



OPEN ACCESS

EDITED BY
Carolina E Reisenman,
University of California, United States

REVIEWED BY
Teruyuki Matsunaga,
The University of Tokyo, Japan
Youssef Dewar,
Agricultural Research Center, Egypt

*CORRESPONDENCE
Youjun Zhang,
zhangyoujun@caas.cn

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

RECEIVED 08 July 2022
ACCEPTED 12 September 2022
PUBLISHED 27 September 2022

CITATION
Yang Y, Hua D, Zhu J, Wang F and
Zhang Y (2022), Chemosensory protein
4 is required for *Bradysia odoriphaga* to
be olfactory attracted to sulfur
compounds released from
Chinese chives.
Front. Physiol. 13:989601.
doi: 10.3389/fphys.2022.989601

COPYRIGHT
© 2022 Yang, Hua, Zhu, Wang and
Zhang. This is an open-access article
distributed under the terms of the
Creative Commons Attribution License
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Chemosensory protein 4 is required for *Bradysia odoriphaga* to be olfactory attracted to sulfur compounds released from Chinese chives

Yuting Yang¹, Dengke Hua², Jiaqi Zhu¹, Fu Wang¹ and
Youjun Zhang^{1,3*}

¹Hubei Engineering Technology Center for Pest Forewarning and Management, Institute of Insect Sciences, Yangtze University, Jingzhou, Hubei, China, ²Institute of Agricultural Quality Standards and Testing Technology Research, Hubei Academy of Agricultural Sciences/Hubei Key Laboratory of Nutritional Quality and Safety of Agro Products, Wuhan, Hubei, China, ³Department of Plant Protection, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China

Bradysia odoriphaga (Diptera: Sciaridae) is a serious pest of Chinese chives cultivated in China. Chemosensory proteins (CSPs) are important components of insect olfactory systems that capture and bind environmental semiochemicals which are then transported to olfactory receptors. Despite their importance, the mechanism of olfaction and related behavioral processes in *B. odoriphaga* have not been characterized. Here, we found that *BodoCSP4* has an important olfactory function. RT-qPCR indicated that *BodoCSP4* expression was highest in the heads (antennae removed) of adult males, followed by the antennae of adult males. Competitive binding assays with 33 ligands indicated that *BodoCSP4* binds well with methyl allyl disulfide, diallyl disulfide, and n-heptadecane; the corresponding dissociation constants (K_i) were as high as 5.71, 5.71, and 6.85 μ M, respectively. 3D-structural and molecular docking indicated that *BodoCSP4* has five α -helices and surrounds the ligand with certain hydrophobic residues including Leu60, Leu63, Leu64, Ala67, Val28, Ile30, Ile33, Leu34, and Val86, suggesting these residues help *BodoCSP4* bind to ligands. Silencing of *BodoCSP4* significantly decreased the attraction of *B. odoriphaga* males to diallyl disulfide and n-heptadecane but not to methyl allyl disulfide in Y-tube olfaction assays. These results increase our understanding of how *BodoCSP4* contributes to host and female localization by *B. odoriphaga* males.

KEYWORDS

Bradysia odoriphaga, chemosensory protein 4, competitive binding assays, RNAi, Y-tube olfaction assay

Introduction

Insects depend on their olfactory systems to perceive chemical signals including host plant volatiles, sex pheromones, and alarm pheromones (Visser, 1986; Hansson, 2002; French et al., 2015). In insect olfactory systems, chemosensory proteins (CSPs) participate in the transmission of semiochemicals through the sensillar lymph fluid to the olfactory receptors (ORs) (Pelosi et al., 2005; Oliveira et al., 2018), and then transduce electrical signals in order to activate physiological functions and regulate insect behavioral responses (Pelosi et al., 2006; Antony et al., 2016). Therefore, insect CSPs have an indispensable role in the recognition of semiochemicals and help ensure that insects make timely and effective behavioral responses to such chemical signals (Pelosi et al., 2018).

Many studies have shown that insect CSPs are found in multiple tissues, suggesting that CSPs have additionally functions to chemosensation (Picimbon, 2003). In addition to being involved in host volatile recognition (Younas et al., 2022), CSPs are also involved in development (Maleszka et al., 2007), reproduction (Cheng et al., 2015; Zeng et al., 2020), circadian cycle regulation (Claridge-Chang et al., 2001), leg regeneration (Kitabayashi et al., 1998), insecticide resistance (Xu et al., 2022), vision (Zhu et al., 2016), and physiological shifts such as transition from the solitary to gregarious phase of *Locusta migratoria* (Chen et al., 2010; Guo et al., 2011). Therefore, studying how insect CSPs function will help to enrich our understanding of the potential roles of CSPs, and advance the development of comprehensive control methods using insect CSPs as molecular targets.

The Gnat *Bradysia odoriphaga* is a pest of both vegetables and mushrooms and is especially damaging to Chinese chives cultivated in Asian countries (Feng and Zheng, 1987). Feeding by *B. odoriphaga* larvae reduces the growth, development, and edibility of Chinese chives (Li et al., 2014). This pest is difficult to control because of its high fecundity and the soil-borne habitat of the larvae (Dang et al., 2001; Ma et al., 2013). At present, *B. odoriphaga* is mainly controlled via the application of insecticides, but overuse of chemical pesticides results in serious problems including insecticide resistance, environmental pollution, pesticide residues on host plants, and negative effects on human health (Yang et al., 2014). As a result, safe and effective non-chemical means to control *B. odoriphaga* are greatly needed.

Previous studies have shown that *B. odoriphaga* uses host plant volatiles to find hosts, mates, and oviposition sites (Yang et al., 2019). Li H. J. et al. (2007) found that *B. odoriphaga* females release n-heptadecane to attract males. Because the larvae have limited dispersal abilities, they rely on the adults to select suitable host plants for larval feeding, growth, and development. Although olfactory proteins are clearly important for regulating the behaviors of *B. odoriphaga* adults, most research concerning the functions of such proteins has

focused on odorant binding proteins (BodoOBPs) (Tang et al., 2019; Yang et al., 2021a; Yang et al., 2021b) rather than on BodoCSPs.

To date, five *BodoCSP* genes have been identified from the *B. odoriphaga* antennae transcriptome (Zhao et al., 2018), but only the function of *BodoCSP1* has been studied (Zhang et al., 2021). As determined by the latter authors, *BodoCSP1* is highly expressed in antennae and is involved in the perception of host plant volatiles. The present work we conducted an extensive study to explore the potential functions of the *CSP* gene, *BodoCSP4*. We found that the *BodoCSP4* expression levels were highest in the heads (without antennae) of males, followed by the antennae of males. To determine the specific physiological functions of *BodoCSP4*, we characterized its binding affinity with Chinese chive volatiles, modeled its protein structure, and investigated its potential binding sites. We also used a Y-tube olfaction assay to measure the behavioral responses of *BodoCSP4*-silenced *B. odoriphaga* adult males to host plant volatiles. These results increase our understanding of chemosensation by *B. odoriphaga* and could identify new molecular targets for controlling this important pest.

Materials and methods

Insect rearing

A population of *B. odoriphaga* was obtained from a Chinese chive field in the ShunYi District of Beijing, in 2019. The population has been maintained and fed on fresh Chinese chives (not treated with insecticides) in a climate-controlled chamber at 25–28°C, 70–80% relative humidity (RH), and a 16:8 h light/dark photoperiod.

RNA extraction and cDNA synthesis

Total RNA was extracted from eggs (n = 100), larvae (n = 10), pupae (n = 10), and adults (females and males that were <2 days old; n = 10 for each sex), and also from the antennae (n = 500), male heads (without antennae; n = 500), abdomens (n = 10), and carcasses (thoraxes, wings, and legs mixed; n = 10) of males and females. Extraction was performed with the Trizol kit (Invitrogen, CA, United States) according to the manufacturer's instructions. The RNA samples were quantified using a Nanodrop ND-2000 spectrophotometer (Nanodrop, Wilmington, DE, United States), and the integrity was confirmed by 2% agarose gel electrophoresis.

