Expression Profile and Functional Characterization Suggesting the Involvement of Three Chemosensory Proteins in Perception of Host Plant Volatiles in Chilo suppressalis (Lepidoptera: Pyralidae)

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Abstract

The high sensitivity of the olfactory system is essential for feeding and oviposition in moth insects, and some chemosensory proteins (CSPs) are thought to play roles in this system by binding and carrying hydrophobic odorants across the aqueous sensillar lymph. In this study, to identify the olfactory CSPs from a repertoire of 21 CSP members in the notorious rice pest Chilo suppressalis (Walker) (Lepidoptera: Pyralidae), tissue expression patterns were firstly examined by quantitative real-time polymerase chain reaction (qPCR). It showed that CSP2 was antennae specific and seven more CSPs (CSP1, 3, 4, 6, 15, 16, and 17) were antennae biased in expression, suggesting their olfactory roles; while other CSPs were multiple-tissue expressed and non-antennae biased, suggesting other functions for these genes. To further determine the ligand binding specificity, three putative olfactory genes (CSP1-3) were expressed in Escherichia coli cells, and binding affinity of these three recombinant CSP proteins were measured for 35 plant volatiles by the ligand binding assays. CSP1 and CSP2 exhibited high binding affinities (Ki ≤ 10.00 µM) for four (2-tridecanone, benzaldehyde, laurinaldehyde and 2-pentadecanone) and two (2-heptanol and (+)-cedrol) host plant volatiles, respectively; the three CSPs also showed moderate binding affinity (Ki = 10.01–20.00 µM) for 16 plant volatiles. Our study suggests that the three CSPs play essential roles in the perception of host plant volatiles, providing bases for the elucidation of olfactory mechanisms in this important pyralid pest.

Key words: chemosensory protein, tissue expression pattern, ligand binding assay, plant volatile

A sensitive olfactory system is essential for insects to locate the food sources, mating partners and oviposition sites. Insect olfaction is mediated by particularly sensory neurons located in the sensilla of antennae and other chemosensory organs (Krieger et al. 2004, Tanaka et al. 2009, Leal 2013). These sensilla are cuticular structures with aqueous lymph surrounding the dendrites of the olfactory neurons, and abound with different types of small soluble olfactory proteins, odorant binding proteins (OBPs) (Mckenna et al. 1994, Pikielny et al. 1994) and chemosensory proteins (CSPs) (Jaccquin-Joly et al. 2001, Calvello et al. 2003, Pelosi et al. 2005). Both OBPs and CSPs are regarded as carriers of pheromones and odorants in insect chemoreception (Pelosi et al. 2018). Similar to OBPs, CSPs consist of around 100–200 residues, but have only four highly conserved cysteines that are attached by two pairs of non-interlocked disulfide bridges (Wanner et al. 2004); both OBPs and CSPs consist of α-helical sections, but the folding conformations were different between OBPs and CSPs (Lartigue et al. 2002, Simona et al. 2006, Jansen et al. 2007). Since the first CSP, olfactory-specific protein p10 was identified two decades ago in the cockroach legs (Nomura et al. 1992). CSP genes have been molecularly identified in many insect species, such as Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) (Maleszka and Stange 1997), Bombyx mori (L.) (Lepidoptera: Bombycidae) (Picimbon et al. 2000), Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) (Liu et al. 2015), Blattella germanica (L.) (Blattodea: Blatellidae) (Niu et al. 2016), and Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) (Wang et al. 2017), and Periplaneta americana (L.) (Blattodea: Blatellidae) (He et al. 2017). However, the olfactory role and the odorant specificity are still unknown for most CSPs. CSPs are extensively expressed from different tissues of insects and thus suggested to play essential roles in various
physiological functions (Pelosi et al. 2006, Lovinella et al. 2013). Some CSPs are highly detected in the antennae and show binding affinity to plant volatiles and sex pheromones, suggesting their olfactory roles (Gu et al. 2012, Lovinella et al. 2013, Zhang et al. 2014); some CSPs are highly detected in non-olfactory tissues, such as the thoraxes (Gu et al. 2012), legs (Wanner et al. 2004, Jacobs et al. 2005, Liu et al. 2010), wings (Ban et al. 2003, Zhou et al. 2008), and abdomens (Gong et al. 2007, Maleszka et al. 2007, Yang et al. 2014), suggesting that they may take a part in other physiological processes. *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), feeds on a variety of crops and has become a serious rice pest in China (Sheng et al. 2003, Chen et al. 2011). The management of the rice striped stem borer has long relied on the spraying of insecticides, which are not only harmful to the environment and people's health but also lead to the evolution of insect resistance to insecticides. Targeting on the sensitive olfactory system, sex pheromones and plant volatiles have been used in the management of this pest (Casagrande 1993, Cork and Basu 1996, Alfaro et al. 2009, Mandras 2013). By transcriptome sequencing and bioinformatics’ analysis, 21 putative CSP genes have been identified (Cao et al. 2014), but the functional study of these genes has not been explored in *C. suppressalis*.

