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Functional analysis of odorant-binding proteins for the parasitic host location to implicate convergent evolution between the grain aphid and its parasitoid *Aphidius gifuensis*

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ABSTRACT

(*E*)-β-farnesene (EBF) is a typical and ecologically important infochemical in tri-trophic level interactions among plant-aphid-natural enemies. However, the molecular mechanisms by which parasitoids recognize and utilize EBF are unclear. In this study, we functionally characterized 8 AgifOBPs in *Aphidifus gifuensis*, one dominant *endo*-parasitoid of wheat aphid as well as peach aphid in China. Among which, AgifOBP6 was the only OBP upregulated by various doses of EBF, and it showed a strong binding affinity to EBF *in vitro*. The lack of homology between AgifOBP6 and EBF-binding proteins from aphids or from other aphid natural enemies supported that this was a convergent evolution among insects from different orders driven by EBF. Molecular docking of AgifOBP6 with EBF revealed key interacting residues and hydrophobic forces as the main forces. AgifOBP6 is widely expressed among various antennal sensilla. Furthermore, two bioassays indicated that trace EBF may promote the biological control efficiency of *A. gifuensis,* especially on winged aphids. In summary, this study reveals an OBP (AgifOBP6) that may play a leading role in aphid alarm pheromone detection by parasitoids and offers a new perspective on aphid biological control by using EBF. These results will improve our understanding of tri-trophic level interactions among plant-aphid-natural enemies.

1. Introduction

Evolutionary adaptation fuels the genetic diversification of living organisms, driving speciation and emergent biodiversity [1,2]. Species with overlapping habitats have typical interaction characteristics, making them excellent subjects for exploring coadaptive evolution. This means that there are more obvious clues of adaptive evolution among multitrophic levels. For example, aphid alarm pheromone is one of the most typical and ecologically important info-chemicals in tritrophic interactions among plants, aphids and their natural enemies. It is contained in aphid cornicle droplets emitted when aphids are physically attacked [3–5]. In addition, it induces behavioral responses in receiving conspecifics [6,7]. Aphids that receive the warning signal typically cease feeding, move away from the signal, and drop off sometime [8,9]. (*E*)-β-farnesene (EBF) is the primary active component of alarm pheromones in most aphid species [10,11]. Interestingly, most research suggested that it also attracts aphid natural enemies such as *Aphidius uzbekistanicus, Coleomegilla maculata, Chrysoperla carnea, Aphidius nigripes, Adalia bipunctata, Episyrphus balteatus, Harmonia axyridis, Aphidius ervi,* and *A. gifuensis* [12–20], although there were a few disagreements [21,22]. In addition, plants are apparently also involved in the interactions. EBF has been identified in volatiles of many plants, such as maize [23] and *Mentha x piperita*, L. [24]. Because EBF is emitted by aphids in very low amounts and is unstable, it was suggested that natural enemies might use plant-derived EBF as a synomone to identify aphid-infested plants *via* an altered plant volatile bouquet [25].

Insect odorant-binding proteins (OBPs) play important roles in peripheral olfactory signal transduction, which connects info-chemicals in

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habitat with olfactory receptors (ORs) located on the olfactory nerve [26–28]. Recently, the aphid EBF recognition mechanism has received extensive attention and has been well-studied. Three EBF-binding proteins (namely, OBP3, OBP7 and OBP9) have been identified in succession thus far in aphids such as *Myzus. persicae* [29], *Sitobion miscanthi* [30], and *Acyrthosiphon. pisum* [9,31,32], *Aphis glycines* [33], *Megoura viciae* [34] and *Rhopalosiphum padi* [35], Which represents the multifunction and cooperation with each other of insect OBPs. There has always been confusion between *Sitobion avenae* (Fabricius) and *S. miscanthi* (Takahashi) in China. Thanks to a systematic study of both aphid species, it was found that *S. avenae* is only distributed in the Yili region of Xinjiang, China, the aphids distributed in other parts of China that were originally named after *S. avenae* were *S. miscanthi* [36,37]. Each of the 3 EBF-binding proteins among aphids is ortholog with high sequence consensus. The positive response to EBF induction by strongly upregulated expression of OBP7 and OBP9 explained the strong olfactory plasticity of aphids [38]. Moreover, OR5, an aphid olfactory receptor, has been demonstrated to be specialized in EBF signal transduction [9].

During predation and parasitism, natural enemies utilize herbivore-induced plant volatiles (HIPVs), green leaf volatiles (GLVs), or volatiles from aphids to locate their hosts [14,39–45]. The recognition mechanism of EBF in natural enemies of aphids also has been investigated within the olfactory system. For instance, CpalOBP10 showed affinities for EBF and green leaf volatiles in *Chrysopa pallens* [43]. HaxyOBP15 displayed a broad binding profile with EBF as well as multiple other odor ligands in ladybeetle *Harmonia axyridis* [44]. Furthermore, one EBF olfactory receptor, EcorOR3, as well as a EBF binding protein EcorOBP15, has been identified to be involved in EBF perception in the hoverfly *Eupeodes corollae* [42].

Aphid parasitoids, as natural enemies, can also detect EBF as well as plants being infested by host aphids [46–50]. *A. gifuensis* is one of the most common endoparasitoids of the tobacco aphid *M. persicae* and the wheat aphid *S. miscanthi* in China [50,51]. It has evolved a powerful peripheral chemosensory system. It distinguishes among healthy, mechanically damaged, and aphid-infested plants and chooses the latter as the one that is most likely to harbor their potential attack targets [39,41]. Both female and male *A. gifuensis* were attracted by EBF as well as plant volatiles, including trans-2-hexenal, methyl salicylate, benzaldehyde, cis-3-hexen-1-ol, and 1-hexanal [14,20,52,53]. In addition, the intense sexual orientation of males to females in distance indicated the existence of sex pheromones [20]. Our previous work incidentally identified two OBPs with medium or weak affinity for EBF in *A. gifuensis* [49]. However, the molecular mechanism underlying the attraction to parasitoids other than *A. gifuensis* of either EBF or any other olfactory cues remains completely unknown.

In this study, we hypothesized that similar as its parasitic host aphids, the EBF recognition of parasitoids could also be strengthened through olfactory plasticity, thus helping with host (aphid) detection and parasitism. EBF induction was performed on *A. gifuensis* to explore the OBPs up-regulated in response (plasticity), and their binding affinities to EBF were verified *in vitro*. Homology modeling, molecular docking and immunolocalization of AgifOBP6 revealed in detail the molecular mechanisms of perceiving EBF in *A. gifuensis*. Meanwhile, data from the *in vivo* EBF application test were obtained to evaluate the promotive effect of EBF on parasitic behavior.

2. Materials and methods

2.1. Plants and insects

Parasitoid wasps (*A. gifuensis*) were originally collected from *M. persicae* mummies in August 2019 in Kunming, Yunnan Province, China, and they had been cultured on *S. miscanthi,* which are parthenogenic clones (Langfang-1) reared on wheat (*T. aestivum* L.) 'AK58' at our laboratory at the Institute of Plant Protection of Chinese Academy of Agricultural Sciences (Beijing, China) for 2 years with an air-conditioned insectary: 23 ± 2 °C with 55 ± 10 % relative humidity and a photoperiod of 16:8 (L: D) h. The detailed rearing methods were described in previous works [20,49].

2.2. EBF induction assay

Olfactory stimulation is an ideal tool for evaluating insect responses to odors at the molecular level, such as in the blowfly *Protophormia terraenovae* [54] and aphid *S. miscanthi* [38]. Here, EBF induction was performed to identify the OBP proteins that respond with upregulated expression in *A. gifuensis.* The protocol mainly followed previous studies [38,55–57]. The mummies were separated individually and waited for emergence. Ten female adults, one day after emergence, were collected into one *Petri* dish (13 cm in diameter and 2 cm in height) at 10:00–11:00 am. Then, each Petri dish was treated with EBF at a certain concentration (a total of 4 concentrations, namely, 0.4, 4, 40, and 400 ng/μL) in triplicates. Their corresponding negative control was set up. A total volume of 10 μL of EBF (Wako, Japan) dissolved in hexene was loaded on filter paper at the bottom of the Petri dish to induce for 0.5 h. Antennae, the olfactory organ, were collected immediately in RNase-free tubes with the bottom immersed in liquid nitrogen and ultimately stored at −80 °C until total RNA extraction.

2.3. Total RNA extraction and cDNA preparation

Antennae were dissected from female adult wasps, which were merged within 24-36 h. In total, there were three replicates for the sampling. For each group, 100 female antennae, control and EBF-treated were collected, respectively. Total RNA was extracted using TRIzol reagent and combined with a micro total RNA extraction kit (Tianmo, Beijing, China) following the manufacturer's instructions. RNA degradation and contamination were monitored on 2 % agarose gels. RNA purity was checked using a Nanodrop ND-1000 spectrophotometer (NanoDrop products, Wilmington, DE, USA). RNA concentration was measured using a spectrophotometer RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). cDNAs were synthesized using the TRUEscript RT kit (LanY Science & Technology, Beijing, China) following the manufacturer's protocol.

2.4. Expression investigation of AgifOBPs

Real-time quantitative PCR (RT–qPCR) with an ABI 7500 real-time PCR system (Applied Biosystems Fosters City CA, USA) was conducted to explore the responses of *AgifOBPs* at the mRNA level after induction with EBF. Primers were described in our previous work [49] and used a 20-μL reaction containing 10 μL of 2 × SuperReal PreMix Plus, 0.6 μL of forward primer (10 μmol/L), 0.6 μL of reverse primer (10 μmol/L), 2 μL of cDNA (50 ng/μL), 0.4 μL of 50 × ROX reference dye, and 6.4 μL of nuclease-free ddH_2O following the instructions provided with the SuperReal PreMix Plus (SYBR Green) kit (FP205) (Tiangen, Beijing, China). The PCR program was as follows: an initial 15-min step at 95 °C, 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 32 s, and elongation at 72 °C for 45 s, and finally a 10-min step at 72 °C. For melting curve analysis, a dissociation step cycle was added automatically. Relative quantification was performed according to the $2^{-\Delta\Delta Ct}$ method [58]. β-actin and NADH were used as reference genes to normalize the data. All qPCR analyses were performed in three technical and biological replicates.

2.5. Heterologous expression and purification of AgifOBPs

AgifOBP(7/9) was obtained in our previous work [49]. Eight OBPs (AgifOBP1-6, AgifOBP8 and AgifOBP11), including those in response to EBF induction, were expressed in prokaryotes. The expression and purification methods were consistent with those in a previous study

[29,49,59]. The PCR products were first cloned into a pEASY-T1 clone vector (Trans, Beijing, China), which was assembled with each AgifOBP to form a fusion protein without a histidine-tagged peptide and then subcloned into the bacterial expression vector pET28a (+) (Novagen, Madison, WI) between *Nde* I and either EcoR I or BamH I restriction sites. Reconstructed plasmids were verified by sequencing. This plasmid protein expression was induced by adding isopropyl-1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 1 mM when the culture had reached an OD_{600} value of 0.6. Cells were incubated for an additional 12 h at 28 °C or 37 °C and then harvested by centrifugation and sonicated in 5 s with a purse at 3 s for 15 min at a low temperature (ice-water mixture). After centrifugation, the obtained bands were checked by 15 % SDS–PAGE for their correspondence to the predicted molecular masses of the proteins. They were solubilized according to protocols for the effective rebuilding of the recombinant OBPs in their active forms [29,32,59]. The soluble proteins in the refolded proteins were then purified by anion-exchange chromatography with a RESOUCE Q15 HP column (GE HEALTH CARE, USA) and finally with two rounds of gel filtration through a Superdex 75 10/300 GL column (GE HEALTH CARE, USA). The concentration of purified protein was determined by a Protein Assay kit (Qubit™ Protein Assay kit, Q33211, Invitrogen), and the purified AgifOBP(1-6/8/11) was analyzed by mass spectrometry (LC–MS). The purity and concentration of the soluble proteins were evaluated using SDS–PAGE. Finally, stock solutions of AgifOBP(1-6/8/11) were collected and kept at −20 °C in Tris–HCl (50 mM, pH 7.4).