Tested ligands

N-phenyl-1-naphthylamine (1-NPN) was used as the competitive fluorescent reporter. The following volatiles were

TABLE 1 Primers used in cloning and expression of CSP4 in *B. odoriphaga*.

Primer name	Sequence (5'-3')
For cloning CSP4 open reading frames	
BodoCSP4-Sense	AGGTCATAACAGTCACAATCACCTTA
BodoCSP4-Anti- sense	AATACTTTTCGGACACACCGATGTAG
For tissue expression of CSP4	
BodoCSP4-Sense	GCACGAAAGAAGGACGTGAAC
BodoCSP4-Anti- sense	GGCCGTCGGCTTCGAATAA
Heterologous expression of CSP4	
BodoCSP4-Sense	CACCCAGGAGTACACGAAGAAATACGATAAC
BodoCSP4-Anti- sense	TTATAGGAAGGATGACCTTTTGTGTA
For dsRNA synthesis	
dsBodoCSP4-Sense	GGATCCTAATACGACTCACTATAGGTGTATGTGCAACTGTGGCAC
dsBodoCSP4-Anti- sense	GGATCCTAATACGACTCACTATAGGCATTTGGAGCAATCTGTTTG
dsGFP-Sense	TAATACGACTCACTATAGGGGTGTTCAATGCTTTTCCCGT
dsGFP-Anti- sense	TAATACGACTCACTATAGGGCAATGTTGTGGCGAATTTTG

used in binding assays with BodoCSP4: alkanes (nonane, dodecane, tetradecane, hexadecane, n-heptadecane); terpenes (ocimene, β -pinene, β -caryophyllene, (R)-(+)-limonene, α -humulene); alcohols ((Z)-3-hexen-1-ol, 1,8-cineole, citronellol, linalool); esters (butyl levulinate, methyl phenylacetate, butyl acrylate); ketones (2-hexanone, beta-Ionone); aldehydes (decanal, valeric aldehyde, octanal, benzaldehyde, heptanal, nonanal); sulfur compounds (diallyl disulfide, methyl allyl disulfide) and others (acetophenone, carvaceol, h-11 indole). All of these compounds and 1-NPN were purchased from Sigma-Aldrich (St. Louis, MO, United States) and had 98% purity. Other background information on the ligands is provided in [Supplementary Table S1](#).

Identification, sequence analysis, and phylogenetic tree construction of *BodoCSP4*

Open reading frames (ORFs), the conserved domains, and N-terminal signal peptides of *B. odoriphaga* RNA were predicted using ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>), SMART software (<http://smart.emblheidelberg.de/>) (Letunic et al., 2015), and SignalP V5.1 (<http://www.cbs.dtu.dk/services/SignalP/>) (Jannick et al., 2004). The ExPASy Proteomics Server (http://cn.expasy.org/tools/pi_tool.html) was used to compute the isoelectric points and molecular weights of the deduced protein sequences. Primers used to validate the *BodoCSP4* sequences are listed in [Table 1](#). Purified PCR products were cloned into the pEASY-T1 vector (TransGen, China) and sequenced.

We used phylogenetic analysis to compare the resulting sequences of the putative CSP4 from *B. odoriphaga* with published orthologous CSP sequences (minus the signal peptides) from three other dipterans (*Drosophila melanogaster*, *Culex quinquefasciatus*, and *Anopheles*

gambiae), two hemipterans (*Adelphocoris lineolatus*, *Aphis gossypii*), one hymenopteran (*Apis mellifera*), and one lepidopteran (*Bombyx mori*). Based on the predicted amino acid sequences, we used the Multithreaded Maximum Likelihood method in MEGA6.0 to construct a phylogenetic tree for each gene; this was done with 1,000 bootstrap replications (Tamura et al., 2013) and with Poisson correction of distances.

Expression of the *BodoCSP4* gene

Reverse transcription quantitative PCR (RT-qPCR) was used to quantify *BodoCSP4* expression in different developmental stages of *B. odoriphaga* (egg, larva, pupa, and adult) and in different tissues of *B. odoriphaga* adults including male heads without antennae, female antennae, male antennae, abdomens, and carcasses (thoraxes, wings, and legs mixed). RNA was extracted according to reagent protocols. Primers designed based on cDNA sequences were used for RT-qPCR ([Table 1](#)). RPL18 and RPS15 were used as reference genes for relative expression analysis of *BodoCSP4* at different life stages, and EF1 and ACT were used as reference genes for expression analysis in different tissues (Shi et al., 2016).

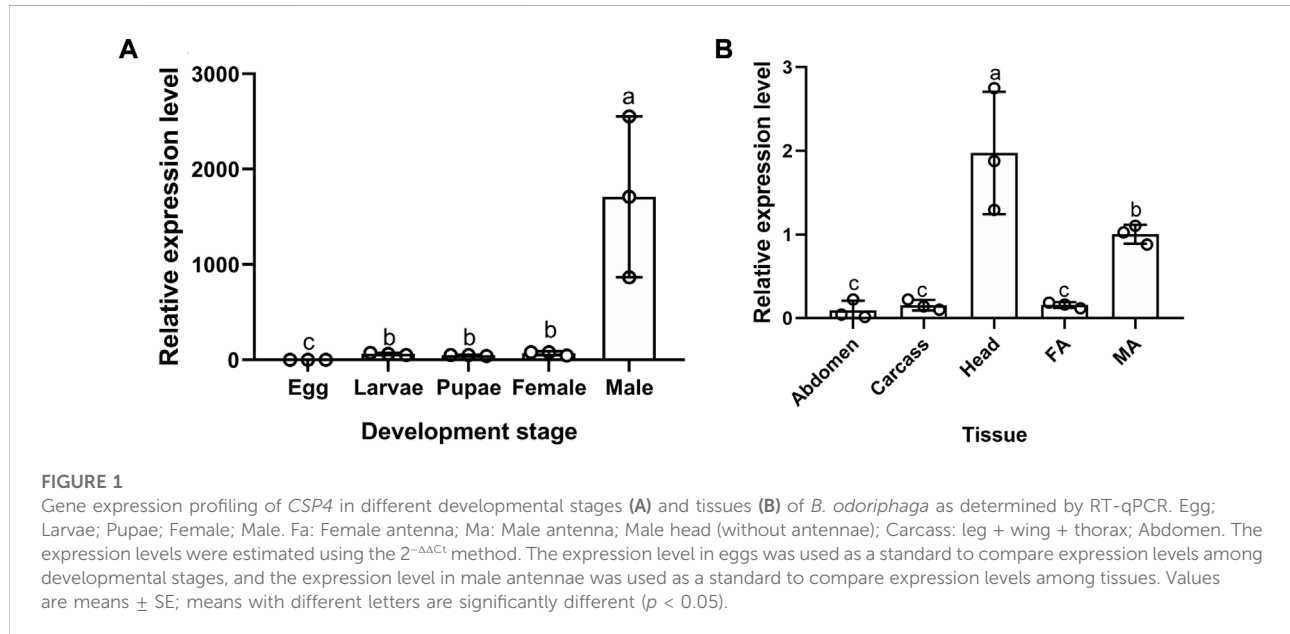
Recombinant protein expression and purification of *BodoCSP4* protein

The *BodoCSP4* cDNA was PCR amplified with specific primers ([Table 1](#)). The PCR products were ligated into the expression vector pBM30 according to the manufacturer's instructions (Biomed, Beijing, China). The ligation products containing the pBM30/*BodoCSP4* sequence were used to transform *Escherichia coli*

TABLE 2 Bioinformatics analysis of odorant-binding protein genes in *B. odoriphaga*.