In this study, to characterize the CSP genes that play olfactory roles, we first examined the tissue and sex expression pattern of 21 reported CSP genes by quantitative real-time polymerase chain reaction (qPCR), and then, in vitro functionally characterized the odorant preference of some antennae specific or highly biased CSPs by ligand binding assays. The results provided important bases for the better understanding of the olfactory mechanisms and for the design of new olfaction-based control strategies for *C. suppressalis*.

### Materials and Methods

**Insect Rearing, Tissue Collections, Total RNA Isolation, and cDNA Synthesis**

The laboratory population of *C. suppressalis* was reared and tissue samples (antenna, head without antenna, leg, and abdomen) for expression analysis were collected as described by Khuhydro et al. (2017b). The collected tissue samples (200 antennae, 40 heads, 80 legs, and 20 abdomens of each sex) were separately placed in liquid nitrogen and stored at −80°C until use. Total RNA isolation and cDNA synthesis were performed as our reported protocols (Khuhydro et al. 2017a,b).

**qPCR and Data Analysis**

Expression pattern of 21 CSPs regarding tissues and sexes were examined by qPCR ABI Prism 7500 (Applied Bio Systems, Foster City, CA) and specific primers (Supp. Table S1) were designed using Beacon Designer 7.6 (PREMIER Biosoft International). The specificity and amplification of each primer pair was validated by using fivelfold cDNA dilution series. The requirement of primer pairs were all within the range as described Khuhro et al. (2017b). The reaction well had a total volume 20-μl mixture containing 10 μl SYBR Green PCR Master mix, 0.4 μl of each primer (10 μM), 0.4 μl ROX Reference Dye II, 1 μl cDNA template (2.0 ng for all tissues), and 7.8 μl sterilized ultrapure water. The PCR conditions were: initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 34 s. For each biological pool; three technical replications were performed. The relative expression levels were calculated by using two different housekeeping genes, i.e., β-actin and GAPDH (Xia et al. 2015), and data was analyzed using the 2−ΔΔCT method (Livak and Schmittgen 2001).

### cDNA Cloning, Sequencing, and Vector Constructions

Based on the results of expression profiles, CSP1, CSP2, and CSP3 that were antennae specifically or predominantly expressed were selected for in vitro functional study. To construct the expression vectors, cDNA encoding mature CSP proteins were amplified by using the gene-specific primers containing the restriction sites (Supp. Table S1). Purified PCR products of three CsupCSPs were subcloned into a pEASY T3 vector (TransGen, Beijing, China), and positive clones were sequenced by the sequencer (GenScript Biology Company, Nanjing, China). To construct CSP expression vectors, the plasmid containing the inserts were digested by restriction enzymes with specific restriction sites BamHI and XhoI (Fermentas, Thermo Fisher Scientific, Waltham, MA). The digested products were ligated into the expression vectors pET-30a (+) (Novagen, Darmstadt, Germany) that previously digested with the same enzymes (Khuhydro et al. 2017b).

### Expression and Purification of Recombinant Proteins

The recombinant proteins were expressed by bacterial expression system and protein purification was accomplished by previously described protocols (Khuhydro et al. 2017a,b). To avoid the possible effects of His-tags on the structures of CSPs, tags were removed by the treatment with a cleaving enzyme (enterokinase), and stored at −80°C for further use.