2.6. Fluorescence competitive binding assays

To investigate the ligand-binding property of AgifOBPs, five groups 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 [11,60]; (ii) main components of the aphid sex pheromone (4aSR,7SR,7aRS)-Nepetalactone); (iii) green leaf volatiles of wheat (Z)-3-hexen-1-ol); (iv) aphid-induced plant volatiles (methyl salicylate and 6-methyl-5-hepten-2-one (MHO)); and (V) an EBF derivative artificial chemical, namely, CAU-II-11 ((E)-3,7-dimethylocta-2,6-dien-1-yl-2-hydroxy-3-methoxybenzoate), which showed a high affinity for aphid EBF-binding proteins (OBP3/7/9) [32]. The classes, CAS numbers, and purity of the chemicals used in this study are listed in Table 2. Fluorescence competition assays were conducted following previous work [29,30,32,35,61]. The decrease in 1-NPN fluorescence due to the ability of different odorants to displace 1-NPN from the binding cavity of AgifOBPs was observed and recorded, and then, the Ki value for each compound was determined. The intensity values corresponding to the maximum fluorescence emissions were plotted against the cumulative 1-NPN concentration to calculate dissociation constants. The amount of bound ligand was calculated from the fluorescence intensity values by assuming that the protein was 100 % active, with a stoichiometry of 1:1 protein:ligand at saturation. The curves were linearized using Scatchard plots. The value of $K_{1\text{-NPN}}$ was estimated on a direct plot by nonlinear regression with an equation corresponding to a single binding site using Prism 9.0 (GraphPad Software, Inc., USA), and the IC_{50} was defined as the concentration of a competitor that caused a 50 % reduction in fluorescence intensity. The dissociation constants of the inhibitors (Ki) were calculated according to the formula $Ki = [IC50]/(1 + [1\text{-NPN}]/K_{1\text{-NPN}})$, in which [1-NPN] represents the free 1-NPN concentration and $K_{1\text{-NPN}}$ represents the dissociation constant for AgifOBPs/1-NPN. Referring to previous work [29], we consider the ligand-binding affinity with AgifOBPs to be high when $Ki < 2$ μM, medium when 2 μM $< Ki < 10$ μM, and weak when $Ki > 10$ μM.

2.7. Sequence analysis and structure prediction

AgifOBP6 was chosen for further homology modeling and molecular docking. First, the amino acid sequences of AgifOBP6 were aligned with those of other EBF-binding proteins, and the sequence consistency was analyzed to determine whether there was homology. Alarm pheromone binding proteins in aphids (OBP3, OBP7, OBP9) and in natural enemies were downloaded from the NCBI website (http://www.ncbi.nlm.nih.gov/) or UniProt database (https://www.uniprot.org/). The amino acid identity of AgifOBP6 with EBF-binding OBPs was analyzed in MEGA11 using the maximum likelihood method with LG+ mode [62]. Values indicated at the nodes are bootstrap values based on 1000 replicates presented with a 95 % cutoff. The orthologous protein sequences from the genomes and transcriptomes of the following species were used in the analysis: *M. persicae* [63], *S. miscanthi* [64], *A. gossypii* [65], *A. pisum* [66], *A. glycines* [33], *M. viciae* [34], *R. padi* [35], LeryOBP3 (AJO61166), LeryOBP7 (AJO61167)) and the OBP protein sequences from parasitoid *A. gifuensis* [20,49]; the predators *H. axyridis* [44], *C. pallens* [67], *Episyrphus balteatus* [68] and *E. corollae* [68,69]. The amino acids of the sequences used are listed in Supplementary file 1. Then, homology modeling was performed using SWISS-MODEL (https://swissmodel.expasy.org/). The amino acid sequence of AgifOBP6 was submitted to the NCBI BLASTp server (http://blast.ncbi.nlm.nih.gov) to search for a proper template in the PDB database. Identity between the template and target protein above 30 % was taken into consideration. Three methods (namely, Verify_3D, Procheck, and ERRAT) were used at UCLA-DOE LAB-SAVES v6.0 (https://saves.mbi.ucla.edu/) to assess the final 3D model of the AgifOBP6 protein [70]. Finally, molecular docking was conducted to investigate the mode of ligand binding. Docking calculations for AgifOBP6 with EBF and its analog CAU-II-11 were performed using the UCSF Dock6.9 protocol in the Yinfo Cloud Computing Platform (https://cloud.yinfotek.com/). The chemical structures of the small molecule EBF and its analog CAU-II-11 were drawn by JSME and converted to a 3D structure with energy minimization in the MMFF94 force field. The crystal/NMR structure of CpalOBP4 was automatically downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/). All redundant atoms except chain A were deleted, and then the protein structure was carefully treated in several steps, including residue repair, protonation, and partial charge assignment in the AMBER ff14SB force field. The DMS tool was employed to build the molecular surface of the receptor using a probe atom with a 1.4 Å radius. The binding pocket was defined by the crystal ligand, and spheres were generated to fill the site by employing the Sphgen module in UCSF Chimera [71]. A box enclosing the spheres was set with a center of (−29.58, −2.026, −13.65) and sizes of (31.592, 31.441, 35.657), within which grids necessary for rapid score evaluation were created by the Grid module. Finally, the DOCK 6.9 [72,73] program was utilized to execute semiflexible docking where 10,000 different orientations were produced. Clustering analysis was performed (RMSD threshold was set 2.0 Å) for candidate poses, and the best-scored poses were output. The top-ranked pose, as judged by the Vina docking score, was subjected to visual analysis using PyMOL v.1.9.0 (http://www.pymol.org/).

2.8. Western blot assay

Rabbit antiserum against a recombinant protein of AgifOBP6 was produced by Xinnuojingke Biotech (Beijing, China). Crude antennal proteins were extracted using RIPA buffer (Solarbio, Beijing). Protein samples were separated by 15 % SDS–PAGE and then transferred to a polyvinylidene fluoride membrane (PVDF) (Millipore, Carrigtwohill, Ireland). The membrane was blocked using 5 % fat-free milk (BD Biosciences, San Jose, CA, USA) in PBS containing 0.05 % Tween-20 (PBST) at 4 °C overnight. After washing three times with PBST (10 min each), the blocked membrane was incubated with rabbit anti-AgifOB6 antiserum (1:5000) at room temperature for 1 h. After three additional washes with PBST, the membrane was incubated with goat anti-rabbit immunoglobulin G (IgG) and HRP-conjugated antibody (CWBIO, Jiangsu, China) (1:5000) at room temperature for 2 h. Finally, the membrane was developed using Immobilon Western Chemiluminescent

HRP Substrate (Merck, Beijing, China) and then exposed and imaged on an ImageQuant LAS 4000 mini (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

2.9. Whole-mount immunolocalization of AgifOBP6

Given that AgifOBP6 strongly binds to EBF, it was chosen to further study the expression characteristics at the subcellular localization. Whole-mount fluorescence was performed to identify the location of AgifOBP6 in antennae according to a previous study [34,74]. Antennae from virgin female specimens were dissected under the microscope after 24 h to 36 h of the adult stage and washed twice with PBS, pH = 7.4. After the washing step, the samples were fixed in 4 % paraformaldehyde in PBS for 2 h and then washed twice with the same buffer. Samples were then incubated for 30 min with PBS containing 2 % BSA (to reduce nonspecific binding) and 0.1 % of the detergent Tween 20 (Sigma) to permeabilize tissues, favoring the entrance of antibodies. Then, samples were incubated for 1 h at room temperature with antisera raised in rabbit. The recombinant protein was used to produce antibodies against all amino acid sequences of AgifOBP6 except for signal peptides. Primary polyclonal anti-AgifOBP6 antibody was omitted or substituted with rabbit preimmune serum (1:200), and sections were treated with a blocking solution containing 0.1 % Tween 20 (Sigma) and incubated only with the secondary antibody in all controls. Antibodies against AgifOBP6 were diluted 1:15000 in Tris-buffered saline with 2 % bovine serum albumin (BSA). Samples were washed with PBS and incubated for 1 h in a dark chamber with the secondary goat anti-rabbit tetramethylrhodamine (TRITC)-conjugated antibody (Jackson, Immuno Research Laboratories Inc., West Grove, PA, United States) diluted 1:200 in blocking solution containing 0.1 % Tween 20. This (TRITC)-conjugated antibody has been previously used in experiments on the aphid *M. viciae* OBPs [34]. Coverslips were mounted with City Fluor (City Fluor Ltd., London, United Kingdom), and immunofluorescence was analyzed using an inverted laser-scanning confocal microscope (LSM880, Carl Zeiss, Germany.) equipped with a Plan APO 40 × 0.95 NA objective. Images were acquired using Zeiss ZEN 2.1 software (emission windows fixed at 561 nm) without saturating any pixels. Fluorescence and bright-field images were combined with Adobe Illustrator 2020 (Adobe Systems Incorporated, San Jose, CA, United States).

2.10. Investigation for the phenotypic host preference

In this section, experiments were performed mainly following previously published work [75,76]. Considering that *A. gifuensis* prefers to parasitize the 2nd instar of *M. persicae* and *S. miscanthi* [77,78], two phenotypes of aphid adults (winged and wingless adults) as well as 2nd star nymphs were employed as three groups to be treated with EBF (4 ng/μl diluted in trimyristoyl triglyceride (TAG, APPLYGEN)). Fifteen aphids were introduced to three wheat seedlings (AK 58) at a density of five per wheat seedling kept in a Petri dish. The roots of wheat seedlings were placed in a 1.5 mL tube and sealed with scraped cotton to moisturize. The introduced aphids were allowed to colonize, and the experiment was started after 24 h. EBF (4 ng/μL, 0.05 μL using a flat-mouth microsyringe with a 5 μL range) was quickly coated on the dorsal abdomen of *S. miscanthi*, and then one female aphidius was placed in each Petri dish. Probing and tapping by antennae of the aphidius were defined as probing. The actions of attacking aphids for oviposition were defined as parasitism. The parameters mentioned above indicate that the behavioral responses within 10 min of aphidius were all recorded [53]. Negative control and blank control were also set up. The aphids of the negative control were treated with trimyristoyl triglyceride, and the aphids of the blank control were not treated anything. A 6 W incandescent lamp was placed 20 cm above the light source to eliminate any light source interference. Fisher LSD one-way ANOVA was used to calculate significant differences ($p < 0.05$).

2.11. Investigation for the foraging behavior of A. gifuensis

We further compared behaviors to aphids of the aphidius with and without EBF treatment to evaluate the effects of nonogram amounts of EBF on both host (aphid) preference and parasitism ratio (0.2 ng/aphids). Both winged and wingless adults and 2nd instar nymphs were separately tested. This work was conducted in a wheat seedling system (Fig. 5F). For details, 10 wheat seedlings (AK58) at their two-leaf period in a pot were placed in a climate chamber (16:8 h L:D; 22 ± 1 °C). Twenty aphids were introduced to the 10 wheat seedlings and covered by a plastic insect cage (13 cm in diameter and 30 cm in height) with screen mesh caps. Remove the newborn aphids after 24 h of colonization of adults. One microliter of EBF solution (4 ng/μL of EBF + trimyristoyl triglyceride (TAG)) was evenly applied to 20 adults or 20 nymphs to the 20 aphids. The same amount of TAG was applied as a negative control group. The blank control group was not treated with any chemical. A single female aphidius merged at 24–36 h was then introduced to EBF-treated aphids as well as their control aphids and allowed to forage and parasitize for 24 h. Experiments were performed in ten replications. The number of mummies was recorded after 12 days [79]. The parasitism rate was defined as the proportion of mummies to the original 20 aphids. Significant differences were analyzed using one-way ANOVA followed by Fisher's LSD test ($p < 0.05$).

2.12. Statistical analyses

For qRT–PCR and bioassays, the differences between the control and treatments of biological replicates were tested using one-way ANOVA followed by multiple comparisons tests regardless of rows and columns using GraphPad Prism version 9.0.0 for Windows (GraphPad, San Diego, California USA, www.graphpad.com). Differences among treatments were distinguished using Tukey's honestly significant difference (HSD) test at the $P < 0.05$ significance level.

3. Results

3.1. Response to EBF induction of AgifOBPs expression

The obtained results show that even without any induction, the expression level among OBPs in antennae varies significantly. Generally, AgifOBP3/5/6/11/15 were highly expressed, while others were expressed at lower levels (Fig. 1S). In this study, EBF application was performed on a 10-fold gradient (4/40/400/4000 ng). The obtained results showed that EBF induced extensive changes in OBP (5 of 14) expression. AgifOBP6, one of the most highly expressed OBPs in the antennae of both sexes (Fig. 1S), was significantly upregulated by various amounts of EBF (from 4 to 4000 ng, Fig. 1), up to 164 %, 157 %, 150 % and 153 %, respectively (Fig. 1P). However, all 4 other EBF-induced OBPs responded only to a certain concentration. Only 4 ng of EBF caused the up-regulation of OBP9 expression to 191 %, and doses of 40 ng and above did not induce any response at the mRNA level (Fig. 1Q). For AgifOBP12, 400 ng of EBF significantly up-regulated its expression to 152 % (Fig. 1M). However, the expression levels of AgifOBP7 and AgifOBP15 were significantly down-regulated by 4000 ng of EBF to 45.20 % and 32.52 %, respectively (Fig. 1G, I). The expression of the remaining OBPs induced by the different doses of EBF was not significant (Fig. 1).