Gene	Acc.No	Length of ORF	Amino acid length	Signal peptide	Full ORF	pI	Mw (kDa)
BodoCSP4	MG544173	357	118	1–18	Yes	9.42	13.58

ORF, open reading frame; pI, isoelectric point; MW, molecular weight.



BL21 (DE3) cells (TransGen Biotech) for protein expression and sequencing. Positive colonies were used for expression and purification of the recombinant BodoCSP4 protein. The results showed that, after sonication and centrifugation, the BodoCSP4 proteins were mainly expressed in insoluble bodies. Protein refolding was performed according to redox methods (Prestwich, 1993). In brief, 50 mM Tris buffer (pH 6.8) containing 0.2% Triton X-100 was used to wash the insoluble inclusion body, which was then dissolved in 6 M guanidine hydrochloride. The refolded protein was then collected and purified by Ni²⁺ ion affinity chromatography (GE-Healthcare, United States). The His-tag was removed by recombinant enterokinase (rEK) (Novagen, Beijing, China). The size and the concentration of BodoCSP4 were determined using SDS-PAGE and the BCA protein assay kit (CoWinbiotech, Beijing, China), respectively.

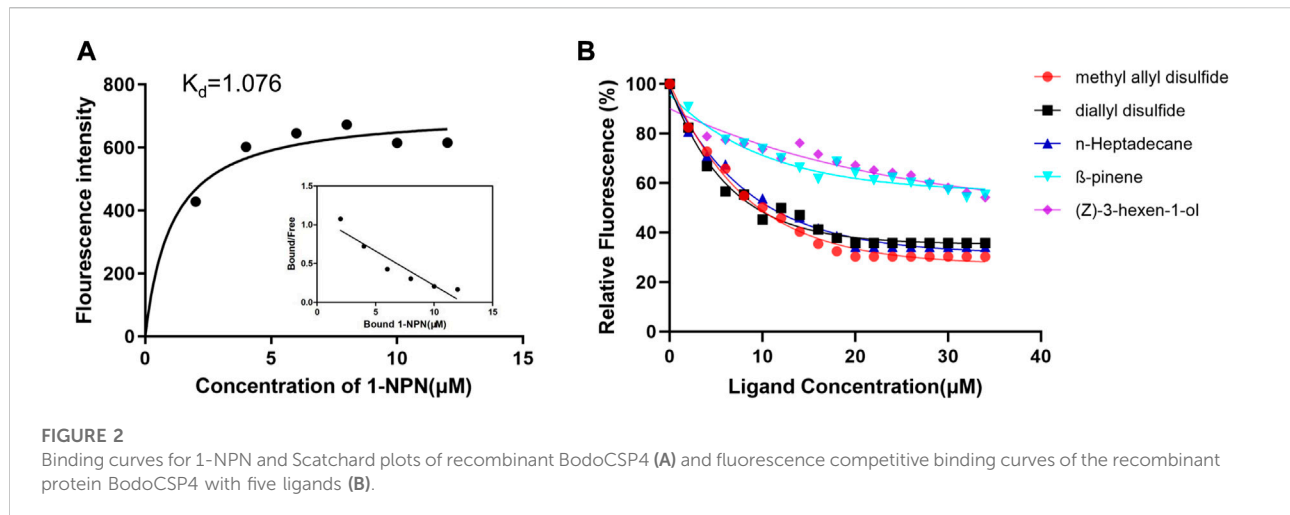
Fluorescence binding assays

An F-380 fluorescence spectrophotometer (Tianjin, China) was used to measure the binding affinity of BodoCSP4 to the selected volatiles, using 1-N-phenyl-naphthylamine (1-NPN) as the

fluorescent probe. 1-NPN and 33 ligands were selected for this assay based on previous studies (Li H. L. et al., 2007; Zhang et al., 2016; Yang et al., 2019) (Supplementary Table S1). Both 1-NPN and the volatile compounds were diluted in chromatographic-grade methanol to make 1 mM stock solutions. Recombinant BodoCSP4 was dissolved in 20 mM Tris-HCl (pH 7.4) and diluted to a 2 μ M stock solution. The dissociation constants (Kd) between 1-NPN and the recombinant BodoCSP4 were calculated by Scatchard analysis and the ligand binding affinity (Ki) was calculated with the following equation: $K_i = [IC_{50}]/(1 + [1-NPN]/K_{1-NPN})$, in which IC₅₀ is the maximum concentration at which the ligand replaces 50% of the fluorescence value of 1-NPN [1-NPN] is the free concentration of 1-NPN; and K_{1-NPN} is the dissociation constant of 1-NPN (Campanacci et al., 2003; Wei et al., 2008).

3D structure and molecular docking of BodoCSP4

The 3D structure of BodoCSP4 was obtained with a template of CSPMbraA6 (1N8V) and Swiss-Model software. The binding cavity of BodoCSP4 was predicted using SYBYL 7.3 software. Based on the results of the fluorescence binding assays, the



ligands diallyl disulfide, methyl allyl disulfide, and n-heptadecane were selected to construct the molecular conformations by Sketch mode and were optimized using the Tripos force field and Gasteiger-Hückel charge. In addition, Surflex-Dock (SYBYL 7.3) was applied to the molecular docking modeling between the BodoCSP4 protein and three ligands.

dsRNA synthesis and Y-tube olfactometer assays

The primer pairs *dsCSP4* and *dsGFP*, containing the T7 promoter sequence, are listed in Table 1, and the T7 RiboMAX™ Express RNAi System was used to synthesize double stranded RNAs (dsRNAs). The dsRNAs were microinjected into 3-day-old pupae of *B. odoriphaga*, and newly eclosed male adults were selected for dsRNA extraction and behavioral assays as previously described (Yang et al., 2021b; Zhang et al., 2021). According to the fluorescence binding results, after *BodoCSP4* was silenced, a Y-tube olfactometer was used to assess the behavioral responses of *B. odoriphaga* male adults to the ligands diallyl disulfide, methyl allyl disulfide, and n-heptadecane as described in our previous report (Yang et al., 2019). In brief, a 10- μ L solution of diallyl disulfide, methyl allyl disulfide, or n-heptadecane in hexane was placed on a strip (4 \times 40 mm) of filter paper; after 10 s, the strip was placed at the end of one of the two Y-tube arms. As a control, a hexane-treated filter paper strip was placed at the end of the other arm. Two streams of clean air were passed through the Y-tube arms to the base, and one adult male that had been previously injected (at the pupal stage, 12 h before emergence) with *dsBodoCSP4* or *dsGFP* was placed in the base of the Y-tube. If the male adult moved at least half way up one of the Y-tube arms, the result was recorded and considered an indication of attraction or repulsion depending on which arm was selected.

Each of six treatment combinations (3 host plant volatiles with controls and *dsBodoCSP4* or *dsGFP* microinjected adults) are represented by 60 replicate assays.