### Ligand Binding Assay

The binding assays were conducted using previously reported protocols (Liu et al. 2012; Liu et al. 2013; Khuhydro et al. 2017a,b) on a Spectra Max M5 Fluorescence Spectrophotometer (Molecular Devices, California, USA) with Greiner Microlon 96-well plates (Greiner Bio-One, Frickenhausen, Germany). The solutions were excited at 337 nm and emission spectra were recorded between 400 and 460 nm. Firstly, affinities of the fluorescent probe N-phenyl-1-naphthylamine (1-NPN) to CSP proteins were measured. The 2 μM CSP solution in 50 mM Tris–HCl (pH 7.4) was titrated with aliquots of 1 mM 1-NPN (dissolved in methanol) to final concentrations of 2–20 μM, and the resulting fluorescence intensities were recorded. Secondly, the binding affinity of each odorant for the CSP protein was measured by binding assays with 1-NPN as the fluorescent reporter. The 250 μl solution (Tris–HCl 50 mM) containing CSP protein (2 μM) and 1-NPN (2 μM) was titrated with aliquots of 1 mM odorant to final odorant concentrations of 2–30 μM. Three replicates were performed for each odorant. Binding data were analyzed using reported methods (Khuhydro et al. 2017a,b).

### Results

**Tissue Expression Profile of CSP Genes**

To predict their potential functions, the expression levels of CSP genes in sensory tissues (antennae and head) and non-sensory tissues (leg and abdomen) were examined by using qPCR (Fig. 1). Results showed that tested CSP genes displayed several tissue expression patterns. Of 21 CSP genes, only CSP2 was antennae specific and seven CSPs (CSP1, 3, 4, 6, 15, 16, and 17) were antennae highly biased in expression. In addition, three CSPs
(CSP8, 12, and 19) were male head biased, showing much higher expression levels in the male head than the female head and other tissues. In contrast, three CSPs (CSP9, 18, and 20) were higher in non-olfactory organ than in olfactory organs, with CSP9 being leg-biased, CSP18 female abdomen biased, and CSP20 male abdomen biased. Other seven CSPs (CSP5, 7, 10, 11, 13, 14, and 21) showed no obvious bias in expression between olfactory and non-olfactory tissues.

Expression and Purification of Recombinant CsupCSPs
To further explore their odorant preference, three antennae-specific or highly biased CSPs (CSP1, 2 and 3) were selected for bacterial expression. CSP1 and 2 were present in bacterial pellets, while CSP3 was detected in the supernatant after sonication (Fig. 2). CSP1 and 2 were further treated with urea to solubilize the protein pellets and re-naturated by extensive dialysis following the previously reported protocol (Liu et al. 2012). Purified proteins were obtained by passing them through a His-tag affinity column followed by a treatment with enterokinase to remove the His-tag (Fig. 2). The purified CSPs (CSP1, 2, and 3) were approximately 19.61, 15.38, and 16.27 kDa, respectively, consistent with the expected molecular weight.

Ligand Binding Properties of Three CsupCSPs
The dissociation constants of CSP1, 2 and 3 to a fluorescent reporter (1-NPN) were calculated as 4.51, 8.88, and 13.97 µM, respectively (Fig. 3A). To determine the binding affinities of CSPs to three sex pheromone components and 35 plant volatiles, competitive binding assays were carried out. According to the criteria of high (Ki ≤ 10.00 µM), moderate (Ki = 10.01–20.00 µM) and low (Ki = 20.01–30.00 µM) binding affinity for plant volatiles. CSP1 displayed high binding affinities for four plant volatiles (Ki = 7.77, 8.90, 9.16, and 9.16 µM) for 2-tridecanone, benzaldehyde, laurinaldehyde,
and 2-pentadecanone, respectively, and moderate bindings for nine and low for two compounds; CSP2 demonstrated high bindings (Ki = 9.22 and 9.25 μM) for two alcohol compounds of (+)-cedrol and 2-heptanol, respectively; moderate for nine and low for six plant volatiles. However, CSP3 did not show high binding affinity for any tested plant volatiles, and only showed moderate binding for three compounds and low binding for four compounds (Fig. 3b and Table 1).