3.2. Expression and purification of AgifOBP(1-6/8/11)

SDS–PAGE showed that AgifOBPs were expressed as inclusion bodies using a prokaryotic expression system (AgifOBPs/pET-28a, (BL21 DE3)), and mature AgifOBPs without fusion tags were purified with serial chromatographic steps on anion-exchange resin RESOUCE15 Q/SP HP columns (GE Healthcare Biosciences, Uppsala, Sweden). After dissolving and refolding treatment, the refolded AgifOBP(1-6/8/11)

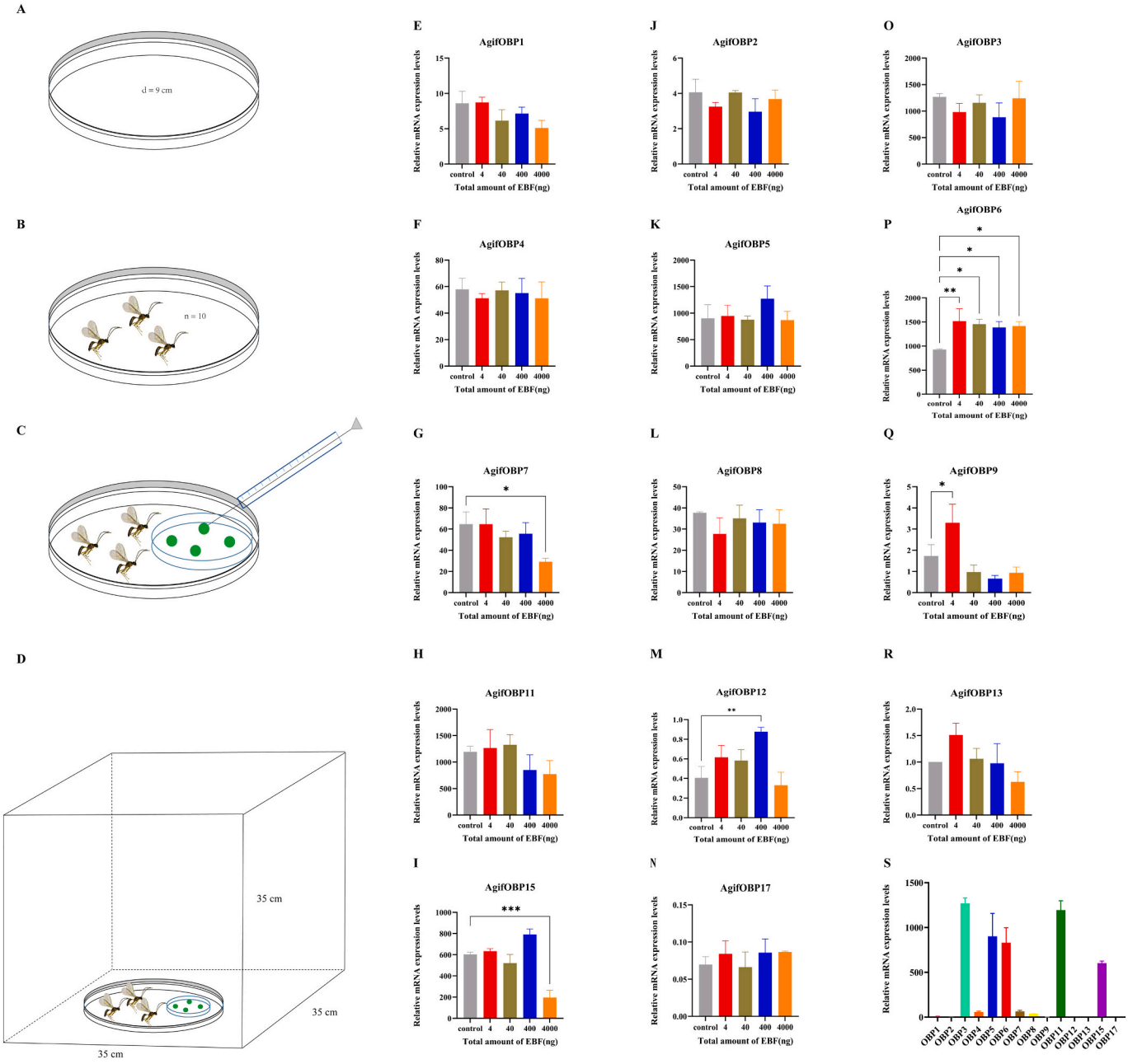


Fig. 1. The induction diagram shows the relative expression levels of *AgifOBPs* after treatment with different dosages of EBF (4–4000 ng) in the antennae. (A)-(D): Diagram of the induction assay with different dosages of EBF dissolved in hexane. (E)-(R): Relative expression levels of *AgifOBPs* in antennae after induction with EBF. (D): Relative expression levels of *AgifOBPs* in the control group. Data are the means of three independent experiments and presented as the mean ± SD. The standard error is represented by the error bar, and the star above each bar denotes significant differences (one-way ANOVA, $P < 0.05$).

were purified as soluble proteins, with yields of 0.21 to 0.63 mg/mL (Supplementary Fig. 1, Fig. 5A), and >15 mg of recombinant AgifOBP(1-6/8/11) was obtained. However, the remaining OBPs were not successfully expressed and purified. The theoretical molecular weight values for AgifOBP(1-6/8/11) were close to the measured values (AgifOBP1, 13.01 kDa; AgifOBP2, 14.15 kDa; AgifOBP3, 13.41 kDa; AgifOBP4, 12.53 kDa; AgifOBP5, 13.18 kDa; AgifOBP6, 13.65 kDa; AgifOBP8, 13.53 kDa; AgifOBP11, 13.90 kDa). The purified protein samples were further identified by LC–MS/MS.

3.3. Fluorescence competitive binding assays

We first tested the binding affinities of AgifOBPs to the fluorescent probe *N*-phenyl-1-naphthylamine (1-NPN). Every single OBP exhibited a regular saturation binding curve to 1-NPN and a linear Scatchard plot (Figs. 2A-2D). The dissociation constants of AgifOBPs are listed in Table 1. Interestingly, in the following fluorescence competitive binding assays, all purified AgifOBPs (AgifOBP1-6/8/11) showed strong binding affinities with CAU-II-11, a previously published EBF analog (Fig. 2, Table 2). Among the 8 AgifOBPs, AgifOBP6 and AgifOBP8 displayed a strong binding affinity for EBF, with Ki values of $6.26E^{-7} \pm 3.82E^{-6}$ μM and 0.51 ± 0.02 μM, respectively (Fig. 2J, Fig. 2K, Table 2). The binding property of AgifOBP6 to diluted EBF (c = 20 nM/L) was still quite strong, with a Ki of 0.48 ± 0.01 μM (Fig. 2J). (−)-α-Pinene, (−)-β-pinene and (+)-limonene are 3 active components of aphid alarm pheromones reported in a few aphids, such as *M. viciae* [60]. AgifOBP8 also showed a strong binding property to (−)-α-pinene, with a Ki value of 1.94 ± 0.02 mM (Fig. 2K, Table 2). AgifOBP1 and AgifOBP4 had a medium binding

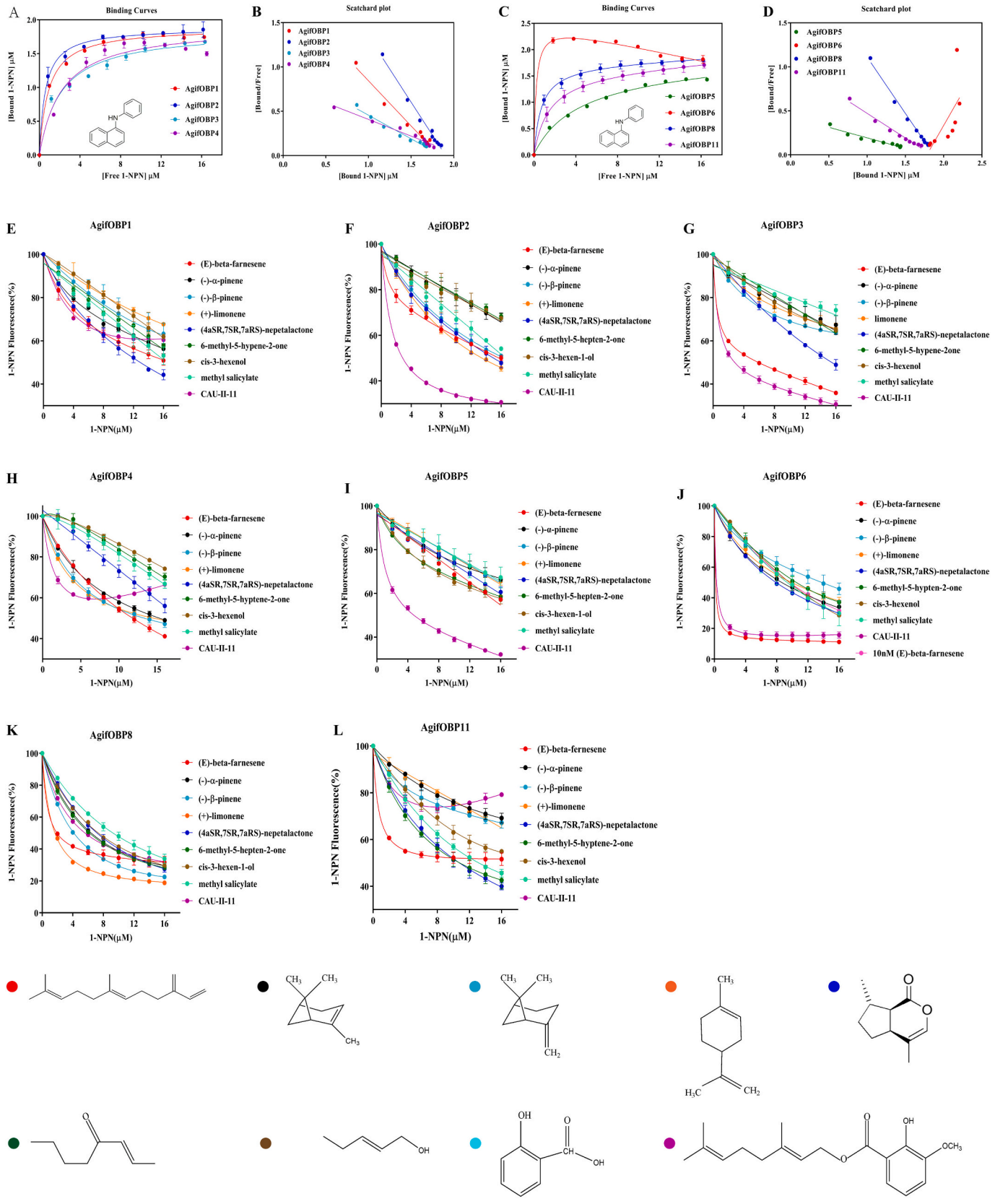


Fig. 2. Competitive binding assays of AgifOBP1-6/8/11 with candidate ligands. AgifOBP1-6/8/11 was 2 μM in Tris buffer (50 mM/L, pH 7.4). Aliquots of 1 mM 1-NPN in methanol solution were added to the protein to final concentrations of 2–16 μM, and the emission spectra were recorded between 350 and 500 nm. A mixture of the recombinant protein and *N*-phenyl-1-naphthylamine (1-NPN) in 50 mM Tris-HCl buffer (pH 7.4) at a concentration of 2 μM was titrated with 1 mM solutions of each competing ligand to a final concentration range of 2–16 μM. Fluorescence values are presented as a percent of the values in the absence of a competitor. Data are presented as the mean ± SD of three independent experiments.

Table 1
Calculated association constants of AgifOBPs/1-NPN probe complexes.

Protein name	AgifOBP1	AgifOBP2	AgifOBP3	AgifOBP4	AgifOBP5	AgifOBP6	AgifOBP8	AgifOBP11
Kd(μM)	0.89 ± 0.09	0.58 ± 0.06	2.11 ± 0.41	2.21 ± 0.56	4.77 ± 0.46	0.21 ± 0.09	0.86 ± 0.06	1.94 ± 0.12

Table 2
Binding affinities of AgifOBPs for candidate ligands evaluated in displacement binding assays using the fluorescent probe 1-NPN.