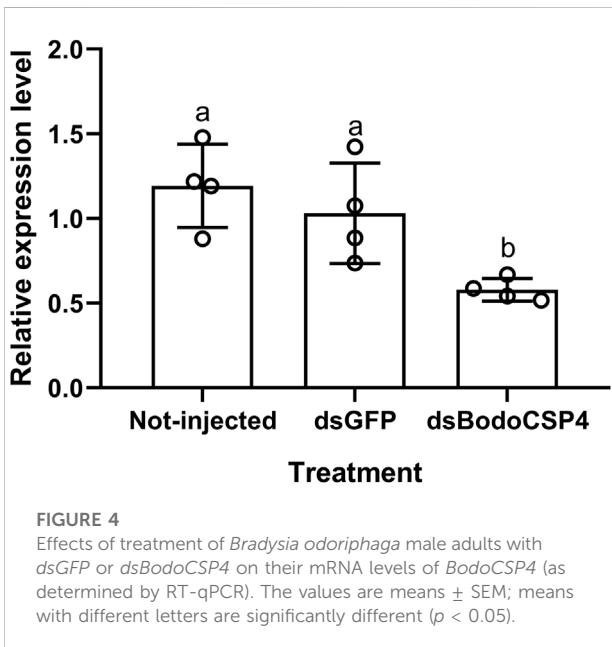
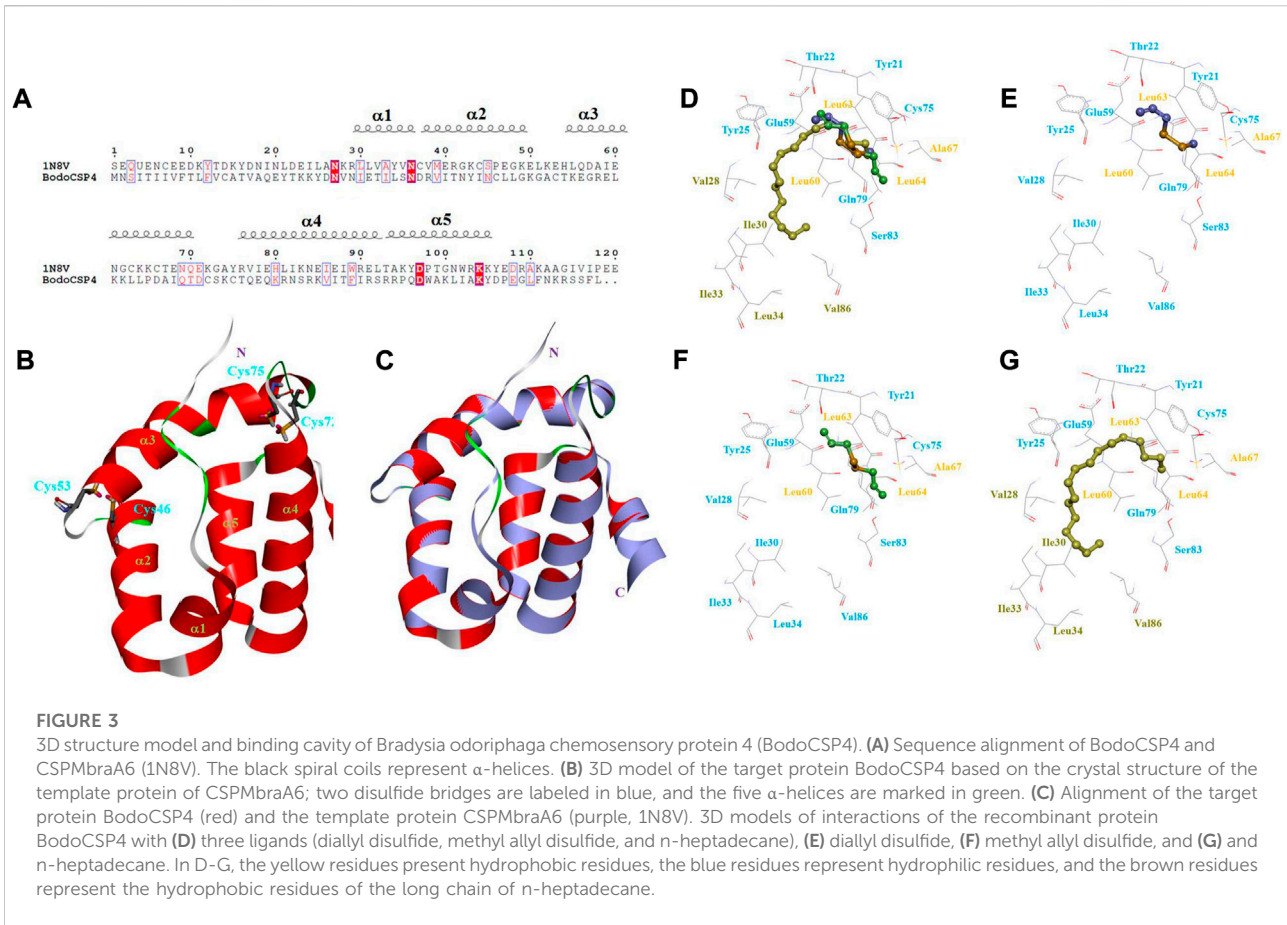
Statistical analysis

All RT-qPCR experiments were analyzed using one-way ANOVA followed by Tukey-HSD tests with SPSS 20.0 software. All RT-qPCR experiments were conducted using three independent biological replicates. In each of the six Y-tube olfactometer assays (3 ligands and controls without ligand \times two types of microinjected *B. odoriphaga* adult males), behavioral responses to filter paper with or without ligand were analyzed by a chi-square test (χ^2).

Results

Identification and phylogenetic analysis of *BodoCSP4* in *B. odoriphaga*

We identified a CSP gene named *BodoCSP4* (Accession number: MG544173) in a previously published *B. odoriphaga* transcriptome dataset, and we verified its identity by RT-PCR (Table 2; Fig. S1A). The full-length *BodoCSP4* sequence contains an ORF of 357 bp. The predicted amino acid sequence has the typical four-cysteine signature (Wanner et al., 2004) with a motif of C₁-X₆₋₈-C₂-X₁₆₋₂₁-C₃-X₂-C₄ (Supplementary Figure S1B) and an 18 amino acid-signal peptide in the N terminus (Supplementary Figure S1B). *BodoCSP4* has a molecular weight of 13.58 kDa and an isoelectric point of 9.42 (Table 2). In a phylogenetic tree of *BodoCSP4* and other insect CSPs, *BodoCSP4* was clustered with the CSPs of the dipterans *D. melanogaster* and *A. gambiae* (Supplementary Figure S2).

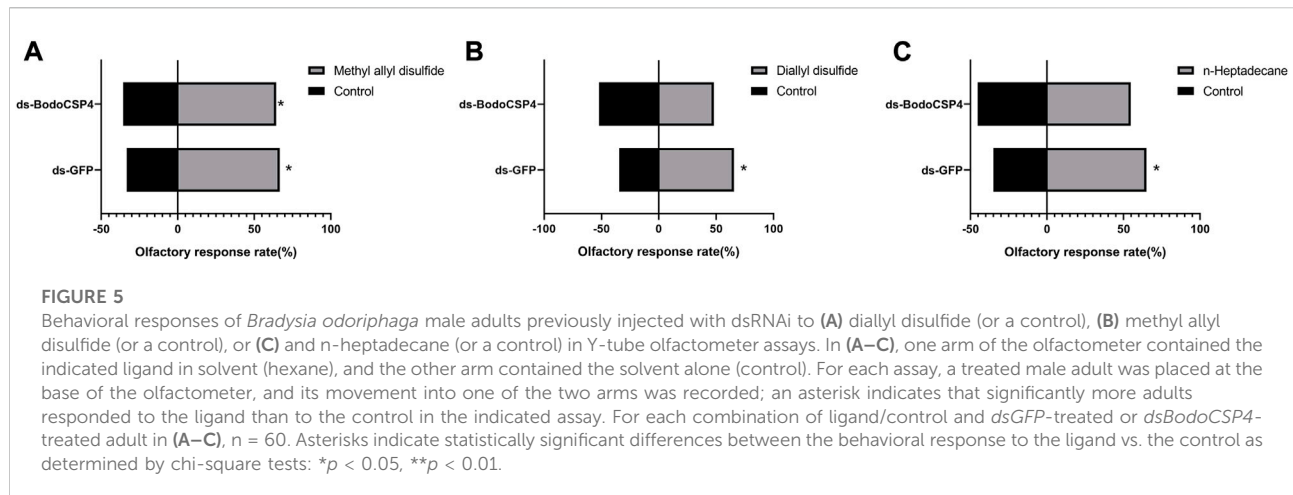


Expression profile analysis of *BodoCSP4*

Among developmental stages, the expression level of *BodoCSP4* was lowest in eggs and highest in adult males ($F = 11.711$, $df = 4,14$, $p = 0.001$) (Figure 1A). Regarding tissue-specific expression analysis in adults, expression levels of *BodoCSP4* were highest in adult male heads (antennae removed), followed by adult male antennae ($F = 14.68$, $df = 4,14$, $p = 0.000$) (Figure 1B).

