrated, such as for eliciting aggregate behaviors in gynoparous females [42]. It also evokes repellant responses at high doses [44] over attractive effect to males as well as gynoparae implied a potentially novel function, repellence, of NEP in virginoparae. Whatever the function is, our present study revealed molecular basis of the active electrophysiological responses to NEP in virginoparous aphids.

The differential expression analysis results based on illumina, TMT and PRM platform were mostly consistent. However, it is not difficult to see from the results that there is variability in the process from gene transcription, translation and expression. For example, the SmisOBP5 was up-regulated in wingless aphid at mRNA level but did not show a specific expression at protein level. Moreover, in TMT (protein level), the abundance values among detected coding SmisOBPs were comparative (from 36.7 to 156.2, Fig. 2). But in RNA-seq (mRNA level), the range of expression abundance values is broad (FPKM from 1.4 to 3299.08, Supplementary Table 3). Take SmisOBP6, SmisOBP9 and SmisOBP10 of winged aphids for example, SmisOBP6 was one of the most abundant SmisOBP although the transcript level is only about 14.7 % of SmisOBP9 and 11.0 % of SmisOBP10 (FPKM 364.1, 2484.7 and 3299.0 respectively, Fig. 2A, B). This indicates a more efficient translation rate of SmisOBP6. All the above further suggests that after transcription, the translation and/or post-transcription regulation is complicated. After being transcribed, cellular abundance of proteins is predominantly controlled at the level of translation process could explain the reason mRNA and protein correlate poorly [45]. This is actually quite common in mammals where changes in protein levels generally are not accompanied by changes in corresponding mRNAs, as gene expression is a complicated process that involves the transcription, translation, turnover of messenger RNAs and proteins. Another fact is that OBPs are secreted by the auxiliary cells such as trichogen and tormogen cells into the extracellular sensillum hemolymph [46–48]. This means that the tissue in which the gene is transcribed does not necessarily correspond to the tissue in which the expressed protein works. Further disassembly of the antennae could result in the loss of a large amount of hemolymph and, therefore, the loss of a high concentration of target OBP dissolved in the hemolymph, and this loss is difficult to evaluate and compare. This could be one reason for the inconsistent results between the transcriptomic and proteomic analysis and is important to report and consider.

### *4.3. Highly expressed and stimulus-sensitive OBP9 without phenotypic difference*

Consistent with the reported function in other aphids, we found strong binding ability of SmisOBP9 to EBF in *S. miscanthi*. In addition, due to the selection of a wide range of ligand types (alarm pheromone, sex pheromone and plant VOCs), we further revealed that OBP9 has a wide range of strong binding capabilities for each type of ligand (Fig. 3). Moreover, OBP9 showed stable and undifferentiated expression at both protein and transcriptome levels (Fig. 2A/B). In previous qPCR tests of OBP9, we also found such a consistent pattern [41]. To sum up, OBP9 responds quickly and strongly to the induction of affinity substances such as EBF in both winged and wingless phenotypes of *S. miscanthi*. In conclusion, SmisOBP9 may function as one fundamental molecule in the basic olfaction of aphids to various odors.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2024.138475.

## CRediT authorship contribution statement

**Jia Fan:** Writing – review & editing, Writing – original draft, Methodology. **Xin Jiang:** Writing – review & editing, Writing – original

draft, Methodology. **Qian Li:** Methodology. **Miaomiao Yu:** Investigation. **Siyu Zhang:** Investigation. **Wenxin Xue:** Investigation. **Frédéric Francis:** Supervision. **Changqing Su:** Methodology, Investigation. **Gudbjorg Inga Aradottir:** Writing – review & editing. **Yanxia Liu:** Resources. **Yong Zhang:** Resources. **Julian Chen:** Formal analysis.

## Declaration of competing interest

The authors have declared no conflict of interest.

## Acknowledgements

National Key R&D Program of China (2024YFD1400400, 2023YFD1400800), The National Natural Science Foundation of China (31871966), Postdoctoral innovation project of Shandong province (SDBX2023059), Open Foundation of Collaborative Innovation Center for Wetland Conservation and Green Development of Hebei Province (No. 2023hbxczx1-2).

## Data availability

Data will be made available on request.