No.	Code	CAS	Purity	OBP1	OBP2	OBP3	OBP4	OBP5	OBP6	OBP8	OBP11
				Ki (μM)							
1	(E)-β-farnesene	18,794–84-8	≥85 %	5.53 ± 0.54	4.08 ± 0.22	2.78 ± 0.07	6.16 ± 0.35	19.48 ± 0.40	6.26*E*-07 ± 3.82E-06	0.51 ± 0.02	6.62 ± 2.23
2	(−)-α-pinene	80–56-8	≥95 %	8.18 ± 1.37	19.20 ± 3.02	–	8.33 ± 0.39	–	16.20 ± 0.07	1.94 ± 0.02	–
3	(−)-β-pinene	19,902–08-0	≥99 %	16.06 ± 5.56	4.07 ± 0.09	24.98 ± 2.83	6.99 ± 0.70	–	1.38 ± 0.35	1.26 ± 0.02	–
4	(+)-limonene	138–86-3	≥95 %	20.24 ± 7.01	3.13 ± 0.17	–	7.39 ± 0.42	–	0.93 ± 0.02	0.39 ± 0.05	–
5	Nepetalactone	21,651–62-7	≥80 %	4.03 ± 0.34	3.66 ± 0.62	8.95 ± 0.69	13.38 ± 0.21	28.47 ± 3.61	0.72 ± 0.01	2.08 ± 0.09	5.21 ± 0.58
6	6-methyl-5-hepten-2-one	110–93-0	≥99 %	9.42 ± 1.50	19.83 ± 2.48	23.04 ± 1.93	–	23.04 ± 2.18	1.00 ± 0.07	1.88 ± 0.10	5.38 ± 0.85
7	cis-3-hexenol	928–96-1	≥97 %	11.44 ± 2.09	10.95 ± 1.06	26.21 ± 1.74	–	20.13 ± 3.28	0.86 ± 0.01	2.18 ± 0.01	10.56 ± 1.11
8	methyl salicylate	119–36-8	≥99 %	6.95 ± 1.69	5.23 ± 0.51	–	27.90 ± 0.3.31	–	0.84 ± 0.19	2.69 ± 0.026	6.69 ± 0.36
9	CU-II-11	–	≥98 %	9.44 ± 0.61	0.65 ± 0.02	1.50 ± 0.20	7.82 ± 1.37	3.35 ± 0.27	1.95 ± 0.01	1.76 ± 0.07	–

affinity to (−)-α-pinene (Fig. 2E, Fig. 2H, Table 2), while the affinities of the remaining OBPs, including AgifOBP6, were weak for (−)-α-pinene. Both AgifOBP6 and AgifOBP8 showed a high affinity for (−)-β-pinene, with Ki values of 1.38 ± 0.35 μM and 1.26 ± 0.02 μM, respectively. For (+)-limonene, AgifOBP6 and AgifOBP8 also showed a strong affinity, with Ki values of 0.93 ± 0.02 μM and 0.39 ± 0.05 μM, respectively (Fig. 2J, Fig. 2K, Table 2). While AgifOBP2 and AgifOBP4 displayed a medium binding affinity for both (−)-β-pinene and (+)-limonene (Fig. 2F, Fig. 2H, Table 2), AgifOBP1, AgifOBP3, AgifOBP5, and AgifOBP11 showed poor binding or no binding properties for the above-mentioned three chemicals (Table 2).

For the main component of the aphid sex pheromone nepetalactone, we found that AgifOBP6 displayed a high affinity for nepetalactone with a Ki value of 0.72 ± 0.01 μM, while AgifOBP1, AgifOBP2, AgifOBP3, AgifOBP8, and AgifOBP11 displayed a medium binding affinity for nepetalactone (Fig. 2J, Table 2). AgifOBP4 and AgifOBP5 showed weak or no binding to the tested ligands.

In addition, both AgifOBP6 and AgifOBP8 exhibited better binding abilities with some wheat volatiles, such as 6-methyl-5-hepten-2-one (MHO), than with other AgifOBPs (Fig. 2J, Fig. 2K, Table 2). However, AgifOBP1, AgifOBP2, AgifOBP3, AgifOBP4, and AgifOBP11 showed medium binding properties (Figs. 2E-2H, Fig. 2L, Table 2), and AgifOBP5 showed weak binding abilities. Only AgifOBP6 had a high affinity with methyl salicylate (MeSA), with a Ki value of 0.84 ± 0.19 μM, while AgifOBP1, AgifOBP2, AgifOBP8, and AgifOBP11 displayed a medium binding affinity (Table 2J, Table 2). For the plant volatile cis-3-hexen-ol, we found that AgifOBP6 had a high affinity with a Ki value of 0.86 ± 0.01 μM, and AgifOBP8 displayed a medium binding affinity (Table 2, Fig. 2).

3.4. Homology modeling and molecular docking

Based on the results of the EBF induction bioassay and fluorescence competitive ligand binding test, AgifOBP6 is the only OBP that was upregulated by EBF and showed a strong binding affinity for EBF. Therefore, we further explored the mechanism of EBF perception in *A. gifuensis*.

No homology in the sequence of amino acids (<30 %) was found between AgifOBP6 and either aphids' OBPs or OBPs from the other natural enemies (Supplementary Fig. S2). Regardless, the crucial binding residue prediction of AgifOBP6 that interacts with EBF is essential for further functional mechanism studies. Considering that no crystal structure of any ortholog is available, we employed a computational procedure to first choose a template for AgifOBP6 and then model the three-dimensional (3D) structure. Sequence alignment analysis using the Protein Data Bank (PDB) database of a web server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that AgifOBP6 shares 33.9 % amino acid identity with CpalOBP4 (PDB ID: 6JPM, has a resolved protein crystal structure) of *C. pallens*, which is greater than the minimum requirement (30 %) for protein modeling. The 3D structure of the AgifOBP6 protein with CpalOBP4 as the template was predicted using the program SWISS-MODELel. As shown in Fig. 3, the structural comparison revealed that the amino acid sequence of AgifOBP6 forms six α-helical structures (α1-α6) (Fig. 3B, C) similar to CpalOBP4, which is similar to the PBP and GOBP-2 in *Bombyx mori* [80]. Moreover, AgifOBP6 had a similar internal cavity structure as CpalOBP4 (Fig. 3A). This result suggested that CpalOBP4 could be a reliable model in subsequent analyses. The models predicted by homology modeling were named Mod-AgifOBP6. For the predicted protein model, VERIFY3D, ERRAT, and Procheck were used to analyze the accuracy and reliability. VERIFY3D (Supplementary Fig. S3), ERRAT (Supplementary File S2), and Procheck (Supplementary File S3) showed that the models of Mod-AgifOBP6 were reasonable. The protein structure of AgifOBP6 was composed of six typical α-helices, forming a hydrophobic binding cavity, which are important features of insect OBPs (Fig. 3C). According to the affinities between recombinant proteins and chemicals, we selected (*E*)-β-farnesene and its analog CAU-II-11 as the target ligand to study the docking conformation and binding energy with the AgifOBP6 protein. The binding energy between AgifOBP6 and EBF and CAU-II-11 was then calculated, and the obtained results showed that the docking binding energies were negative and that the total values were −35.116863 and −48.893936, respectively. The distances of all potential interaction residues were <4 Å (Fig. 3F, I), indicating that there was a strong interaction of AgifOBP6 with EBF and CAU-II-11. For AgifOBP6, seven

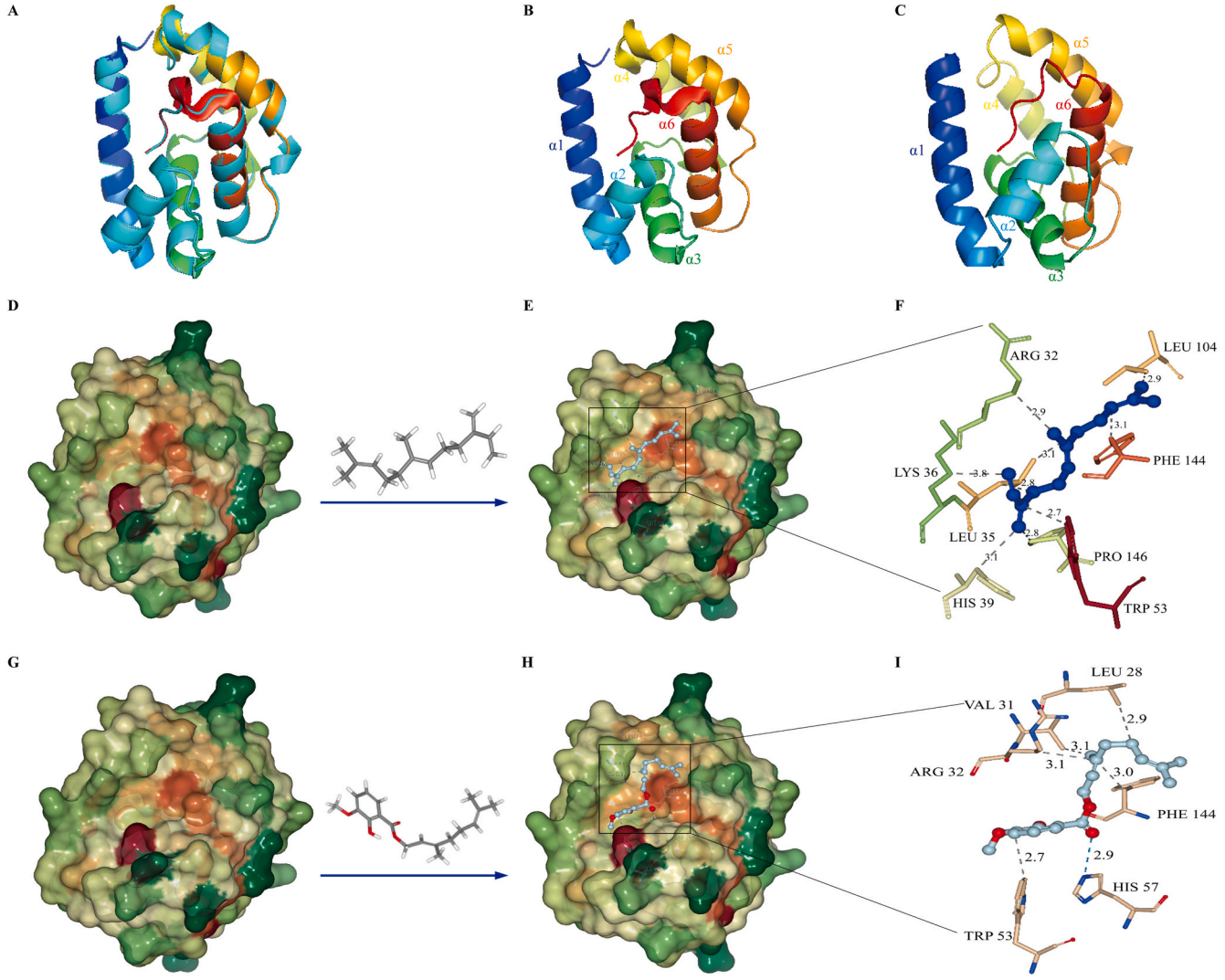


Fig. 3. Structural modeling and pattern analysis of AgifOBP6 for molecular interactions with *E*-β-farnesene and its analog CAU-II-11. (A): Superposition of the two odorant-binding proteins (OBPs) from the matching panels B and C in the same orientation. (B): CpalOBP4 (PDB ID: 6JPM). (C): AgifOBP6. (D), (G): Surface hydrophobicity of AgifOBP6; (E), (H): Surface hydrophobicity of AgifOBP6 binding with E-β-farnesene and its analog CAU-II-11 (dodger green, most hydrophilic; orange, intermediately hydrophobic; red, most hydrophobic). Key residues within 4 Å of E-β-farnesene and its analog CAU-II-11 are presented in F and I, respectively.

residues, including ARG32, LEU35, LYS36, HIS39, TRP53, PHE144, and PRO146, were critical for binding affinity to EBF based on hydrophobic interactions. LEU28, VAL31, ARG32, TRP5339, HIS57, and PHE144 were critical for binding affinity to CAU-II-11. Hydrophobic interactions were the important linkages of AgifOBP6 with EBF and its analog CAU-II-11.