For sex pheromone components, CSP2 and CSP3 showed Ki values of 8.89 and 6.23 μM for Z11–16:Ald, and of 9.55 and 8.86 μM for Z9–16:Ald, respectively (Fig. 3D and Table 1). However, according to the criteria for sex pheromone components, these Ki values indicated only low binding affinities. For sex pheromones, it is recognized as high, moderate and low binding affinity when the Ki value is ≤2.00, 2.01–5.00, and 5.01–10.00 μM, respectively.

**Discussion**

Insects mostly use antennae to detect odorants from the external environments (Schneider 1957). Various sensory sensilla in the antennae play essential roles for insects to locate the food, shelter, mate, and threats (Kaisling 2001, Hallem and Carlson 2006, Hansson and Stensmyr 2011). CSPs are thought to function as carriers of external odorants, and thus play crucial roles in insect olfaction. However, only few CSPs are specifically or predominantly expressed in olfactory organs, while most CSPs are extensively expressed in a wide range of sensory and non-sensory tissues, suggesting that these CSPs play other functions. RNAi or other in vivo functional studies have evidenced some non-olfactory functions, such as limb regeneration by a CSP (p10) in *P. americana* (L.) (Nomura et al. 1992, Kitabayashi et al. 1998), embryo development by CSP5 in *Apis mellifera* (L.) (Hymenoptera: Apidae) (Maleszka et al. 2007), and cuticle growth and ecdysis by CSP9 (a homologue of AmelCSP5) in *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae) (Cheng et al. 2015).

In order to understand functions of CSP in *C. suppressalis*, tissue expression profiles were characterized by qPCR. As expected, 21 CSPs showed various tissue expression patterns and thus suggested different functions, consistent with studies in other insects (Gong et al. 2007, Gu et al. 2012, Qiao et al. 2013, Zhang et al. 2014, Li et al. 2015). First of all, eight CSPs (CSP1–4, 6, and 15–17) were specifically or predominantly expressed in the antennae of both male and females. Since antennae are primary olfactory tissue in adult insects, these CSPs may play important roles in perceiving host plant volatile and odorant from other sources, similar to CSP19 in *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) (Zhang et al. 2014) and CSP6 in *H. armigera* (Hübner) (Lepidoptera: Noctuidae) (Li et al. 2015). CSPs highly expressed in antennae were also reported in other insects, such as *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) (Gu et al. 2012), and *Choubia cunea* (Yang) (Hymenoptera: Eulophidae) (Zhao et al. 2016). Secondly, we found that 4 CSPs (CSP5, 8, 12, and 19) were expressed predominantly in heads. Although antennae have been removed from the tested heads, there is still the proboscis, a major gustatory organ and minor olfactory organ (Scott et al. 2001, Kwon et al. 2006). Therefore, these CSP genes might be involved in the recognition of non-volatile and volatile chemicals especially from the food sources. In addition, CSP9 transcripts were mainly detected in the legs of both sexes, suggesting their possible gustatory roles, considering that adult legs bear gustatory sensilla and even olfactory sensilla in some insects (Nayak and Singh 1983, Stocker 1994). On the other hand, some CSPs (CSP18, 20, and 21) were highly detected in both male and female abdomen, a clearly non-olfactory tissue, implying the importance of these CSPs in other physiological/developmental functions. In many other insects, abdomen highly expressed CSPs were also reported, such as CSP18 in testes and CSP12 in ovaries from *B. mori* (Gong et al. 2007), and CSP5 in ovaries from the *A. mellifera* (Maleszka et al. 2007).