## References

[1] D. Marsh, Sex pheromone in the aphid *Megoura viciae*, Nat. New Biol. 238 (1972) 31–32, https://doi.org/10.1038/newbio238031a0.
[2] G.W. Dawson, D.C. Griffiths, N.F. Janes, A. Mudd, J.A. Pickett, L.J. Wadhams, Christine M. Woodcock, Identification of an aphid sex pheromone, Nature 325 (1987) 614–616, https://doi.org/10.1038/325614a0.
[3] C. Sims, M.A. Birkett, N.J. Oldham, R.A. Stockman, D.M. Withall, Pea aphid odorant-binding protein ApisOBP6 discriminates between aphid sex pheromone components, aphid alarm pheromone and a host plant volatile, Insect Biochem. Mol. Biol. 162 (2023) 104026, https://doi.org/10.1016/j.ibmb.2023.104026.
[4] W.S. Bowers, L.R. Nault, R.E. Webb, S.R. Dutky, Aphid alarm pheromone: isolation, identification, synthesis, Science 177 (1972) 1121–1122, https://doi.org/10.1126/science.177.4054.1121.
[5] F. Francis, S. Vandermoten, F. Verheggen, G. Lognay, E. Haubruge, Is the (E)-β-farnesene only volatile terpenoid in aphids? J. Appl. Entomol. 129 (2005) 6–11, https://doi.org/10.1111/j.1439-0418.2005.00925.x.
[6] P. Pelosi, J.J. Zhou, L.P. Ban, M. Calvello, Soluble proteins in insect chemical communication, Cell. Mol. Life Sci. 63 (2006) 1658–1676, https://doi.org/10.1007/s00018-005-5607-0.
[7] J. Fan, F. Francis, Y. Liu, J.L. Chen, D.F. Cheng, Review an overview of odorant-binding protein functions in insect peripheral olfactory reception, Genet. Mol. Res. 10 (2011) 3056–3069, https://doi.org/10.4238/2011.December.8.2.
[8] R. Vogt, L. Riddiford, Pheromone binding and inactivation by moth antennae, Nature 293 (1981) 161–163, https://doi.org/10.1038/293161a0.
[9] J.J. Zhou, F.G. Vieira, X.L. He, C. Smadja, R. Liu, J. Rozas, et al., Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid *Acyrthosiphon pisum*, Insect Biochem. Mol. Biol. 19 (2010) 113–122, https://doi.org/10.1111/j.1365-2583.2009.00919.x.
[10] W. Xue, J. Fan, Y. Zhang, Q. Xu, Z. Han, J. Sun, et al., Identification and expression analysis of candidate odorant-binding protein and chemosensory protein genes by antennal transcriptome of *Sitobion avenae*, PloS One 11 (2016) e0161839, https://doi.org/10.1371/journal.pone.0161839.
[11] S.H. Gu, K.M. Wu, Y.Y. Guo, L.M. Field, J.A. Pickett, Y.J. Zhang, 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 8 (2013) e73524, https://doi.org/10.1371/journal.pone.0073524.
[12] L. Wang, Y.D. Bi, M. Liu, W. Li, M. Liu, S.F. Di, et al., Identification and expression profiles analysis of odorant-binding proteins in soybean aphid, *Aphis glycines* (hemiptera: aphididae), Insect. Sci. 27 (2020) 1019–1030, https://doi.org/10.1111/1744-7917.12709.
[13] H. Qiao, E. Tuccori, X. He, A. Gazzano, L. Field, J.J. Zhou, et al., Discrimination of alarm pheromone (E)-β-farnesene by aphid odorant-binding proteins, Insect Biochem. Mol. Biol. 39 (2009) 414–419, https://doi.org/10.1016/j.ibmb.2009.03.004.
[14] Y.F. Sun, F. De Biasio, H.L. Qiao, I. Iovinella, S.X. Yang, Y. Ling, et al., Two odorant-binding proteins mediate the behavioral response of aphids to the alarm pheromone (E)-β-farnesene and structural analogues, PloS One 7 (2012) e32759, https://doi.org/10.1371/journal.pone.0032759.
[15] J. Fan, W. Xue, H. Duan, X. Jiang, Y. Zhang, W. Yu, et al., Identification of an intraspecific alarm pheromone and two conserved odorant-binding proteins associated with (E)-β-farnesene perception in aphid *Rhopalosiphum padi*, J. Insect Physiol. 101 (2017) 51–160, https://doi.org/10.1016/j.jinsphys.2017.07.014.
[16] Q. Wang, J.T. Liu, Y.J. Zhang, J.L. Chen, S.H. Gu, Coordinative mediation of the response to alarm pheromones by three odorant binding proteins in the green peach aphid *Myzus persicae*, Insect Biochem. Mol. Biol. 130 (2021) 103528, https://doi.org/10.1016/j.ibmb.2021.103528.
[17] R. Zhang, B. Wang, G. Grossi, P. Falabella, Y. Liu, S. Yan, et al., Molecular basis of alarm pheromone detection in aphids, Curr. Biol. 27 (2017) 55–61, https://doi.org/10.1016/j.cub.2016.10.013.
[18] T. Zhong, J. Yin, S. Deng, K. Li, Y. Cao, Fluorescence competition assay for the assessment of green leaf volatiles and trans-β-farnesene bound to three odorant binding proteins in the wheat aphid *Sitobion avenae* (Fabricius), J. Insect Physiol. 58 (2012) 771–781, https://doi.org/10.1016/j.jinsphys.2012.01.011.
[19] Y.G. Qin, Z.K. Yang, D.L. Song, Q. Wang, S.H. Gu, W.H. Li, et al., Bioactivities of synthetic salicylate-substituted carboxyl (E)-β-farnesene derivatives as ecofriendly agrochemicals and their binding mechanism with potential targets in aphid olfactory system, Pest Manag. Sci. 76 (2020) 2465–2472, https://doi.org/10.1002/ps.5787.