3.5. Western blot and immunocytochemical localization of AgifOBP6

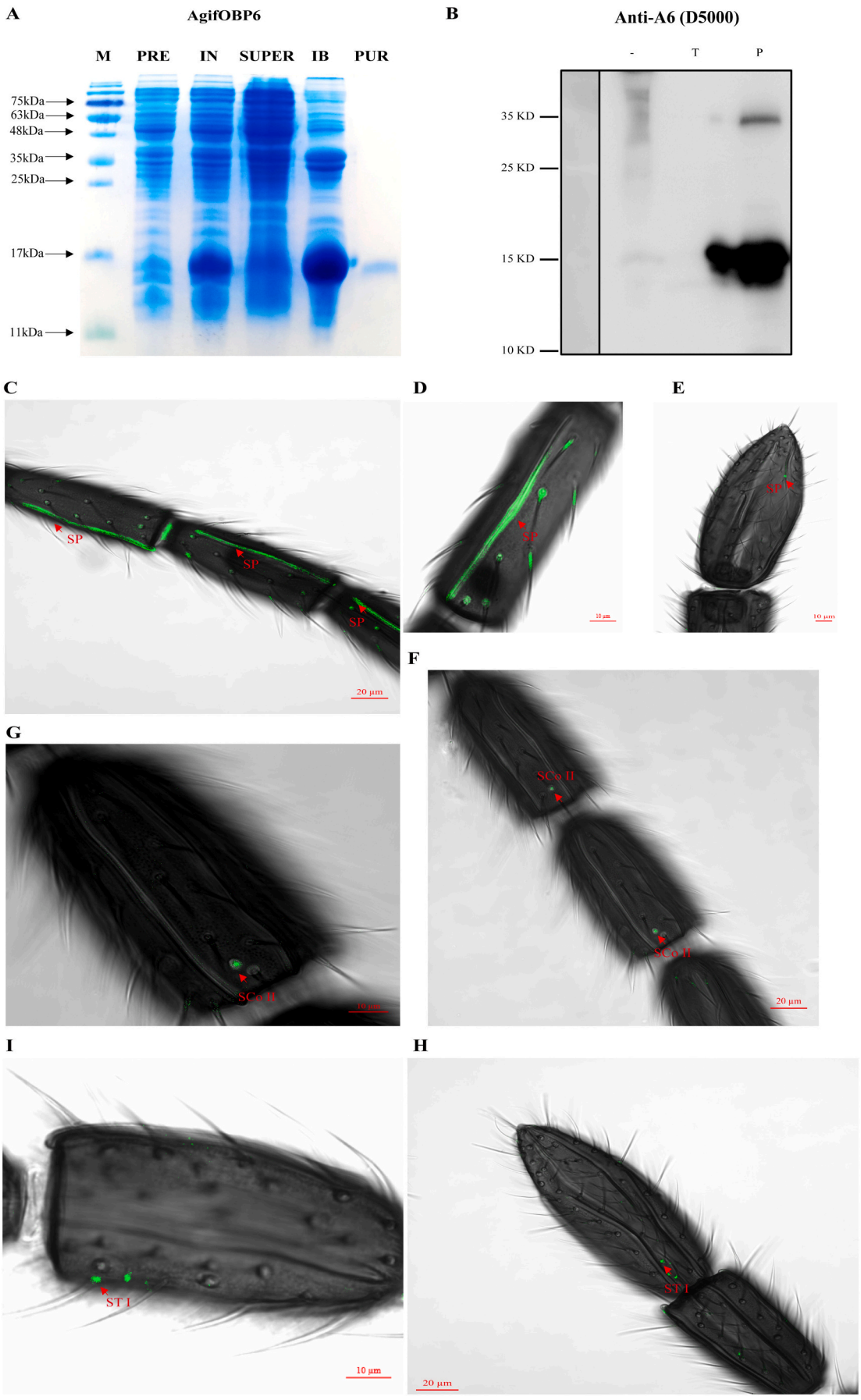
Previous works have revealed that AgifOBP6 is mainly expressed in the antennae of both sexes of *A. gifuensis* at the transcript level [49]. In the Western blot examination, staining of recombinant OBP6 and antennal extract with anti-AgifOBP6 antiserum revealed a prominent band at 15 kDa, which was comparable in size to the expected AgifOBP6 (approximately 13.65 kDa) (Fig. 4B). This finding was further complemented by whole-mount immunolocalization investigations (Fig. 4A). The obtained results showed the sensillum distribution in detail for AgifOBP6. It is commonly abundant in the lymph surrounding the sensory dendrites of the olfactory sensilla. In particular, sensilla placodea are equally distributed around all flagellomeres except the first one (Fig. 4C, Fig. 4D) and on the antennal tip (Fig. 4E); in addition, AgifOBP6 is expressed in sensilla coeloconica presented on each antenna segment (Fig. 4F, Fig. 4G) as well as in the sensilla trichaidea, which are found on all segments except the radicula (Fig. 4H, Fig. 4I).

3.6. EBF-induced interactions between S. miscanthi and A. gifuensis

The behavior traits were grouped and separately collected from aphidius. Data are listed in terms of probe times and attacking times. The probing results showed less interest of aphidius in winged aphids of aphidius than in nymphs or wingless adults. However, there were no significant differences between the nymph and wingless adults (Fig. 5D). Furthermore, EBF did not significantly stimulate the already high level of probing times of both nymph and wingless aphids to a higher level (Fig. 5D). Aphidius showed no additional differences on probes between EBF-treated winged aphids and nymphs as well as wingless aphids. Hence, EBF treatment significantly increased the probe times on winged aphids. Therefore, it reached the level of no significant difference with EBF-treated wingless adults and nymphs as well as their controls.

Interestingly, the subsequent attacks on aphids (parasitism) corresponded to the probes. Aphidius were more excited (more probes) to EBF-treated aphids than trimyristoyl triglyceride (solvent) and the blank control.

In the aphid group, after 24–36 h of free walking, the final parasitism



(caption on next page)

Fig. 4. Heterologous expression, western blot and immunolocalization analysis of AgifOBP6. (A): The mature AgifOBP6 protein was heterologously expressed and purified. Lane M: molecular weight PR1910 (11–180 kDa); PRE: Noninduced pET-28a (+)/AgifOBP6; IN: Induced pET-28a (+)/AgifOBP6; Super: pET-28a (+)/AgifOBP6 Supernatant; IB: pET-28a (+)/AgifOBP6 Supernatant; Pur: Purified pET-28a (+)/AgifOBP6 without His-tag. (B): Western blot analysis of AgifOBP6. The line "-": Negative control; Line "T": the antennae crude of female *A. gifuensis*; Line "P": the recombinant AgifOBP6. (C), (D): Immunolocalization of AgifOBP6 expressed in Sensilla placodea equally distributed around all flagellomeres of *A. gifuensis* females, except the first flagellomere and on the antennal tip (E); (F), (G): Antiserum of anti-AgifOB6 was detected on Sensilla coeloconica presented on each antenna segment; (H), (I): Antiserum of anti-AgifOB6 was detected on Sensilla trichaidea, which are found on all segments except the radicula of *A. gifuensis*. Bars in (C), (F), (H): 20 μm; bars in (D), (E), (G), (I): 10 μm.

rate was calculated using data collected on the 12th day, and all offspring were removed during the entire investigation period. The percentage of mummies in winged adults was the lowest at 35 %, and mummies of both nymphs and wingless aphids were 78.5 % and 90 %, respectively. They were all significantly higher than winged adults but not significantly different from each other (Fig. 5G). The mummy rate of EBF-treated winged adults significantly increased compared with that of the blank control, which is consistent with the results of the investigation on probe and attacking (Fig. 5G). Another investigation after 12 d of free walking with all 2nd generation offspring retained showed that: 1. There was a significantly higher survival number of winged adults than both nymph and wingless adults (Fig. 5H). 2. For EBF-treated winged adults, aphidius finally reduced their populations to a level that was the same as that of wingless adults, although their parasitoid rate was lower than 53.5 % (Fig. 5H).

4. Discussion

4.1. Screening for EBF-binding proteins

Aphid OBPs extensively respond to EBF with complex patterns, which shows strong olfactory plasticity among aphid species. However, related studies on their parasitoids are scarce. In this work, there were up to 5 OBPs in total that responded to EBF induction. Interestingly, AgifOBP6 was the only OBP that responded to EBF in a wide range of doses from low to high (4 ng to 4000 ng) by up-regulation. The affinity of AgifOBP6 for EBF was further confirmed by a subsequent competitive ligand binding test, which indicated that AgifOBP6 may play a role in the molecular basis of EBF recognition in *A. gifuensis*. However, the up-regulation of AgifOBP9 and AgifOBP12 was limited to specific doses of 4 and 400 ng EBF, respectively, and the down-regulation of AgifOBP7 and AgifOBP15 was limited to a high dosage of 4000 ng. Similar to AgifOBP6, AgifOBP7 and AgifOBP9 were identified as having affinity for EBF as well, although their affinities were much weaker (*i.e.*, weak and intermediate, respectively). However, the affinity data of AgifOBP12 and AgifOBP15 with EBF could not be obtained because they were not able to be successfully purified. The olfactory perception in insects as well as other animals is generally dose-dependent. Dose beyond the threshold range, either too high or too low, will lead to a decrease in response [81]. Thus, the up-regulation induced by low-dose EBF, *i.e.*, 40 ng, suggested that AgifOBP9 was associated with the high sensitivity of aphidius to EBF. Therefore, AgifOBP9 could collaborate with AgifOBP6 to ensure the sensitivity of *A. gifuensis* to low doses of EBF. Once the EBF dose was above the threshold, AgifOBP7/15, the two OBPs down-regulated by EBF induction may neutralize AgifOBP6 by down-regulating their expression, and the response to excessively high doses of EBF was achieved through the cooperation of three OBPs (AgifOBP6, AgifOBP7 and AgifOBP15 at 4000 ng).

In summary, a basic pattern was identified for EBF-induced OBPs. AgifOBP6 was always up-regulated by EBF induction, and there were two more up-regulated EBF-binding proteins interacting with AgifOBP6 at lower concentrations. At higher doses of 4000 ng, down-regulated EBF-binding proteins may interact with AgifOBP6.

In addition, we noticed that AgifOBP7 and AgifOBP9, the other two EBF-binding OBPs reported in our previous works, are mainly expressed in the legs of females instead of antennae [47]. This suggests the possibility of physiological functions other than olfaction. Although numerous experimental settings have been previously reported [29,49,82], we failed to obtain AgifOBP12, AgifOBP13, AgifOBP15, and AgifOBP17.

The most notable binding affinity was between AgifOBP6 and EBF, which demonstrated the best binding property with Ki values of $6.26E^{-7} \pm 3.82E^{-6}$ μM (Fig. 2J, Table 2). Even after diluting 100 times (c = 20 nM/L), AgifOBP6 still showed a strong binding property with EBF (Ki = 0.48 ± 0.01 μM) (Fig. 2J). Western blotting and immunocytochemical localization of AgifOBP6 further validated the expression in antennae. AgifOBP6 was found in sensilla placodea (Fig. 4C, D). There are many multiple pores on the surface of sensilla placodea (SP), which is consistent with the putative theory that sensilla placodea likely has an olfactory function [83]; AgifOBP6 is also labeled in sensilla coeloconica presented on each antenna segment (Fig. 4F, G), which is consistent with the discovery that SCo I and SCo II are thought to have olfactory or thermos functions [84,85]. Furthermore, the antiserum of AgifOBP6 was detected on the sensilla trichaidea (Fig. 4H, I), which is prevalent on all segments except the radicula of *A. gifuensis*. We hypothesize that sensilla trichaidea may have and additional function in female antennae beyond the putative mechanoreceptive function in male and female *A. gifuensis* [86].

The binding mechanisms of AgifOBP6 with two ligands (EBF and its analog CAU-II-11) that displayed high binding affinities were explored. Because the three-dimensional (3D) structure of AgifOBP6 (Fig. 4C) was employed by a computational procedure, the docking results revealed that negative docking binding energies were the main force to maintain such binding properties. Aphid EBF-binding OBPs shared orthologous genes among species. However, there was clearly no homology of EBF-binding OBPs between aphids and aphidius or between aphidius and other aphid natural enemies such as *E. balteatus* and *Chrysopa pallens* [42,43]. It is clear that when they diverged into different species, aphids acquired homologous EBF-binding proteins from their common ancestor. Since then, natural enemies coevolved with aphids and gradually acquired the ability to detect EBF. Thus, their EBF-binding proteins independently evolved from each other and were driven by convergent evolution.

4.2. EBF effects on parasitism of A. gifuensis

First, the probing times of aphidius wasps to aphids with different phenotypes or at different developmental stages were different. Once aphidius arrive near their prey, they generally begin frequent, excited probing. Subsequent more probing of wingless adults and nymph may be stimulated by the detection of residual EBF on the abdomen surface by the first few probing. However, for winged aphids, the wing tissue covers the abdomen, preventing antennae from detecting residual EBF. The wing tissue interferes with the contact between antennae and the residual EBF, which may lead to a lack of interest in winged aphids, thus, the probing times are less than those of wingless adults and nymphs. Consequently, the parasitic rate of the winged adult is lower than that of the wingless adult and the nymph, although a consistent result in the number of attacks, that is, the parasitism times was not found. This result suggested that the wing tissue may prevent aphids from being parasitized to the correct site, thus reducing the successful parasitism rate.