Recombinant protein expression and purification of *BodoCSP4*

The recombinant *BodoCSP4* protein was successfully expressed and purified in *Escherichia coli*, yielding a high concentration (up to 0.68 mg/ml). *BodoCSP4* was mainly expressed in insoluble bodies and its molecular weight is < 15 kDa, which was consistent with the predicted results (Supplementary Figure S3).



Fluorescence binding assays with BodoCSP4

To determine the function of the recombinant protein, we measured the ligand-binding affinity of recombinant CSP4 in a competitive fluorescence binding assay with 33 ligands (Figure 2 and Supplementary Table S2). The dissociation constant (K_d) of BodoCSP4/1-NPN was 1.076 μM (Figure 2A). The binding affinity results showed that BodoCSP4 specifically bound to 3 of the 33 ligands: diallyl disulfide (5.71 μM), methyl allyl disulfide (5.71 μM), and n-heptadecane (6.85 μM) (Figure 2B). The binding affinities between BodoCSP4 and the other ligands are listed in Supplementary Table S2.

3D model structuring and molecular docking of BodoCSP4

Based on the high alignment and high sequence identity (42%) between BodoCSP4 and the template protein CSPMbraA6 (Figure 3A), BodoCSP4 has five α -helices located between residues Asn29-Asn36 (α 1), Asp37-Lys50 (α 2), Thr54-Thr70 (α 3), Cys75-Arg93 (α 4), and Arg94-Asp106 (α 5) (Figure 3B).

To better understand the potential key amino acid residues involved in the interaction between the BodoCSP4 protein and a ligand, we examined BodoCSP4 and ligand complexes by molecular docking (Figure 3). Some hydrophobic residues of BodoCSP4 surround the ligands (diallyl disulfide, methyl allyl disulfide, and n-heptadecane), including Leu60, Leu63, Leu64, and Ala67 (Figure 3C). Because diallyl disulfide, methyl allyl disulfide, and n-heptadecane have similar structures and conformations (diallyl disulfide and methyl allyl disulfide in particular have the same disulfide bond group), they have almost the same BodoCSP4 binding site (Figures 3D, E). Unlike diallyl disulfide and methyl allyl disulfide, however, n-heptadecane occupied a narrow and hydrophobic tunnel of

BodoCSP4 owing to the ligand's long hydrophobic tail; the hydrophobic tunnel included residues Val28, Ile30, Ile33, Leu34, Val86, among others (Figure 3F). The following hydrophobic residues of BodoCSP4 surround the three ligands and are likely binding sites: Leu60, Leu63, Leu64, Ala67, Val28, Ile30, Ile33, Leu34, and Val86.

Behavioral effect of BodoCSP4 knockdown by RNAi

To further determine whether *BodoCSP4* is important for recognizing the above three ligands, we silenced the *BodoCSP4* gene and measured the attraction of dsBodoCSP4- and dsGFP-treated adult males to each ligand or to a control without ligand in Y-tube olfaction assays. Treatment of adult males with dsBodoCSP4, but not with dsGFP, significantly reduced *BodoCSP4* expression ($F = 7.952, df = 2, 11, p = 0.01$) (Figure 4). The dsGFP-treated adults were attracted to methyl allyl disulfide (Figure 5A; $\chi^2 = 5.952, df = 1, p = 0.025$), diallyl disulfide (Figure 5B; $\chi^2 = 4.849, df = 1, p = 0.028$), and n-heptadecane (Figure 5C; $\chi^2 = 4.604, df = 1, p = 0.032$). The dsBodoCSP4-treated adults were attracted to methyl allyl disulfide (Figure 5A; $\chi^2 = 3.998, df = 1, p = 0.046$), however no olfactory attraction to diallyl disulfide (Figure 5B; $\chi^2 = 0.08, df = 1, p = 0.777$) and n-heptadecane (Figure 5C; $\chi^2 = 0.501, df = 1, p = 0.479$) was observed. These results suggest that *BodoCSP4* is important for the behavioral response of *B. odoriphaga* adults to diallyl disulfide and n-heptadecane but not to methyl allyl disulfide.

Discussion

Like odorant-binding proteins (OBPs), CSPs are important components of insect olfactory systems, and are required by

insects to search for and detect host plants, suitable oviposition sites, and mates (Vogt et al., 1985; Field et al., 2000; Sun et al., 2014). Previous research clarified the physiology and function of *BodoCSP1*, but the physiology and function of other CSPs in *B. odoriphaga* remained unknown prior to the current study.

That CSPs are widely distributed on insect chemosensory and non-chemosensory organs suggests that they have diverse functions (Jacquin-Joly et al., 2001). The current study with *B. odoriphaga* revealed that *BodoCSP4* is mainly expressed in the heads (without antennae) of adult males, followed by the antennae of adult males. The broad expression profile of *BodoCSP4* among *B. odoriphaga* tissues indicates that *CSP4* may have other functions besides olfaction in this insect. Previous studies showed that OBP genes were highly expressed in insect heads, suggesting that they contribute to the detection of host plant volatiles and sex pheromones (Chang et al., 2015; Liu et al., 2015; Wang et al., 2016). The following studies also showed that the indicated CSPs are highly expressed in the male antennae of the following insects: *CSP11* of *Plutella xylostella* (Fu et al., 2020), *CSP8* of *Nilaparvata lugens* (Waris et al., 2018), *CSP1* of *Cylas formicarius* (Hua et al., 2021), and *CSP3* of *Cnaphalocrocis medinalis* (Zeng et al., 2018). High expression of CSP in male antennae suggests that they may be more involved in the perception of sex pheromones than plant volatiles. Based on the current results with *B. odoriphaga*, we speculate that *BodoCSP4* is involved not only in the searching and localization of host plants by males, but also in finding females.

To explore the role of *BodoCSP4*, we determined the binding affinity of *BodoCSP4* to 33 ligands. The results indicated that *BodoCSP4* displayed high binding affinities ($K_i < 10 \mu\text{M}$) to two sulfur-containing volatiles, diallyl disulfide and methyl allyl disulfide, and to n-heptadecane. Liu et al. (2020) found that sulfur-containing secondary isothiocyanates (ITCs) affected the preference of *P. xylostella* for *Arabidopsis thaliana*; the latter study also reported that knockdown of *PxylOr35* or *PxylOr49* reduced the effect of ITCs on *P. xylostella* selection of oviposition sites, and that knockdown of both genes reduced the attraction of *P. xylostella* to ITCs. In addition, researchers recently reported that ITCs are not only chemical signals used by adult *Scaptomyza flava* to find host plants, but are also involved in the evolution of the olfactory receptor *SflaOr67bs* (Matsunaga et al., 2022). Our previous results showed that the sulfur-containing volatiles from Chinese chives, diallyl disulfide and methyl allyl disulfide, elicit strong electrophysiological responses from the antennae of both male and female *B. odoriphaga* (Yang et al., 2019). In the current study, however, the expression levels of *BodoCSP4* were highest in male heads followed by male antennae. We therefore hypothesized that diallyl disulfide and methyl allyl disulfide are chemical cues that are specifically recognized by the olfactory gene *BodoCSP4* and may help male *B. odoriphaga* adults locate Chinese chives. Interestingly, the current results indicated that *BodoCSP4* also displayed a high binding affinity

with n-heptadecane, which was obtained as an extract from the abdomen of *B. odoriphaga* females and elicits a behavioral response in male adults (Li H. J. et al., 2007). This finding is similar to that for *CSPA6* of *Mamestra brassicae* (Jacquin-Joly et al., 2001) and *SinfCSP19* of *Sesamia inferen* (Zhang et al., 2014), suggesting that *BodoCSP4* may be involved in the recognition of sex pheromones by *B. odoriphaga*.