[20] G. Zhang, Aphids in Agriculture and Forestry of Northwest China, 1st ed., China Environmental Science, Beijing, 1999.
[21] X. Jiang, Q. Zhang, Y. Qin, S. Zhang, Q. Li, Y. Zhang, et al., A chromosome-level draft genome of the grain aphid *Sitobion miscanthi*, GigaScience 8 (2019) 1–8, https://doi.org/10.1093/gigascience/giz101.
[22] G. Kunert, S. Otto, U.S.R. Rose, J. Gershenzon, W.W. Weisser, Alarm pheromone mediates production of winged dispersal morphs in aphids, Ecol. Lett. 8 (2005) 596–603, https://doi.org/10.1111/j.1461-0248.2005.00754.x.
[23] J. Fan, Y. Zhang, F. Frédéric, D. Cheng, J. Sun, J. Chen, Orco mediates olfactory behaviors and winged morph differentiation induced by alarm pheromone in the grain aphid, *Sitobion avenae*, Insect. Biochem. Molec. 64 (2015) 16–24, https://doi.org/10.1016/j.ibmb.2015.07.006.
[24] A.K. Bromley, J.A. Dunn, M. Anderson, Ultrastructure of the antennal sensilla of aphids. I. Coeloconic and placoid sensilla, Cell Tissue Res. 203 (1979) 427–442, https://doi.org/10.1007/BF00233272.
[25] Y. Du, F. Yan, X. Han, G. Zhang, Olfaction in host plant selection of the soybean aphid *Aphis glyines*, Acta. Entomologica. Sinica. 99 (1994) 1423–1425, https://doi.org/10.5334/pia.159.
[26] J. Pickett, L. Wadhams, C. Woodcock, J. Hardie, The chemical ecology of aphids, Annu. Rev. Entomol. 37 (1992) 67–90, https://doi.org/10.1146/ANNUREV.EN.37.010192.000435.
[27] Y. Du, J. Tangm, Structure and function of antennal olfactory sensilla of soybean aphid *Aphis glycines*, Acta Entomol. Sin. 38 (1995) 7.
[28] R.J. Wensler, Mode of host selection by an aphid, Nature 195 (1962) 830–831.
[29] G. Powell, J. Hardie, J.A. Pickett, Behavioural evidence for detection of the repellent polygodial by aphid antennal tip sensilla, Physiol. Entomol. 20 (2010) 141–146, https://doi.org/10.1111/j.1365-3032.1995.tb00810.x.
[30] A. Mortazavi, B.A. Williams, K. Mccue, L. Schaeffer, B. Wold, Mapping and quantifying mammalian transcriptomes by RNA-Seq, Nat. Methods 5 (2008) 621–628, https://doi.org/10.1038/nmeth.1226.
[31] J. Wisniewski, A. Zougman, N. Nagaraj, M. Mann, Universal sample preparation method for proteome analysis, Nat. Methods 5 (2009) 6, https://doi.org/10.1038/NMETH.1322.
[32] G.D. Prestwich, Bacterial expression and photoaffinity labeling of a pheromone binding protein, Protein Sci. 2 (1993) 420–428, https://doi.org/10.1002/pro.5560020314.
[33] L. Ban, A. Scaloni, C. D'Ambrosio, L. Zhang, Y. Yan, P. Pelosi, Biochemical characterization and bacterial expression of an odorant-binding protein from *Locusta migratoria*, Cell. Mol. Life. Sci. 60 (2003) 390–400, https://doi.org/10.1007/s000180300032.
[34] X. Song, Y.G. Qin, Y. Yin, Z.X. Li, Identification and behavioral assays of alarm pheromone in the vetch aphid *Megoura viciae*, J. Chem. Ecol. 47 (2021) 740–746, https://doi.org/10.1007/s10886-021-01297-4.
[35] J. Fan, J.L. Chen, D.F. Cheng, J.R. Sun, Cloning and eukaryotic expression of olfaction-related Gqα-protein gene in English grain aphid (*Sitobion avenae*), Chin. J. Agric. Biotechnol. 6 (2009) 111–117, https://doi.org/10.1017/S1479236209990180.
[36] P. Majovsky, C. Naumann, C.W. Lee, I. Lassowskat, M. Trujillo, N. Dissmeyer, W. Hoehenwarter, Targeted proteomics analysis of protein degradation in plant signaling on an LTQ-Orbitrap mass spectrometer, J. Proteome Res. 13 (2014) 4246–4258, https://doi.org/10.1021/pr500164j.
[37] L.R. Nault, L.J. Edwards, W.E. Styer, Aphid alarm pheromones: secretion and reception, Environ. Entomol. 2 (1973) 101–105, https://doi.org/10.1093/ee/2.1.101.
[38] Y.F. Shun, J.H. Visser, Electroantennogram responses of the cereal aphid *Sitobion avenae* to plant volatile components, Physiol. Entomol. (1982), https://doi.org/10.1111/j.1365-3032.1980.tb00218.x.
[39] F.D. Biasio, L. Riviello, D. Bruno, A. Grimaldi, T. Congiu, Y.F. Sun, et al., Expression pattern analysis of odorant-binding proteins in the pea aphid *Acyrthosiphon pisum*, Insect. Sci. 22 (2015) 220–234, https://doi.org/10.1111/1744-7917.12118.
[40] B. Daniele, G. Gerarda, S. Rosanna, S. Andrea, F. Donatella, G. Annalisa, et al., Sensilla morphology and complex expression pattern of odorant binding proteins in the vetch aphid *Megoura viciae* (hemiptera: aphididae), Front. Physiol. 9 (2018) 777, https://doi.org/10.3389/fphys.2018.00777.
[41] S. Zhang, Q. Zhang, X. Jiang, Q. Li, Y. Qin, W. Wang, et al., Novel temporal expression patterns of ebf-binding proteins in wing morphs of the grain aphid *Sitobion miscanthi*, Front. Physiol. 12 (2021) 732578, https://doi.org/10.3389/fphys.2021.732578.