EBF promoted the efficiency of biological control, especially on winged aphids. Our results showed that the application of EBF on the dorsal abdomen of winged aphids caused more frequent probing by the aphidius wasp, which was as frequent as that of the wingless adult and

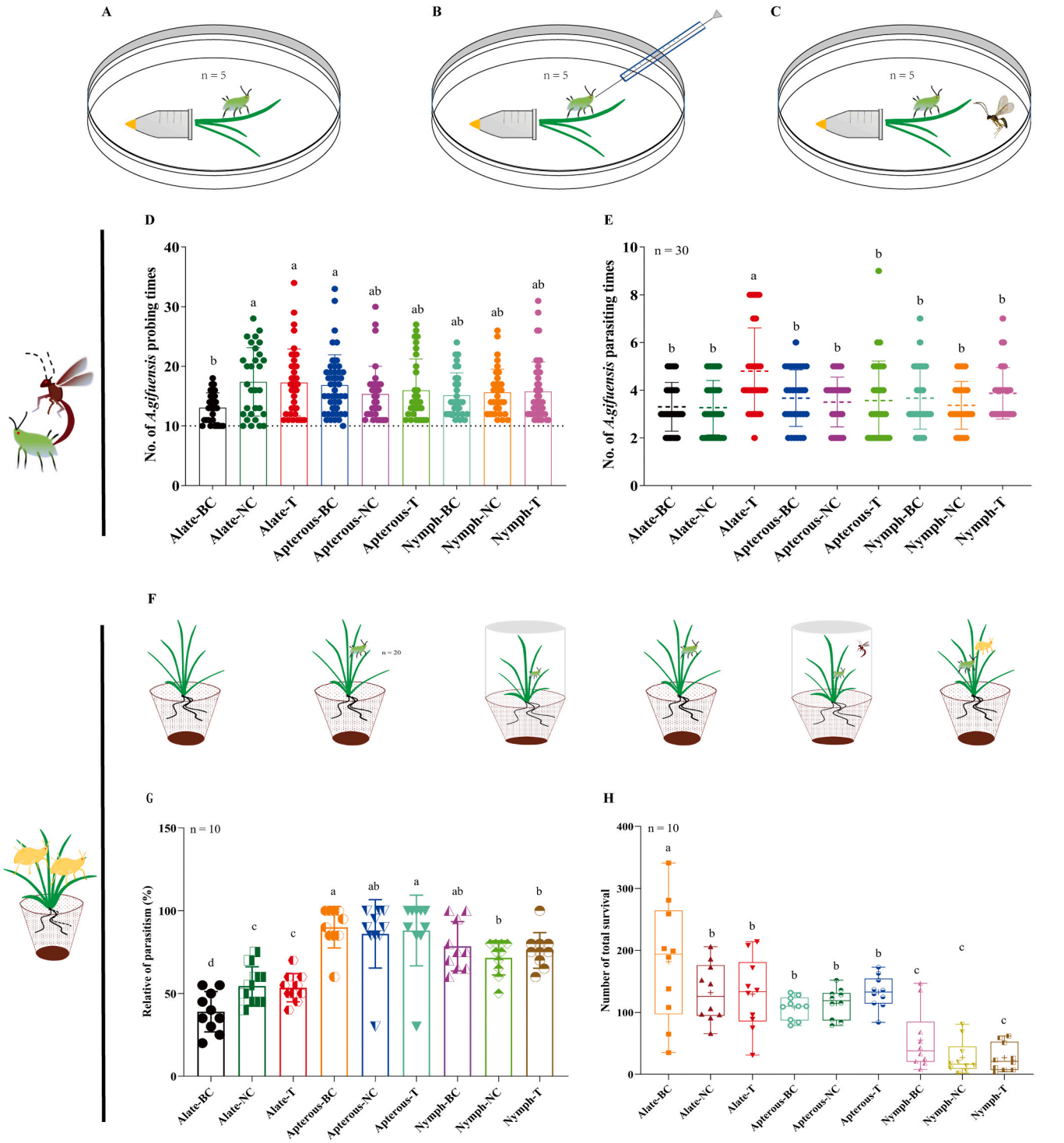


Fig. 5. Schematic of coating low concentrations of E-β-farnesene on the pronotum of *S. miscanthi* and the foraging behavior of *A. gifuensis* responses. (A)-(C): Diagram of coating low concentrations of E-β-farnesene on the pronotum of *S. miscanthi*; (D): Number of *A. gifuensis* probing times, $n \geq 30$; (E): Number of *A. gifuensis* parasitizing times, $n \geq 30$; (F): Diagram of *A. gifuensis* foraging behavior based on coating EBF on *S. miscanthi*. (G): Relative *S. miscanthi* parasitism. (H): Total number of surviving aphids. Nymph-NC: nymph negative control, nymph-T: nymph treatment; apterous-BC: apterous blank control, apterous-NC: apterous negative control, apterous-T: apterous treatment; alate-BC: alate blank control, alate-NC: alate negative control, alate-T: alate treatment. The different lowercase letters (a, b, c, d) indicate significant differences (one-way ANOVA followed by Tukey's HSD multiple comparison test, $P < 0.05$).

nymph aphid. This supported the idea that there was remains of EBF on the surface of aphids that attracted the aphidius' attention and made it excited and probe frequently. EBF was detected from the fresh and dried cornicles of *S. avenae* [12], and recent work also found that the extract from the surface of *A. pisum* contains EBF, which serves as a short-range guide for the larvae of *E. corollae* [42]. The length of the wing tissue covers the entire abdomen and extends beyond the end of the abdomen. This may physically interfere with the antennal detection of aphidius to abdominal EBF of aphid, thus reducing the interest of aphidius in winged adults. However, when EBF was applied to winged aphids, a portion of the wing tissue that covered the abdomen was coated by EBF. This could explain why trace EBF significantly increased the frequency

of winged aphid probes and attacks, further increasing the rate of mummies. Although EBF significantly increased the parasitism rate of winged aphids, it was still significantly lower than that of wingless aphids and nymphs, which may also be due to the obstruction of wing tissue. For nymph and wingless aphids, compared with body size, it is more difficult for the aphidius to accurately attack the smaller aphid. This may be the reason result in a difference in the parasitism rate between them. The total survival of the winged aphid was significantly higher than that of the wingless and nymph in TB groups (Fig. 5H); a reasonable explanation is that the winged aphid is also divided into colonized aphids and migratory aphids, and when the winged aphid is colonized, the flight muscle is decomposed in its body, and it has the ability to transform into a reproductive aphid [87]. A large number of aphids were parasitized in the wingless and nymph groups, while the winged aphid had a much lower rate than them. As a result, the winged aphids that survived produced a considerably higher number of offspring nymphs than the wingless and nymphs. Meanwhile, the 2–3 instar nymph started producing aphid 3–4 days later on average than other adults groups, which was why the number of surviving aphids in the nymph groups (BC, NC, T) was much lower than in the winged and wingless adult groups.

EBF is a critical infochemical in the tri-trophic level interactions among plant-aphid-natural enemies, and its biological significance to aphids seems to be more likely continuation of the aphid population through a few escapes than overall survival of the current generation. The majority of aphid species respond to EBF at a low dosage of 1 ng to 10 ng in the field [88]. However, aphids typically emit cornicle droplets only after being physically attacked [5], resulting in emitter escape in approximately 10 % of attacks [4,89]. Furthermore, within an aphid colony, a single or few aphids are generally attacked at the same time, and the signal is not amplified by the emission of neighboring aphids [90]. Of note, EBF applied in this study was dissolved in triglycerides (TICs). We chose TIC because the major component of aphid droplets secreted from cornicles is TIC [91]. Although the solubility of EBF in different solvents is different, the amount of EBF volatilized out is definitely not the same as the amount we applied to insects, which was not identified in this study.

Although odors, electromagnetic radiation, sounds and visual cues, such as the color and shape of the aphids [92,93], may contribute to target detection of aphidius to some extent, olfactory stimuli are predominant [90,94–98]. Several investigations have also reported the involvement of host conditions and predator affects parasitism. For example, parasitized aphid colonies are less attractive than healthy ones [99]. When the species richness of parasitoid wasps increases, the parasitism ratio decreases [100]. Furthermore, both host density and natal host significantly affected the self superparasitism of *A. gifuensis* on *S. miscanthi* [51], and the presence of aphid predator chemical cues (such as *H. axyridis*-related cues) influenced the foraging behavior and activity of *A. gifuensis* [75]. EBF triggers attack behavior in predators [101] and parasitoids [12,48], clearly serving as a stimulant for host/prey finding and attacking. For instance, the release of alarm pheromones by *S. avenae* is attractive for *A. rhopalosiphi* parasitoids [12], while the emission of cornicle secretions by *A. pisum* stimulates a strong oviposition attack response from *A. ervi* females [48]. In addition, in this study in the lab, we also found that the biological significance to natural enemies seems to be taking advantage of EBF to ensure every full meal. However, wasp parasitism rates are relatively low when evaluated in an open ecosystem like the field. For example, the highest parasitism rate of aphids in maize fields was only 1.79 % in Jilin Province, China [102], and the calculation of the parasitism ratio of *S. miscanthi* is estimated to be between 10 % and 15 % [103–105]. *A. gossypii* showed a similar phenomenon, approximately between 10 % and 20 % [100]. Therefore, our work is instructive to increase the parasitism rate and promote the biological control efficiency of *A. gifuensis*, especially for winged aphids, by coating trace levels of EBF on the dorsal abdomen of *S. miscanthi*.

5. Conclusion

In this study, our results demonstrated a high plasticity of OBPs on EBF recognition in *A. gifuensis*. AgifOBP6 could be the leading one among 5 EBF-binding OBPs, and hydrophobic interactions were the main forces between AgifOBP6 and EBF or EBF analog. It also showed that AgifOBP6 is expressed in the peripheral sensilla organs of antennae by whole-mount immunolocalization, such as the sensilla placodea, sensilla coeloconica, and sensilla trichaidea. And EBF-binding OBPs evolved separately in the aphidius *A. gifuensis* and its host aphid, and were eventually driven to a common biological function by convergent evolution.

Then, it was confirmed that EBF could promote the biological control efficiency of *A. gifuensis*, especially on winged aphids. Our findings provide a new perspective on aphid biological control by using EBF to reduce the initial population size of migratory biotype aphids from the source areas: promoting the biological control efficiency of parasitoids against winged aphids before migration by using low concentration of EBF. which will aid in the strengthening and better utilization of *A. gifuensis* as a powerful and natural biocontrol strategy.

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CRediT authorship contribution statement

XJ collected the samples for RNA extraction, HIC (immuno-histochemical) as well as Western blot and further performed the molecular detection, bioassay and bioinformatic prediction. JJ, MMY and SYZ participated in part implementation of the above works. XJ, JF and YX raised the insects and established a laboratory population for the study. XJ and JF discussed and analyzed the data, and wrote the draft. YQ, JF and JC conceived and organized the project. JC and FF directed the project. All authors contributed to the article and approved the submitted version.

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Declaration of competing interest

On behalf of all authors, I clarify that the authors whose names are listed below have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

The original contributions presented in the study are included in the article/Supplementary Material, and further inquiries can be directed to the corresponding author.

References

[1] S. Lamichhaney, J. Berglund, M.S. Almén, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerová, C.J. Rubin, C. Wang, N. Zamani, B.R. Grant, P.R. Grant, M. T. Webster, L. Andersson, Evolution of Darwin's finches and their beaks revealed