Focussing on the olfactory CSPs, binding properties of three antennae specific or highly biased CSPs (CSP1, 2, and 3) were further determined by competitive binding assays, with 35 host plant volatiles from rice (Du et al. 2005, Lou et al. 2005, Feng et al. 2010, Fujii et al. 2010) and other plants (Bengtsson et al. 2001, Dudaeva et al. 2004, Mei et al. 2007), and three sex pheromone components of *C. suppressalis* (Neshitt et al. 1975). The binding assay showed high bindings of three CSPs for the fluorescent reporter (1-NPN), with dissociation constant of 4.51, 8.88, and 13.97 μM for CSP1, 2, and 3, respectively. However, the straight lines on the graph insets for CSP2 and 3 did not fit the data very well.
well. The upward-curving Scatchard plots might be caused by negative interaction between different binding sites in a CSP molecule or with heterogeneous binding sites, and thus the binding constants reported in this study were not so accurate, and could be improved by using a different binding model that takes into account the complexity of the NPN binding to the CSPs. CSP1 and CSP2 exhibited high binding affinities for four and two plant volatiles respectively, whereas CSP3 did not show high binding affinity for any tested plant volatiles. These six host plant volatiles and several other volatiles of moderate affinity for CSP1 and CSP2 are previously reported to be able to elicit strong electroantennographic (EAG) responses in adult C. suppressalis (Wei et al. 2013, Khuhro et al. 2017a), showing consistency between binding assay and the EAG assay. However, we noted that farnesene elicited significant EAG response (Khuhro et al. 2017a), but did not show high or moderate binding affinity with any of three CSPs tested here and recently studied CsupOBPs (Khuhro et al. 2017b). It is expected that other CSPs or/and OBPs are responsible for farnesene binding which needs further exploration.

Several studies reported that CSPs displayed high binding affinity for sex pheromones (Ki < 2.00 µM), such as CSP19 in S. inferens (Ki = 0.49–1.78 µM) (Zhang et al. 2014), and CSP6 in H. armigera (Ki = 0.92–1.38 µM) (Li et al. 2015), suggesting that these CSPs play roles in perception of the sex pheromones. In the present study, we also measured the binding affinities of three CSPs for the sex pheromones. However, none of the three CSPs showed high binding for the sex pheromones according to the criteria. In contrast, high binding affinity for three sex pheromone components (Ki < 2.00 µM) was demonstrated with pheromone binding proteins (PBPs) (Chang et al. 2015) and general odorant binding proteins (GOBPs) (Gong et al. 2009, Khuhro et al. 2017a). Therefore, in C. suppressalis, PBPs and GOBPs are responsible for transporting the sex pheromones, and CSPs (at least the three ones tested here) are not involved in the sex pheromone perception.

In summary, we found different tissue expression patterns among 21 CSPs, suggesting the functional diversity of CSPs and in particular, eight CSPs showed antennae biased expression, indicating their involvement in olfaction. Further ligand binding assay of three antennae highly biased CSPs revealed that CSP1 and CSP2 have high binding affinities for four and two host plant volatiles, respectively.
Table 1. Binding affinities of tested ligands to three *Chilo suppressalis* chemosensory proteins