Homology modeling and molecular docking have been used to explore specific ligand-binding features (Liu et al., 2019). In the previous study, the modeled *BodoCSP4* only had five α -helices, which is similar to *SfurCSP5* (Chen et al., 2018). *BodoCSP4*, however, differs from other insect CSPs, which contain six α -helices (Campanacci et al., 2003; Tomaselli et al., 2006; Jean-François, 2014); as a result, it will be necessary to confirm or modify the actual 3D structure of *BodoCSP4* in future studies. Kulmuni and Havukainen (2013) found that insect CSPs with five α -helices are mostly involved in searching for mates. We therefore suggest that *BodoCSP4* may be involved in finding females and in other olfactory functions.

Binding models in the current study showed that *BodoCSP4* bound to the ligands of methyl allyl disulfide, diallyl disulfide, and n-heptadecane near many *BodoCSP4* hydrophobic residues (including Leu60, Leu63, Leu64, and Ala67, Val28, Ile30, Ile33, Leu34, and Val86) but not the most polar hydrophobic residues (Figure 3). Mármol et al. (2021) reported that interactions with hydrophobic residues contribute to odor molecule binding and thereby mediate ligand recognition. In addition, previous studies have reported that leucine is the key amino acid for ligand binding, as was observed for *Mamestra brassicae* *CSP6* (Campanacci et al., 2003), *SfurCSP5* (Chen et al., 2018), and *BminCSP3* (Cui et al., 2022). The hydrophobic residues of *BodoCSP4* interacted with all three ligands, indicating their key involvement in ligand binding of *BodoCSP4*. This feature is consistent with other OBPs and CSPs (Tomaselli et al., 2006; Zheng et al., 2015; Northey et al., 2016). However, molecular docking can only assist in predicting binding mechanisms, and as such we will use site-directed mutagenesis to further investigate the specific functions of these hydrophobic amino acids. Based on both the homology modeling, molecular docking, and competitive binding results, we conclude that *CSP4* can bind to both sulfur volatiles and sex pheromones, and that *CSP4* therefore participates in multiple physiological functions in *B. odoriphaga*.

In the present study we used RNA interference and behavioral assays to further confirm the physiological functions of *BodoCSP4* in *B. odoriphaga*. The results indicated that silencing of the *BodoCSP4* gene significantly decreased the behavioral response of *B. odoriphaga* male adults to diallyl disulfide and n-heptadecane but did not decrease the behavioral response to methyl allyl disulfide. In contrast, Zhang et al. (2021) reported that silencing of *BodoCSP1* reduced the behavioral response of *B. odoriphaga* male adults

to diallyl disulfide and methyl allyl disulfide. In addition, previous reports indicated that *BodoOBP1*, *BodoOBP2* (Tang et al., 2019), *BodoOBP5* (Yang et al., 2021a), and *BodoOBP8* (Yang et al., 2021b) bind well to diallyl disulfide and methyl allyl disulfide. We suggest that *BodoCSP4*, however, may work together with other OBPs or CSPs to recognize methyl allyl disulfide methyl allyl disulfide. For example, Sun et al. (2016) found that *CmedOBP2* and *CmedOBP3* co-regulate odorant recognition by *C. medinalis* adults, and Maguire et al. (2022) found that *AgamOR2* and *ORCO* can together alter the olfactory preferences of *Anopheles mosquitoes*. Based on the current results, we speculate that diallyl disulfide and n-heptadecane but not methyl allyl disulfide can be specifically recognized by *BodoCSP4*, and that diallyl disulfide and n-heptadecane are the main chemical signals perceived by *BodoCSP4* in host plant recognition and localization by males, as well as female localization.

Overall, the results of this study suggest that *BodoCSP4* may be involved in the recognition and localization of host plants and mates. Determining whether diallyl disulfide and n-heptadecane function as attractants to regulate *B. odoriphaga* behavior will require additional research.

Data availability statement

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

Author contributions

ZY and YY designed the experiments. YY performed the experiment. ZJ, WF and HD contributed reagents/materials. YY wrote the manuscript. All authors gave final approval for publication.

References

- Antony, B., Soffan, A., Jakše, J., Abdelazim, M. M., Aldosari, S. A., Aldawood, A. S., et al. (2016). Identification of the genes involved in odorant reception and detection in the palm weevil *Rhynchophorus ferrugineus*, an important quarantine pest, by antennal transcriptome analysis. *BMC Genomics* 17, 69. doi:10.1186/s12864-016-2362-6
- Campanacci, V., Lartigue, A., Hallberg, B. M., Jones, T. A., Giudici-Ortoni, M. T., Tegoni, M., et al. (2003). Moth chemosensory protein exhibits drastic conformational changes and cooperativity on ligand binding. *Proc. Natl. Acad. Sci. U. S. A.* 100, 5069–5074. doi:10.1073/pnas.0836654100
- Chang, H. T., Liu, Y., Yang, T., Pelosi, P., Dong, S. L., and Wang, G. R. (2015). Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. *Sci. Rep.* 5, 13093. doi:10.1038/srep13093
- Chen, S., Yang, P., Jiang, F., Wei, Y., Ma, Z., and Kang, L. (2010). De novo analysis of transcriptome dynamics in the migratory locust during the development of phase traits. *PLoS One* 5, e15633. doi:10.1371/journal.pone.0015633
- Chen, G. L., Pan, Y. F., Ma, Y. F., Wang, J., He, M., and He, P. (2018). Binding affinity characterization of an antennae-enriched chemosensory protein from the white-backed planthopper, *Sogatella furcifera* (Horváth), with host plant

Funding

This work was funded by the National Natural Science Foundation of China (Grant Nos. 32102202) to YY.; China Agriculture Research System (CARS-24-C-02) to ZY.

Acknowledgments

We would like to thanks to the Professor Hongxia Duan from China Agricultural University for her Homology modeling and molecular docking technical assistance in the laboratory.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2022.989601/full#supplementary-material>

volatiles.finity characterization of an antennae-enriched chemosensory protein from the white-backed planthopper, *Sogatella furcifera* (Horváth), with host plant volatiles. *Pestic. Biochem. Physiol.* 152, 1–7. doi:10.1016/j.pestbp.2018.09.006

Cheng, D., Lu, Y., Zeng, L., Liang, G., and He, X. (2015). Si-CSP9 regulates the integument and moulting process of larvae in the red imported fire ant. *Solenopsis invicta*. *Sci. Rep.* 5, 1–14. doi:10.1038/srep09245

Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N., and Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32, 657–671. doi:10.1016/s0896-6273(01)00515-3

Cui, Z. Y., Liu, Y. P., Wang, G. R., and Zhou, Q. (2022). Identification and functional analysis of a chemosensory protein from *Bactrocera minax* (Diptera: Tephritidae). *Pest Manag. Sci.* 78, 3479–3488. doi:10.1002/ps.6988

Dang, Z. H., Dong, J. Z., Gao, Z. L., Jia, H. M., Zhang, K. J., and Pan, W. L. (2001). Biology and injury of *Bradysia odoriphaga* on leek in different types of cultivation. *J. Agric. Univ. Hebei.* 24, 65–68.

Feng, H. Q., and Zheng, F. Q. (1987). Studies of the occurrence and control of *Bradysia odoriphaga* Yang et Zhang. *J. Agri UnivK Shandong* 18, 71–80.