[42] J. Hardie, J.R. Storer, F.J. Cook, C.A.M. Campbell, L.J. Wadhams, R. Lilley, et al., Sex pheromone and visual trap interactions in mate location strategies and aggregation by host-alternating aphids in the field, Physiol. Entomol. 21 (1996) 97–106, https://doi.org/10.1111/j.1365-3032.1996.tb00841.

[43] K.C. Park, J. Hardie, Functional specialisation and polyphenism in aphid olfactory sensilla, J. Insect Physiol. 48 (2002) 527–535, https://doi.org/10.1016/S0022-1910(02)00082-3.

[44] G.M. Fernández-Grandon, C.M. Woodcock, G.M. Poppy, Do asexual morphs of the peach-potato aphid, *Myzus persicae*, utilise the aphid sex pheromone? Behavioural and electrophysiological responses of *M. persicae* virginoparae to (4aS,7S,7aR)-nepetalactone and its effect on aphid performance, Bull. Entomol. Res. 103 (2013) 466–472, https://doi.org/10.1017/S0007485313000126.

[45] B. Schwanhusser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, et al., Corrigendum: global quantification of mammalian gene expression control, Nature 495 (2013) 126–127, https://doi.org/10.1038/nature10098.

[46] R.A. Steinbrecht, M. Ozaki, G. Ziegelberger, Immunocytochemical localization of pheromone-binding protein in moth antennae, Cell Tissue Res. 270 (1992) 287–302, https://doi.org/10.1007/BF00328015.

[47] R.A. Steinbrecht, Experimental morphology of insect olfaction: tracer studies, X-ray microanalysis, autoradiography, and immunocytochemistry with silkmoth antennae, Microsc. Res. Tech. 22 (1992) 336–350, https://doi.org/10.1002/jemt.1070220404.

[48] R. Maida, A. Steinbrecht, G. Ziegelberger, P. Pelosi, The pheromone binding protein of *Bombyx mori*: purification, characterization and immunocytochemical localization, Insect Biochem. Mol. Biol. 23 (1993) 243–253, https://doi.org/10.1016/0965-1748(93)90005-D.