| Ligand name                              | CSP1        | CSP2        | CSP3        |
|------------------------------------------|-------------|-------------|-------------|
| Sex pheromones                           |             |             |             |
| Z11-Hexadecenal (Z11–16:Ald)             | 43.59 ± 0.68| 20.89       | 16.16       |
| Z9-Hexadecenal (Z9–16:Ald)               | 44.79 ± 2.25| 17.45       | 13.50       |
| Z13-Octadecenal (Z13–18:Ald)             | 51.06 ± 1.62| >30         | -           |
| Aldehyde compounds                       |             |             |             |
| Laurinaldehyde                           | 37.43 ± 2.76| 11.84       | 9.16        |
| Benzaldehyde                             | 34.25 ± 2.35| 11.51       | 8.90        |
| Z2-Hexenal                               | 43.07 ± 2.14| 15.79       | 12.21       |
| E2-Hexenal                               | 56.72 ± 0.97| >30         | -           |
| 1-Nonaldehyde                            | 74.08 ± 4.97| >30         | -           |
| Alcohols compounds                       |             |             |             |
| (+)-Cedrol                               | 47.17 ± 1.16| 21.15       | 16.35       |
| Farnesol                                 | 50.77 ± 0.79| >30         | -           |
| Nerolidol                                | 44.30 ± 2.10| 14.95       | 11.56       |
| Hexyl alcohol                            | 63.95 ± 4.34| >30         | -           |
| E3-Hexenol                               | 66.89 ± 1.48| >30         | -           |
| Z3-Hexenol                               | 77.00 ± 1.25| >30         | -           |
| Linalool                                 | 48.21 ± 0.96| 20.76       | 16.05       |
| 2,4-hexadienol                           | 85.76 ± 1.92| >30         | -           |
| Benzy l alcohol                          | 67.48 ± 2.24| >30         | -           |
| E2-hexenol                               | 88.97 ± 2.02| >30         | -           |
| 2-Heptanol                               | 48.87 ± 0.63| 20.93       | 16.19       |
| Ketones compounds                        |             |             |             |
| 2-Pentadecanone                          | 27.92 ± 2.11| 11.84       | 9.16        |
| 2-Tridecanone                            | 24.81 ± 1.61| 10.05       | 7.77        |
| 2-Methylacetoephene                      | 47.17 ± 0.80| 22.99       | 17.78       |
| 2-Heptanone                              | 49.31 ± 1.89| 19.95       | 15.43       |
| 3-Acetoephene                            | 42.92 ± 4.17| 18.54       | 14.34       |
| Terpenes compounds                       |             |             |             |
| Myrcene                                  | 55.29 ± 5.11| >30         | -           |
| (R)-(+)-Limonene                         | 49.77 ± 2.33| 26.34       | 21.63       |
| Farnesene                                | 49.72 ± 1.29| 27.56       | 23.29       |
| Ocimene                                  | 73.90 ± 1.70| >30         | -           |
| α-Phellandrene                           | 72.99 ± 2.46| >20         | -           |
| β-Ionone                                 | 47.59 ± 0.91| 22.51       | 17.14       |
| Esters and benzoates                     |             |             |             |
| Methyl Salicylate                        | 72.58 ± 2.60| >30         | -           |
| Ethyl benzoates                          | 82.50 ± 3.39| >30         | -           |
| E2-Hexenyl acetate                       | 83.08 ± 0.86| >30         | -           |
| Z3-Hexenyl acetate                       | 85.80 ± 2.19| >30         | -           |
| Carboxylic acids                         |             |             |             |
| Linoleic acid                            | 55.58 ± 3.45| >30         | -           |
| Oleic acid                               | 58.86 ± 2.59| >30         | -           |
| Lauric acid                              | 73.89 ± 2.72| >30         | -           |
| Palmitic acid                            | 78.25 ± 0.93| >30         | -           |

*Intensity %* means the 1-NPN fluorescence (%) at the highest ligand concentration tested. ‘>30’ for IC$_{50}$ means that the IC$_{50} > 30$ µM and the accurate values cannot be directly calculated with the ligand concentration range tested in the assay.

*<sup>a</sup>Rice plant volatiles according to the literature.

Taken together, we suggest that these CSPs play essential functions in the perception of host plant volatiles and thus, in finding host plants for food, habitat and other purposes.

**Supplementary Data**

Supplementary data are available at *Journal of Insect Science* online.

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McKenna, M. P., D. S. Hekmat-Scafe, P. Gaines, and J. R. Carlson. 1994. Putative Drosophila pheromone-binding proteins expressed in a subregion of the olfactory system. J. Biol. Chem. 269: 16340–16347.

Mei, W. L., Y. B. Zeng, J. Liu, and H. F. Dai. 2007. GC-MS analysis of volatile constituents from five different kinds of Chinese eaglewood. Zhong Yao Cai 30: 531–535.

Nayak, S. V., and R. N. Singh. 1983. Sensilla on the tarsal segments and mouthparts of adult Drosophila melanogaster Meigen (Diptera: Drosophilidae). Int. J. Insect Morphol. Embryol. 12: 273–291.

Niu, D. J., Y. Liu, X. T. Dong, and S. L. Dong. 2016. Transcriptome-based identification and tissue expression profiles of chemosensory genes in Blattella germanica (Blattaria: Blattidae). Comp. Biochem. Physiol. Part D. Genomics Proteomics. 18: 30–43.

Nomura, A., K. Kawasaki, T. Kubo, and S. Natori. 1992. Purification and localization of p10, a novel protein that increases in nymphal regenerating legs of Periplaneta americana (American cockroach). Int. J. Dev. Biol. 36: 391–398.

Pelosi, P., M. Calvello, and L. Ban. 2005. Diversity of odorant-binding proteins and chemosensory proteins in insects. Chem. Senses 30(Suppl 1): i291–i292.