- Field, L. M., Pickett, J. A., and Wadhams, L. J. (2000). Molecular studies in insect olfaction. *Insect Mol. Biol.* 9, 545–551. doi:10.1046/j.1365-2583.2000.00221.x
- French, A., Ali Agha, M., Mitra, A., Yanagawa, A., Sellier, M. J., and Marion-Poll, F. (2015). Drosophila bitter taste(s). *Front. Integr. Neurosci.* 9, 207. doi:10.3389/fnint.2015.00058
- Fu, S. H., Li, F. Y., Yan, X. Z., and Hao, C. (2020). Expression profiles and binding properties of the chemosensory protein PxlCSP11 from the Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Insect Sci.* 20, 17–11. doi:10.1093/jisesa/ieaa107
- Guo, W., Wang, X., Ma, Z., Xue, L., Han, J., Yu, D., et al. (2011). CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. *PLoS Genet.* 7, e1001291. doi:10.1371/journal.pgen.1001291
- Hansson, B. S. (2002). A bug's smell-research into insect olfaction. *Trends Neurosci.* 25, 270–274. doi:10.1016/s0166-2236(02)02140-9
- Hua, J. F., Pan, C., H. Y. M., Li, Y. Q., Li, H. F., Wu, C. R., et al. (2021). Functional characteristic analysis of three odorant-binding proteins from the sweet potato weevil (*Cylas formicarius*) in the perception of sex pheromones and host plant volatiles. *Pest Manag. Sci.* 77, 300–312. doi:10.1002/ps.6019
- Jacquin-Joly, E., Vogt, R. G., Francois, M. C., and Nagnan-Le, M. P. (2001). Functional and expression pattern analysis of chemosensory proteins expressed in antennae and pheromonal gland of *Mamestra brassicae*. *Chem. Senses* 26, 833–844. doi:10.1093/chemse/26.7.833
- Jannick, D. B., Henrik, N., and Gunnar von, H. J. (2004). Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* 16, 783–795. doi:10.1016/j.jmb.2004.05.028
- Jean-François, P. (2014). Renaming *Bombyx Mori* chemosensory proteins. *Int. J. Bioorg. Chem. Mol. Biol.* 2, 1–4. doi:10.19070/2332-2756-140005e
- Kitabayashi, A. N., Arai, T., Kubo, T., and Natori, S. (1998). Molecular cloning of cDNA for p10, a novel protein that increases in the regenerating legs of *Periplaneta americana* (American cockroach). *Insect biochem. Mol. Biol.* 28, 785–790. doi:10.1016/s0965-1748(98)00058-7
- Kulmuni, J., and Havukainen, H. (2013). Insights into the evolution of the CSP gene family through the integration of evolutionary analysis and comparative protein modeling. *PLoS One* 8, e63688. doi:10.1371/journal.pone.0063688
- Letunic, L., Doerks, T., and Bork, P. (2015). Smart: Recent updates, new developments and status in 2015. *Nucleic Acids Res.* 43, 257–260. doi:10.1093/nar/gku949
- Li, H. J., He, X. K., Zeng, A. J., Liu, Y. J., and Jiang, S. R. (2007a). *Bradysia odoriphaga* copulatory behavior and evidence of a female sex pheromone. *J. Agri. Urban Entomol.* 24, 27–34. doi:10.3954/1523-5475-24.1.27
- Li, H. L., Lou, B., Cheng, J. A., and Gao, Q. (2007b). The chemosensory protein of Chinese honeybee, *Apis cerana cerana*: Molecular cloning of cDNA, immunocytochemical localization and expression. *Chin. Sci. Bull.* 52, 1355–1364. doi:10.1007/s11434-007-0210-4
- Li, W. X., Yang, Y. T., Xie, W., Wu, Q. J., Xu, B. Y., Wang, S. L., et al. (2014). Effects of temperature on the age-stage, two-sex life table of *Bradysia odoriphaga* (Diptera: Sciaridae). *J. Econ. Entomol.* 108, 126–134. doi:10.1093/jeetou011
- Liu, N. Y., Yang, K., Liu, Y., Xu, W., Anderson, A., and Dong, S. L. (2015). Two general-odorant binding proteins in *Spodoptera litura* are differentially tuned to sex pheromones and plant odorants. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 180, 23–31. doi:10.1016/j.cbpa.2014.11.005
- Liu, H. W., Duan, H. X., Wang, Q., Xiao, Y., Wang, Q., Xiao, Q., et al. (2019). Key amino residues determining binding activities of the odorant binding protein AlucOBP22 to two host plant terpenoids of *Apolygus lucorum*. *J. Agric. Food Chem.* 67, 5949–5956. doi:10.1021/acs.jafc.8b05975
- Liu, X. L., Zhang, J., Yan, Q., Miao, C. L., Han, W. K., Hou, W., et al. (2020). The molecular basis of host selection in a crucifer-specialized moth. *Curr. Biol.* 30, 4476–4482. doi:10.1016/j.cub.2020.08.047
- Ma, J., Chen, S. L., and Maurice, M. (2013). Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the chive gnat, *Bradysia odoriphaga*. *J. Pest Sci.* 86, 556–561. doi:10.1007/s10340-013-0497-7
- Maguire, S. E., Afify, A., Goff, L. A., and Potter, C. J. (2022). Odorant-receptor-mediated regulation of chemosensory gene expression in the malaria mosquito *Anopheles gambiae*. *Cell Rep.* 38, 110494. doi:10.1016/j.celrep.2022.110494
- Maleszka, J., Foret, S., Saint, R., and Maleszka, R. (2007). RNAi-induced phenotypes suggest a novel role for a chemosensory protein CSP5 in the development of embryonic integument in the honeybee (*Apis mellifera*). *Dev. Genes Evol.* 217, 189–196. doi:10.1007/s00427-006-0127-y
- Mármol, J. del, Yedlin, M. A., and Ruta, V. (2021). The structural basis of odorant recognition in insect olfactory receptors. *Nature* 597, 126–131. doi:10.1038/s41586-021-03794-8
- Matsunaga, T., Reisenman, C. E., Goldman-Huertas, B., Brand, P., Miao, K., Suzuki, H. C., et al. (2022). Evolution of olfactory receptors tuned to mustard oils in herbivorous drosophilidae. *Mol. Biol. Evol.* 39, msab362. doi:10.1093/molbev/msab362
- Northey, T., Venthur, H., De Biasio, F., Chauviac, F. X., Cole, A., Ribeiro, K. A. L., et al. (2016). Crystal structures and binding dynamics of odorant-binding protein 3 from two aphid species *Megoura viciae* and *Nasonovia ribisnigri*. *Sci. Rep.* 6, 24739. doi:10.1038/srep24739
- Oliveira, D. S., Brito, N. F., Franco, T. A., Moreira, M. F., Leal, W. S., and Melo, A. C. A. (2018). Functional characterization of odorant binding protein 27 (RproOBP27) from *Rhodnius prolixus* antennae. *Front. Physiol.* 9, 1175. doi:10.3389/fphys.2018.01175
- Pelosi, P., Calvello, M., and Ban, L. (2005). Diversity of odorant-binding proteins and chemosensory proteins in insects. *Chem. Senses* 30, 291–292. doi:10.1093/chemse/bjh229
- Pelosi, P., Zhou, J. J., Ban, L. P., and Calvello, M. (2006). Soluble proteins in insect chemical communication. *Cell. Mol. Life Sci.* 63, 1658–1676. doi:10.1007/s00018-005-5607-0
- Pelosi, P., Iovinella, I., Zhu, J., Wang, G., and Dani, F. R. (2018). Beyond chemoreception: Diverse tasks of soluble olfactory proteins in insects. *Biol. Rev. Camb. Philos. Soc.* 93, 184–200. doi:10.1111/brv.12339
- Picimbon, J. (2003). *Biochemistry and evolution of OBP and CSP proteins*. Elsevier, 539–566.
- Prestwich, G. D. (1993). Bacterial expression and photo affinity labeling of a pheromone binding protein. *Protein Sci.* 2, 420–428. doi:10.1002/pro.5560020314
- Shi, C. H., Yang, F. S., Zhu, X., Du, E. X., Yang, Y. T., Wang, S. L., et al. (2016). Evaluation of housekeeping genes for quantitative real-time PCR analysis of *Bradysia odoriphaga* (Diptera: Sciaridae). *Int. J. Mol. Sci.* 17, 1034. doi:10.3390/ijms17071034
- Sun, H., Guan, L., Feng, H., Yin, J., Cao, Y., Xi, J., et al. (2014). Functional characterization of chemosensory proteins in the scarab beetle, *Holotrichia oblitra* Faldermann (Coleoptera: Scarabaeida). *PLoS One* 9, e107059. doi:10.1371/journal.pone.0107059
- Sun, X., Zeng, F. F., Yan, M. J., Zhang, A., Lu, Z. X., and Wang, M. Q. (2016). Interactions of two odorant-binding proteins influence insect chemoreception. *Insect Mol. Biol.* 25, 712–723. doi:10.1111/imb.12256
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi:10.1093/molbev/mst197
- Tang, B. W., Tai, S. L., Dai, W., and Zhang, C. N. (2019). Expression and functional analysis of two odorant-binding proteins from *Bradysia odoriphaga* (Diptera: Sciaridae). *J. Agric. Food Chem.* 67, 3565–3574. doi:10.1021/acs.jafc.9b00568
- Tomaselli, S., Crescenzi, O., Sanfelice, D., Ab, E., Wechselberger, R., Angeli, S., et al. (2006). Solution structure of a chemosensory protein from the desert locust *Schistocerca gregaria*. *Biochemistry* 45, 10606–10613. doi:10.1021/bi060998w
- Visser, J. H. (1986). Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* 31, 121–144. doi:10.1146/annurev.en.31.010186.001005
- Vogt, R. G., Riddiford, L. M., and Prestwich, G. D. (1985). Kinetic properties of a sex pheromone-degrading enzyme: The sensillar esterase of *Antheraea polyphemus*. *Proc. Natl. Acad. Sci. U. S. A.* 82, 8827–8831. doi:10.1073/pnas.82.24.8827
- Wang, R., Zhang, X. M., Li, F. Q., Wu, F., Li, H. L., and Luo, C. (2016). Cloning and prokaryotic expression of odorant binding protein OBP8 in MED cryptic species *Bemisia tabaci* and the binding characteristics with plant volatiles. *J. Plant Prot.* 43, 32–39.
- Wanner, K. W., Willis, L. G., Theilmann, D. A., Isman, M. B., Feng, Q., and Plettner, E. (2004). Analysis of the insect os-d-like gene family. *J. Chem. Ecol.* 30, 889–911. doi:10.1023/b:joec.0000028457.51147.d4
- Waris, M. I., Younas, A., ul Qamar, M. T., Hao, L., Ameen, A., Ali, S., et al. (2018). Silencing of chemosensory protein gene NlugCSP8 by RNAi induces declining behavioral responses of *Nilaparvata lugens*. *Front. Physiol.* 9, 379. doi:10.3389/fphys.2018.00379
- Wei, Y., Brandazza, A., and Pelosi, P. (2008). Binding of polycyclic aromatic hydrocarbons to mutants of odorant-binding protein: A first step towards biosensors for environmental monitoring. *Biochim. Biophys. Acta* 1784, 666–671. doi:10.1016/j.bbapap.2008.01.012
- Xu, H. F., Pan, Y., Li, J. Y., Yang, F. T., Chen, X. W., Gao, X. W., et al. (2022). Chemosensory proteins confer adaptation to the ryanoid anthranilic diamide insecticide cyantraniliprole in *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* 184, 105076. doi:10.1016/j.pestbp.2022.105076
- Yang, Y. T., Li, W. X., Xie, W., Wu, Q. J., Xu, B. Y., Wang, S. L., et al. (2014). Development of *Bradysia odoriphaga* (Diptera: Sciaridae) as affected by humidity:

An age-stage, two-sex life table study. *Appl. Entomol. Zool.* 50, 3–10. doi:10.1007/s13355-014-0295-6

Yang, Y. T., Su, Q., Shi, L. L., Chen, G., Zeng, Y., Shi, C. H., et al. (2019). Electrophysiological and behavioral responses of *Bradysia odoriphaga* (Diptera: Sciaridae) to volatiles from its host plant, Chinese Chives (*Allium tuberosum* Rottler ex Spreng). *J. Econ. Entomol.* 112, 1638–1644. doi:10.1093/jee/toz057

Yang, Y. T., Hua, D. K., Shi, C. H., Xie, W., and Zhang, Y. J. (2021a). Molecular and binding characteristic of OBP5 of *Bradysia odoriphaga* (Diptera: Sciaridae). *J. Econ. Entomol.* 114, 1509–1516. doi:10.1093/jee/toab095

Yang, Y. T., Luo, L., Tian, L. X., Zhao, C. W., Niu, H. L., Hu, Y. F., et al. (2021b). Function and characterization analysis of BodoOBP8 from *Bradysia odoriphaga* (Diptera: Sciaridae) in the recognition of plant volatiles and sex pheromones. *Insects* 12, 879. doi:10.3390/insects12100879

Younas, A., Waris, M. I., Shaaban, M., Qamar, M. T., and Wang, M. Q. (2022). Appraisal of MsepCSP14 for chemosensory functions in *Mythimna separate*. *Insect Sci.* 29, 162–176. doi:10.1111/1744-7917.12909

Zeng, F. F., Liu, H., Zhang, A., Lu, Z. X., Leal, W. S., Abdelnabby, H., et al. (2018). Three chemosensory proteins from the rice leaf folder *Cnaphalocrocis medinalis* involved in host volatile and sex pheromone reception. *Insect Mol. Biol.* 27, 710–723. doi:10.1111/imb.12503

Zeng, Y., Merchant, A., W, Q. J., Wang, S. L., Kong, L., Zhou, X., et al. (2020). A chemosensory protein BtabCSP11 mediates reproduction in *Bemisia tabaci*. *Front. Physiol.* 11, 709. doi:10.3389/fphys.2020.00709

Zhang, Y. N., Ye, Z. F., Yang, K., and Dong, S. L. (2014). Antenna-predominant and male-biased CSP19 of *Sesamia inferens* is able to bind the female sex pheromones and host plant volatiles. *Gene* 536, 279–286. doi:10.1016/j.gene.2013.12.011

Zhang, Z. J., Li, W. X., He, M., Zhu, X. D., Lin, W. C., Li, X. W., et al. (2016). Behavioral responses of female *Bradysia odoriphaga* Yang et Zhang to volatiles of conspecific larvae, pupae and eggs. *Chin. J. Appl. Entom.* 53, 1198–1204.

Zhang, C. N., Tang, B. W., Zhou, T. L., Yu, X. T., Hu, M. F., and Dai, W. (2021). Involvement of chemosensory protein BodoCSP1 in perception of host plant volatiles in *Bradysia odoriphaga*. *J. Agric. Food Chem.* 69, 10797–10806. doi:10.1021/acs.jafc.1c02807

Zhao, Y., Ding, J., Zhang, Z., Liu, F., Zhou, C., and Mu, W. (2018). Sex- and tissue-specific expression profiles of odorant binding protein and chemosensory protein genes in *Bradysia odoriphaga* (Diptera: Sciaridae). fic expression profiles of odorant binding protein and chemosensory protein genes in *Bradysia odoriphaga* (Diptera: Sciaridae). *Front. Physiol.* 9, 107. doi:10.3389/fphys.2018.00107

Zheng, J., Li, J., Han, L., Wang, Y., Wu, W., Qi, X., et al. (2015). Crystal structure of the *Locusta migratoria* odorant binding protein. *Biochem. Biophys. Res. Commun.* 456, 737–742. doi:10.1016/j.bbrc.2014.12.048

Zhu, J., Iovinella, I., Romana Dani, F., Liu, Y. L., Huang, L. Q., Liu, Y., et al. (2016). Conserved chemosensory proteins in the proboscis and eyes of Lepidoptera. *Int. J. Biol. Sci.* 12, 1394–1404. doi:10.7150/ijbs.16517