by genome sequencing, Nature 518 (2015) 371–375, https://doi.org/10.1038/nature14181.
[2] M. Simões, L. Breitkreuz, M. Alvarado, S. Baca, J.C. Cooper, L. Heins, K. Herzog, B.S. Lieberman, The evolving theory of evolutionary radiations, Trends Ecol. Evol. 1 (2016) 27–34, https://doi.org/10.1016/j.tree.2015.10.007.
[3] M. Büsgen, Der honigtau biologische studien an pflanzen und pflanzenlaüsen, Jena. Zeits, Naturwiss, 1891.
[4] A.F.G. Dixon, The escape responses shown by certain aphids to the presence of the coccinellid Adalia decempunctata (L.), Trans.R.Entomol.Soc.Lond. 110 (1958) 319–334, https://doi.org/10.1111/j.1365-2311.1958.tb00786.x.
[5] L.R. Nault, P.L. Phelan, Alarm pheromones and sociality in pre-social insects, in: Chemical Ecology of Insects, Springer, Boston, MA, 1984, pp. 237–256, https://doi.org/10.1007/978-1-4899-3368-3_9.
[6] C.J. Kislow, L.J. Edwards, Repellent odour in aphids, Nature 235 (1972) 108–109, https://doi.org/10.1038/235108a0.
[7] F.P. Müller, Differential alarm pheromone responses between strains of the aphid Acyrthosiphon pisum, Entomol. Exp. Appl. 34 (1983), 347e348, https://doi.org/10.1111/j.1570-7458.1983.tb03348.x.
[8] J.A. Pickett, L.J. Wadhams, C.M. 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.
[9] R. Zhang, B. Wang, G. Grossi, P. Falabella, Y. Liu, S. Yan, J. Lu, J. Xi, G. Wang, Molecular basis of alarm pheromone detection in aphids, Curr. Biol. 27 (2017) 55–61, https://doi.org/10.1016/j.cub.2016.10.013.
[10] 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.
[11] 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.
[12] S.G. Micha, U. Wyss, Aphid alarm pheromone (E)-β-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphididae), Chemoecology 7 (1996) 132–139, https://doi.org/10.1007/BF01245965.
[13] J. Zhu, A.A. Cossé, J.J. Obrycki, K.S. Boo, T.C. Baker, Olfactory reactions of the twelve-spotted lady beetle, Coleomegilla maculata and the green lacewing, Chrysoperla carnea to semiochemicals released from their prey and host plant: electroantennogram and behavioral responses, J. Chem. Ecol. 25 (1999) 1163–1177, https://doi.org/10.1023/A:1020846212465.
[14] R. Buitenhuis, J.N. McNeil, G. Boivin, J. Brodeur, The role of honeydew in host searching of aphid hyperparasitoids, J. Chem. Ecol. 30 (2004) 273–285, https://doi.org/10.1023/B: JOEC.0000017977.39957.97.
[15] F. Francis, G. Lognay, E. Haubruge, Olfactory responses to aphid and host plant volatile releases:(E)-β-farnesene an effective kairomone for the predator Adalia bipunctata, J. Chem. Ecol. 30 (4) (2004) 741–755, https://doi.org/10.1023/B:JOEC.0000028429.13413.a2.
[16] F. Francis, T. Martin, G. Lognay, E. Haubruge, Role of (E)-beta-farnesene in systematic aphid prey location by Episyrphus balteatus larvae (Diptera: Syrphidae), Eur. J. Entomol. 102 (2005) 431–436, https://doi.org/10.14411/eje.2005.061.
[17] F.J. Verheggen, Q. Fagel, S. Heuskin, G. Lognay, F. Francis, E. Haubruge, Electrophysiological and behavioral responses of the multicolored Asian lady beetle, Harmonia axyridis Pallas, to sesquiterpene semiochemicals, J. Chem. Ecol. 33 (2007) 2148–2155, https://doi.org/10.1007/s10886-007-9370-6.
[18] F.J. Verheggen, L. Arnaud, S. Bartram, M. Gohy, E. Haubruge, Aphid and plant volatiles induce oviposition in an aphidophagous hoverfly, J. Chem. Ecol. 34 (2008) 301–307, https://doi.org/10.1007/s10886-008-9434-2.
[19] S. Heuskin, S. Lorge, B. Godin, P. Leroy, I. Frère, F.J. Verheggen, E. Haubruge, J. P. Wathelet, M. Mestdagh, T. Hance, G. Lognay, Optimisation of a semiochemical slow-release alginate formulation attractive towards Aphidius ervi Haliday parasitoids, Pest Manag. Sci. 68 (2012) 127–136, https://doi.org/10.1002/ps.2234.
[20] J. Fan, Q. Zhang, Q. Xu, W. Xue, Z. Han, J. Sun, J. Chen, Differential expression analysis of olfactory genes based on a combination of sequencing platforms and behavioral investigations in Aphidius gifuensis, Front. Physiol. 9 (2018) 1679, https://doi.org/10.3389/fphys.2018.01679.
[21] C. Joachim, W.W. Weisser, Does the aphid alarm pheromone (E)-β-farnesene act as a kairomone under field conditions? J. Chem. Ecol. 41 (2015) 267–275, https://doi.org/10.1007/s10886-015-0555-0.
[22] J.C. Joachim, I. Vosteen, W.W. Weisser, The aphid alarm pheromone (E)-β-farnesene does not act as a cue for predators searching on a plant, Chemoecology 25 (2015) 105–113, https://doi.org/10.1007/s00049-014-0176-z.
[23] C. Schnee, T.G. Köllner, J. Gershenzon, J. Degenhardt, The maize gene terpene synthase 1 encodes a sesquiterpene synthase catalyzing the formation of (E)-β-farnesene, (E)-nerolidol, and (E, E)-farnesol after herbivore damage, Plant Physiol. 130 (2002) 2049–2060, https://doi.org/10.1104/pp.008326.
[24] J. Crock, M. Wildung, R. Croteau, Isolation and bacterial expression of a sesquiterpene synthase cDNA clone from peppermint (Mentha x piperita, L.) that produces the aphid alarm pheromone (E)-β-farnesene, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 12833–12838, https://doi.org/10.1073/pnas.94.24.12833.
[25] I. Vosteen, W.W. Weisser, G. Kunert, Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? Ecol. Entomol. 41 (2016) 1–12, https://doi.org/10.1111/een.12271.
[26] R.G. Vogt, L.M. Riddiford, Pheromone binding and inactivation by moth antennae, Nature 293 (1981) 161–163, https://doi.org/10.1038/293161a0.
[27] 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.
[28] W.S. Leal, Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes, Annu. Rev. Entomol. 58 (2013) 373–391, https://doi.org/10.1146/annurev-ento-120811-153635.
[29] Q. Wang, J. Liu, Y. Zhang, J. Chen, X. Li, P. Liang, X. Gao, J. Zhoue, S. 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.
[30] 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.
[31] H. Qiao, E. Tuccori, X. He, A. Gazzano, L. Field, J. Zhou, P. Pelosi, 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.
[32] Y. Qin, Z. Yang, D. Song, Q. Wang, S. Gu, W. Li, H. Duan, J. Zhou, X. Yang, 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.
[33] L. Wang, Y. Bi, M. Liu, W. Li, M. Liu, S. Di, S. Yang, C. Fan, L. Bai, Y. Lai, 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.
[34] D. Bruno, G. Grossi, R. Salvia, A. Scala, D. Farina, A. Grimaldi, J. Zhou, S.A. Bufo, H. Vogel, E. Grosse-Wilde, B.S. Hansson, P. Falabella, 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.
[35] J. Fan, W. Xue, H. Duan, X. Jiang, Y. Zhang, W. Yu, S. Jiang, J. Sun, J. Chen, 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) 151–160, https://doi.org/10.1016/j.jinsphys.2017.07.014.
[36] Z. Liu, X. Huang, L. Jiang, G. Qiao, The species diversity and geographical distribution of aphids in China (Hemiptera, Aphidoidea), Acta Zootaxon.Sin. 34 (2009) 277–291.
[37] X. Jiang, Q. Zhang, Y. Qin, H. Yin, S. Zhang, Q. Li, Y. Zhang, J. Fan, J. Chen, A chromosome-level draft genome of the grain aphid Sitobion miscanthi, Gigascience 8 (2019), giz101, https://doi.org/10.1093/gigascience/giz101.
[38] S. Zhang, Q. Zhang, X. Jiang, Q. Li, Y. Qin, W. Wang, J. Fan, J. Chen, 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.
[39] W. Dong, F. Zhang, Y. Fang, Z. Zhang, Electroantennogram responses of aphid parasitoid Aphidius gifuensis to aphid pheromones and host-plant volatiles, Chin. J. Ecol. 27 (2008) 591–594.
[40] C.M. De Moraes, W.J. Lewis, P.W. Paré, H.T. Alborn, J.H. Tumlinson, Herbivore-infested plants selectively attract parasitoids, Nature 393 (1998) 570–573, https://doi.org/10.1038/31219.
[41] S. Yang, R. Xu, S. Yang, R. Kuang, Olfactory responses of Aphidius gifuensis to odors of host plants and aphid-plant complexes, Insect Sci. 16 (2009) 503–510, https://doi.org/10.1111/j.1744-7917.2009.01282.x.
[42] B. Wang, W. Dong, H. Li, C. D'Onofrio, P. Bai, R. Chen, L. Yang, J. Wu, X. Wang, B. Wang, D. Ai, W. Knoll, P. Pelosi, G. Wang, Molecular basis of (E)-β-farnesene-mediated aphid location in the predator Eupeodes corollae, Curr. Biol. 32 (2022) 951–962, https://doi.org/10.1016/j.cub.2021.12.054.
[43] Z. Li, S. Zhang, X. Cai, J. Luo, S. Dong, J. Cui, Z. Chen, Three odorant binding proteins may regulate the behavioural response of Chrysopa pallens to plant volatiles and the aphid alarm pheromone (E)-β-farnesene, Insect Mol. Biol. 26 (2017) 255–265, https://doi.org/10.1111/imb.12295.
[44] C. Qu, Z. Yang, S. Wang, H. Zhao, F. Li, X. Yang, C. Luo, Binding affinity characterization of four antennae-enriched odorant-binding proteins from Harmonia axyridis (Coleoptera: Coccinellidae), Front. Physiol. 13 (2022), 829766, https://doi.org/10.3389/fphys.2022.829766.
[45] Y. Du, G.M. Poppy, W. Powell, L.J. Wadhams, Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid Aphidius ervi (Hymenoptera: Braconidae), J. Insect Behav. 10 (1997) 509–522, https://doi.org/10.1007/BF02765374.
[46] Y. Du, G.M. Poppy, W. Powell, J.A. Pickett, L.J. Wadhams, C.M. Woodcock, Identification of semiochemicals released during aphid feeding that attract parasitoid Aphidius ervi, J. Chem. Ecol. 24 (1998), 1355e1368, https://doi.org/10.1023/A:1021278816970.
[47] E. Guerrieri, G.M. Poppy, W. Powell, E. Tremblay, F. Pennacchio, Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of Aphidius ervi, J. Chem. Ecol. 25 (1999) 1247–1261, https://doi.org/10.1023/A:1020914506782.
[48] D. Battaglia, F. Pennacchio, G. Marincola, A. Tranfaglia, Cornicle secretion of Acyrthosiphon pisum (Homoptera: Aphididae) as a contact kairomone for the parasitoid Aphidius ervi (Hymenoptera: Braconidae), Eur. J. Entomol. 90 (1993), 423-423.
[49] X. Jiang, Y. Qin, J. Jiang, Y. Xu, F. Francis, J. Fan, J. Chen, Spatial expression analysis of odorant binding proteins in both sexes of the aphid parasitoid