Pelosi, P., J. J. Zhou, I. P. Ban, and M. Calvello. 2006. Soluble proteins in insect chemical communication. Cell. Mol. Life Sci. 63: 1638–1676.

Picimbon, J. F., K. Dietrich, S. Angeli, A. Scaloni, J. Krieger, H. Breer, and P. Pelosi. 2000. Purification and molecular cloning of chemosensory proteins from Bombyx mori. Arch. Insect Biochem. Physiol. 44: 120–129.

Pikieley, C. W., G. Hasan, F. Rouyer, and M. Rosbash. 1994. Members of a family of Drosophila putative odorant-binding proteins are expressed in different subsets of olfactory hairs. Neuron. 12: 35–49.

Qiao, H. L., P. Y. Deng, D. D. Li, M. Chen, Z. J. Jiao, Z. C. Liu, Y. Z. Zhang, and Y. C. Kan. 2013. Expression analysis and binding experiments of chemosensory proteins indicate multiple roles in Bombyx mori. J. Insect Physiol. 59: 667–675.

Schneider, D. 1987. Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. Cell Mol. Life Sci. 13: 89–91.

Scott, K., R. Brady, Jr., A. Cravchik, P. Morozov, A. Rzhetsky, C. Zuker, and R. Axel. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in Drosophila. Cell. 104: 661–673.

Sheng, C. F., H. T. Wang, S. S. Yu, L. D. Gao, and X. W. Jian. 2003. Pest status and loss assessment of crop damage caused by the rice borer, Chilo suppressalis and Tryporyza incertulas in China. Entomol. Knowle. 40: 289–294.

Simona, T., O. Crescenzi, D. Sanfelice, E. Ab, R. Wechselberger, S. Angeli, A. Scaloni, R. Boelens, T. Tancredi, P. Pelosi, et al. 2006. Solution structure of a chemosensory protein from the desert locust Schistocerca gregaria. Biochemistry. 45: 10606–10613.

Stockner, R. F. 1994. The organization of the chemosensory system in Drosophila melanogaster: a review. Cell Tissue Res. 275: 3–26.

Tanaka, K., Y. Uda, Y. Ono, T. Nakagawa, M. Suwa, R. Yamaoka, and K. Touhara. 2009. Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Curr. Biol. 19: 881–890.

Wang, R., F. Li, W. Zhang, X. Zhang, C. Qu, G. Tetreau, L. Sun, C. Luo, and J. Zhou. 2017. Identification and expression profile analysis of odorant binding protein and chemosensory protein genes in Bemisia tabaci MED by head transcriptome. PLoS One 12: e0171739.

Wanner, K. W., L. G. Willis, D. A. Theilmann, M. B. Isman, Q. Feng, and E. Plettner. 2004. Analysis of the insect ox-d-like gene family. J. Chem. Ecol. 30: 889–911.

Wei, D., Z. Ye, J. Gao, and S. L. Dong. 2013. Molecular cloning and functional identification of a Mmus-C odorant binding protein from the rice striped stem borer, Chilo suppressalis (Lepidoptera: Pyralidae). Acta Entomol. Sin. 56: 734–746.

Xia, Y. H., Y. N. Zhang, X. Q. Hou, F. Li, and S. L. Dong. 2016. Large number of putative chemoreception and pheromone biosynthesis genes revealed by analyzing transcriptome from ovipositor-pheromone glands of Chilo suppressalis. Sci. Rep. 5: 7888.

Yang, K., P. He, and S. L. Dong. 2014. Different expression profiles suggest functional differentiation among chemosensory proteins in Nilaparvata lugens (Hemiptera: Delphaciidae). J. Insect. Sci. 14: 1–18.

Zhang, Y. N., Z. F. Ye, K. Yang, and S. L. Dong. 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.

Zhao, Y., F. Wang, X. Zhang, S. Zhang, S. Guo, G. Zhu, Q. Liu, and M. Li. 2016. Transcriptome and expression patterns of chemosensory genes in antennae of the parasitoid wasp Chouioia canea. PLoS One 11: e0148159.

Zhou, S. H., J. Zhang, S. G. Zhang, and L. Zhang. 2008. Expression of chemosensory proteins in hairs on wings of Locusta migratoria (Orthoptera: Acrididae). J. Appl. Entomol. 132: 439–450.