Aphidius gifuensis and their ligand binding properties, Front. Physiol. 13 (2022), 877133, https://doi.org/10.3389/fphys.2022.877133.
[50] I. Ohta, K. Honda, Use of Sitobion akebiae (Hemiptera: Aphididae) as an alternative host aphid for a banker-plant system using an indigenous parasitoid, Aphidius gifuensis (Hymenoptera: Braconidae), Appl. Entomol. Zool. 45 (2010) 233–238, https://doi.org/10.1303/aez.2010.233.
[51] M. Pan, T. Liu, C. Nansen, Avoidance of parasitized host by female wasps of Aphidius gifuensis (Hymenoptera: Braconidae): the role of natal rearing effects and host availability? Insect Sci. 25 (2018) 1035–1044, https://doi.org/10.1111/1744-7917.12496.
[52] L. Zhang, G. Lu, X. Huang, H. Guo, X. Su, L. Han, Y. Zhang, Z. Qi, Y. Xiao, H. Cheng, Overexpression of the caryophyllene synthase gene GhTPS1 in cotton negatively affects multiple pests while attracting parasitoids, Pest Manag. Sci. 76 (2020) 1722–1730, https://doi.org/10.1002/ps.5695.
[53] Y. Song, C. Liu, P. Cai, W. Chen, Y. Guo, J. Lin, S. Zhang, Host-seeking behavior of Aphidius gifuensis (Hymenoptera: Braconidae) modulated by chemical cues within a tritrophic context, J. Insect Sci. 21 (2021), https://doi.org/10.1093/jisesa/ieab036.
[54] I.T. Barbarossa, P. Muroni, M.D. Setzu, A.M. Angioy, Dose-dependent nonassociative olfactory learning in a fly, Chem. Senses 32 (2007) 535–541, https://doi.org/10.1093/chemse/bjm022.
[55] L. Pan, M. Guo, X. Jin, Z. Sun, H. Jiang, J. Han, Y. Wang, C. Yan, M. Li, Full-length transcriptome survey and expression analysis of parasitoid wasp Chouioia cunea upon exposure to 1-dodecene, Sci. Rep. 9 (2019) 1–11, https://doi.org/10.1038/s41598-019-54710-0.
[56] L. Hu, B. Chen, K. Liu, G. Yu, Y. Chen, J. Dai, X. Zhao, G. Zhong, Y. Zhang, J. Shen, OBP2 in the midlegs of the male Bactrocera dorsalis is involved in the perception of the female-biased sex pheromone 4-allyl-2, 6-dimethoxyphenol, J. Agric. Food Chem. 69 (2021) 126–134, https://doi.org/10.1021/acs.jafc.0c05945.
[57] Y. Ma, T. Huang, B. Tang, B. Wang, L. Wang, J. Liu, Q. Zhou, Transcriptome analysis and molecular characterization of soluble chemical communication proteins in the parasitoid wasp Anagrus nilaparvatae (Hymenoptera: Mymaridae), Ecol. Evol. 12 (2022), e8661, https://doi.org/10.1002/ece3.8661.
[58] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[59] 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.
[60] 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.
[61] 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.
[62] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (2021) 3022–3027, https://doi.org/10.1093/molbev/msab120.
[63] Q. Wang, J. Zhou, J. Liu, G. Huang, W. Xu, Q. Zhang, J. Chen, Y. Zhang, X. Li, S. Gu, Integrative transcriptomic and genomic analysis of odorant binding proteins and chemosensory proteins in aphids, Insect Mol. Biol. 28 (2019) 1–22, https://doi.org/10.1111/imb.12513.
[64] W. Xue, J. Fan, Y. Zhang, Q. Xu, Z. Han, J. Sun, J. Chen, 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.
[65] S. Gu, K. Wu, Y. Guo, L.M. Field, J.A. Pickett, Y. Zhang, J. Zhou, 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.
[66] J. Zhou, F.G. Vieira, X. He, C. Smadja, R. Liu, J. Rozas, L.M. Field, Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid Acyrthosiphon pisum, Insect Mol. Biol. 19 (2010) 113–122, https://doi.org/10.1111/j.1365-2583.2009.00919.x.
[67] Z. Li, S. Zhang, Y. Ma, J. Luo, C. Wang, L. Lv, S. Dong, J. Cui, First transcriptome and digital gene expression analysis in Neuroptera with an emphasis on chemoreception genes in Chrysopa pallens (Rambur), PLoS One 8 (2013), e67151, https://doi.org/10.1371/journal.pone.0067151.
[68] B. Wang, Y. Liu, G.R. Wang, Chemosensory genes in the antennal transcriptome of two syrphid species, Episyrphus balteatus and Eupeodes corollae (Diptera: Syrphidae), BMC Genomics 18 (2017) 1–15, https://doi.org/10.1186/s12864-017-3939-4.
[69] H. Jia, Y. Sun, S. Luo, K. Wu, Characterization of antennal chemosensilla and associated odorant binding as well as chemosensory proteins in the Eupeodes corollae (Diptera: Syrphidae), J. Insect Physiol. 113 (2019) 49–58, https://doi.org/10.1016/j.jinsphys.2018.08.002.
[70] R.A. Laskowski, J.A.C. Rullmann, M.W. MacArthur, R. Kaptein, J.M. Thornton, AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR, J. Biomol. NMR 8 (1996) 477–486, https://doi.org/10.1007/BF00228148.
[71] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E. C. Meng, T.E. Ferrin, UCSF Chimera-a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605–1612, https://doi.org/10.1002/jcc.20084.
[72] P.T. Lang, S.R. Brozell, S. Mukherjee, E.F. Pettersen, E.C. Meng, V. Thomas, R. C. Rizzo, D.A. Case, T.L. James, I.D. Kuntz, DOCK 6: combining techniques to model RNA-small molecule complexes, RNA 15 (2009) 1219–1230, https://doi.org/10.1261/rna.1563609.
[73] S. Muk Mukherjee, T.E. Balius, R.C. Rizzo, Docking validation resources: protein family and ligand flexibility experiments, J. Chem. Inf. Model. 50 (2010) 1986–2000, https://doi.org/10.1021/ci1001982.
[74] Q. Li, Y. Fu, X. Liu, J. Sun, M. Hou, Y. Zhang, J. Chen, Activation of wheat defense response by Buchnera aphidicola-derived small chaperone protein GroES in wheat aphid saliva, J. Agric. Food Chem. 70 (2022) 1058–1067, https://doi.org/10.1021/acs.jafc.1c07046.
[75] P.L. Xia, X.L. Yu, Z.T. Li, Y. Feng, The impacts of Harmonia axyridis cues on foraging behavior of Aphidius gifuensis to Myzus persicae, J. Asia Pac. Entomol. 24 (2021) 278–284, https://doi.org/10.1016/j.aspen.2020.12.008.
[76] M. Rezaei, A.A. Talebi, Y. Fathipour, J. Karimzadeh, M. Mehrabadi, Foraging behavior of Aphidius matricariae (Hymenoptera: Braconidae) on tobacco aphid, Myzus persicae nicotianae (Hemiptera: Aphididae), Bull. Entomol. Res. 109 (2019) 840–848, https://doi.org/10.1017/S0007485319000166.
[77] S. Wu, Z. Zhou, S. Peng, X. Shan, W. Zeng, H. Cai, F. Zhou, Parasitic selectivity of Aphidius gifuensis on different ages of Myzus persicae and development of their offsprings (in Chinese), J.Henan Agric.Sci. 46 (2017) 84–88.
[78] M. Pan, H. Cao, T. Liu, Effects of winter wheat cultivars on the life history traits and olfactory response of Aphidius gifuensis, BioControl. 59 (2014) 539–546, https://doi.org/10.1007/s10526-014-9594-7.
[79] Z. Bi, Z. Ji, Biological study of Aphidius gifuensis Ashmead: I. The developmental process and nymph morphology (in Chinese), J.Hebei Agric.Univ. 16 (1993) 1–8.
[80] A. Scaloni, M. Monti, S. Angeli, P. Pelosi, Structural analysis and disulfide-bridge pairing of two odorant-binding proteins from Bombyx mori, Biochem. Biophys. Res. Commun. 266 (1999) 386–391, https://doi.org/10.1006/bbrc.1999.1791.
[81] C. Yao, L. Du, Q. Liu, X. Hu, W. Ye, T.C.J. Turlings, Y. Li, Stemborer-induced rice plant volatiles boost direct and indirect resistance in neighboring plants, New Phytol. (2022), https://doi.org/10.1111/nph.18548.
[82] T. Northey, H. Venthur, F. De Biasio, F.X. Chauviac, A. Cole, K.A.L.R. Junior, G. Grossi, P. Falabella, L.M. Field, N.H. Keep, J. Zhou, Crystal structures and binding dynamics of odorant-binding protein 3 from two aphid species Megoura viciae and Nasonovia ribisnigri, Sci. Rep. 6 (2016) 1–13, https://doi.org/10.1038/srep24739.
[83] S.A. Ochieng, K.C. Park, J.W. Zhu, T.C. Baker, Functional morphology of antennal chemoreceptors of the parasitoid Microplitis croceipes (Hymenoptera: Braconidae), Arthropod Struct. Dev. 29 (2000) 231–240, https://doi.org/10.1016/S1467-8039(01)00008-1.
[84] D. Bourdais, P. Vernon, L. Krespi, J. Le Lannic, J. Van Baaren, Antennal structure of male and female Aphidius rhopalosiphi DeStefani-peres (Hymenoptera: Braconidae): description and morphological alterations after cold storage or heat exposure, Microsc. Res. Tech. 69 (2006) 1005–1013.
[85] Y. Xi, X. Y, X. Li, C. Zhu, Y. Zhang, Scanning electron microscopy studies of antennal sensilla of Lysiphlebus fabarum (Marshall) (Hymenoptera: Braconidae), Acta Ecol. Sin. 53 (2010) 936–942.
[86] P. Das, L. Chen, K.R. Sharma, H.Y. Fadamiro, Abundance of antennal chemosensilla in two parasitoid wasps with different degree of host specificity may explain sexual and species differences in their response to host-related volatiles, Microsc. Res. Tech. 74 (2011) 900–909, https://doi.org/10.1002/jemt.20974.
[87] Y. Bai, X. Pei, N. Ban, N. Chen, S. Liu, S. Li, T. Liu, Nutrition-dependent juvenile hormone sensitivity promotes flight-muscle degeneration during the aphid dispersal-reproduction transition, Development 149 (2022), dev200891, https://doi.org/10.1242/dev.200891.
[88] X. Jing-Gong, F. Zhang, Y. Fang, W. Kan, G. Zhang, Z. Zhang, Behavioural response of aphids to the alarm pheromone component (E)-β-farnesene in the field, Physiol. Entomol. 27 (2002) 307–311, https://doi.org/10.1046/j.1365-3032.2002.00302.x.
[89] J.S. Edwards, Defence by smear: supercooling in the cornicle wax of aphids, Nature 211 (1966) 73–74, https://doi.org/10.1038/211073a0.
[90] E. Hatano, G. Kunert, S. Bartram, W. Boland, J. Gershenzon, W.W. Weisser, Do aphid colonies amplify their emission of alarm pheromone? J. Chem. Ecol. 34 (2008) 1149–1152, https://doi.org/10.1007/s10886-008-9527-y.
[91] R.K. Callow, A.R. Greenway, D.C. Griffiths, Chemistry of the secretion from the cornicles of various species of aphids, J. Insect Physiol. 19 (1973) 737–748, https://doi.org/10.1016/0022-1910(73)90146-7.
[92] S.B. Vinson, Host selection by insect parasitoids, Annu. Rev. Entomol. 21 (1976) 109–133, https://doi.org/10.1146/annurev.en.21.010176.000545.
[93] J.P. Harmon, J.E. Losey, A.R. Ives, The role of vision and color in the close proximity foraging behavior of four coccinellid species, Oecologia 115 (1998) 287–292, https://doi.org/10.1007/s004420050518.
[94] D.P. Read, P.P. Feeny, R.B. Root, Habitat selection by the aphid parasite Diaeretiella rapae (Hymenoptera: Braconidae) and hyperparasite Charips brassicae (Hymenoptera: Cynipidae), Can. Entomol. 102 (1970) 1567–1578, https://doi.org/10.4039/Ent1021567-12.
[95] R. Singh, T.B. Sinha, Bionomics of Trioxys (Binodoxys) indicus Subba Rao & Sharma, an aphidiid parasitoid of Aphis craccivora Koch, J. Appl. Entomol. 93 (1982) 64–75, https://doi.org/10.1111/j.1439-0418.1982.tb03571.x.
[96] M.G.V. Wickremasinghe, H.V. Emden, Reactions of adult female parasitoids, particularly Aphidius rhopalosiphi, to volatile chemical cues from the host plants

of their aphid prey, Physiol. Entomol. 17 (1992) 297–304, https://doi.org/10.1111/j.1365-3032.1992.tb01025.x.
[97] W. Powell, F. Pennacchio, G.M. Poppy, E. Tremblay, Strategies involved in the location of hosts by the parasitoid Aphidius ervi Haliday (Hymenoptera: Braconidae: Aphidiinae), Biol. Control 11 (1998) 104–112, https://doi.org/10.1006/bcon.1997.0584.
[98] A. Storeck, G.M. Poppy, H.F. van Emden, W. Powell, The role of plant chemical cues in determining host preference in the generalist aphid parasitoid Aphidius colemani, Entomol. Exp. Appl. 97 (2000) 41–46, https://doi.org/10.1046/j.1570-7458.2000.00714.x.
[99] A. Pineda, I. Morales, M.A. Marcos-García, A. Fereres, Oviposition avoidance of parasitized aphid colonies by the syrphid predator Episyrphus balteatus mediated by different cues, Biol. Control 42 (2007) 274–280, https://doi.org/10.1016/j.biocontrol.2007.05.017.
[100] F. Yang, B. Liu, Y. Zhu, K.A. Wyckhuys, W. van der Werf, Y. Lu, Species diversity and food web structure jointly shape natural biological control in agricultural landscapes, Commun. Biol. 4 (2021) 1–11, https://doi.org/10.1038/s42003-021-02509-z.
[101] P. Kindlmann, K. Houdkova, A.F.G. Dixon, Aphid Biodiversity Under Environmental Change, 2010. Dordrecht, The Netherlands.
[102] Y. Zhang, M. Zhao, J. Cheng, S. Liu, H. Yuan, Population dynamics and species composition of maize field parasitoids attacking aphids in northeastern China, PLoS One 15 (2020), e0241530, https://doi.org/10.1371/journal.pone.0241530.
[103] F. Yang, L. Xu, Y. Wu, Q. Wang, Z. Yao, V. Žikić, Ž. Tomanović, M. Ferrer-Suay, J. Selfa, J. Pujade-Villar, M. Traugott, N. Desneux, Y. Lu, Y. Guo, Species composition and seasonal dynamics of aphid parasitoids and hyperparasitoids in wheat fields in northern China, Sci. Rep. 7 (2017), 13989, https://doi.org/10.1038/s41598-017-14441-6.
[104] Q. Xu, S. Hatt, Z. Han, F. Francis, J. Chen, Combining E-β-farnesene and methyl salicylate release with wheat-pea intercropping enhances biological control of aphids in North China, Biocontrol Sci. Tech. 28 (2018) 883–894, https://doi.org/10.1080/09583157.2018.1504885.
[105] J. Liu, X. Zhao, Y. Zhan, K. Wang, F. Francis, Y. Liu, New slow release mixture of (E)-β-farnesene with methyl salicylate to enhance aphid biocontrol efficacy in wheat ecosystem, Pest Manag. Sci. 77 (2021) 3341–3348, https://doi.org/10.1002/ps.6378.