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Research article

Identification and functional characterization of odorant-binding proteins 69a and 76a of *Drosophila suzukii*

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ABSTRACT

The fruit fly *Drosophila suzukii* is a fruit crop pest that causes a severe economic threat to soft summer fruit worldwide. The male sex pheromone, cis-vaccenyl acetate (cVA) has multiple functions in intra-species communication in *Drosophila melanogaster*, which is required in male to suppress male-male courtship. *D. suzukii* males do not produce cVA; however, the odorant receptor for cVA (Or67d) is still functional. The lack of cVA in *D. suzukii* casts the question of whether this pheromone might have been replaced by another compound similar to cVA that disrupts mating in *D. suzukii*. In order to address this question, we cloned two *D. suzukii* adult antenna-specific odorant-binding proteins (OBPs) DsOBP69a and DsOBP76a and aligned with their *D. melanogaster* orthologues. Moreover, we examined the binding properties of the newly identified recombinant proteins against 26 potential ligands including cVA, using the fluorescence-based ligand binding assay. The alignment showed that DsOBP69a and DsOBP76a, have six conserved cysteines and belong to the classic OBP family. Furthermore, our results revealed that cVA did not bind to DsOBP69a or DsOBP76a proteins. Interestingly, the floral odorant β-ionone and the bitter substance berberine chloride and coumarin displayed high binding ability. It is also worth noting that DsOBP69a and DsOBP76a have different affinities to (Z)-7-Tricosene that may reflect different functional roles. These findings suggest that *DsOBP69a* and *DsOBP76a* are potentially involved in olfaction and gustation of *D. suzukii*.

1. Introduction

The fruit fly *Drosophila suzukii* (also known as Spotted Wing *Drosophila*) is an important polyphagous insect pest infesting many fruit crops, causing damage to soft-skinned fruits such as strawberries, cherries, grapes and others. *D. suzukii* prefers to lay eggs in fresh and ripening fruits, while most other *Drosophila* species, such as *Drosophila melanogaster*, only deposit eggs on decaying, fermented fruits (Mitsui et al., 2006). The fly was first observed in Mainland (Honshu) Japan in 1916, and then it broke out in Europe and the United States in 2008 (Rota-Stabelli et al., 2013). Currently, it is widely distributed in Asia, Europe, and America, and may spread to Africa and Australia (dos Santos et al., 2017; Asplen et al., 2015). If proper control measures are not taken, the annual loss to the American fruit industry from *D. suzukii* could reach $500 million (Goodhue et al., 2011; Walsh et al., 2011). At present, the main method for reducing the economic losses caused by *D. suzukii* is the frequent use of chemical pesticides (Farnsworth et al., 2017). However, because the eggs and larvae are inside the fruit, the effectiveness of chemical control is very limited, and it is not conducive to environmental protection and food safety. Therefore, developing of species-specific baits that may provide a successful management method for the control of *D. suzukii*, is still required. According to the scent of fresh and fermented apple juice, a mixture of five compounds is more effective and more specifically targeted for attracting *D. suzukii* (Feng et al., 2018).

Pheromones are important targets for pest monitoring. There are two pheromonal systems known in *D. melanogaster*: cuticular hydrocarbons (CHCs) produced by oenocytes, which are located directly under the cuticle of the abdomen, and cis-vaccenyl acetate (cVA) produced by the

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male ejaculatory bulb (Brieger and Butterworth, 1970). cVA is the most thoroughly studied pheromone in fruit flies. The cVA promotes mating of the female and it is transferred to the female during the mating process. After mating, the cVA in the female will inhibit further male mating (Kurtovic et al., 2007; Jallon, 1984; Billeter et al., 2009; Ruta et al., 2010). cVA also promotes aggression between the males of *D. melanogaster* (Wang and Anderson, 2010). Adult males of *D. Suzuki* do not produce cVA due to atrophy of the ejaculation tube, and artificial application of cVA to males will inhibit their mating (Dekker et al., 2015; Snellings et al., 2018).

Drosophila senses pheromone signals through the olfactory and taste sensory neurons in the sensilla. And the neurons are bathed in an aqueous phase called sensory lymph. The odorant molecules bind to the odorant binding proteins (OBPs) in the sensory lymph, and they are transported to the sensory neuron dendrites where they bind to the receptors, stimulating the neuron to transmit sensory signals to the central nervous system (Leal, 2013; Kohl et al., 2015; Joseph and Carlson, 2015). Some *D. suzukii* OBPs, like *D. melanogaster* OBPs (Hekmat-Scafe et al., 2002; Larter et al. 2016; Rihani et al., 2019), are expressed in the olfactory sensilla on the antenna and taste sensilla of the labellum and leg (Ahn et al., 2020; Crava et al., 2019; Ramasamy, 2016). *D. melanogaster* perception of cVA requires OBP76a (also known as Lush) to promote cVA transport for binding through OR67d and SNMP proteins (Laughlin et al., 2008; Gomez-Diaz et al., 2013). Recently, OBP69a has also been reported to be involved in the response of *D. melanogaster* to cVA (Bentzur et al., 2018). However, whether cVA or some other substances bind directly to OBP69a remains unclear.

The olfactory system of *D. suzukii* has changed to adapt to its unique ecological niche. There are 53 OBPs in *D. suzukii* (Chiu et al., 2013; Ramasamy et al., 2016) while there are 52 OBPs in *D. melanogaster* (Hekmat-Scafe et al., 2002; Larter et al. 2016), and four OBPs show signs of different selective properties and positive selection, including DsOBP69a in *D. suzukii* (Ramasamy et al., 2016). There is no functional research on OBPs reported for *D. suzukii*, except for our earlier report on DsOBP56h (Li et al., 2019). In this study, we identified DsOBP69a and DsOBP76a as orthologs to *D. melanogaster* OBP69a and OBP76a. We also evaluated the binding properties of DsOBP69a and DsOBP76a proteins to a panel of potential ligands using the fluorescence competition assay. cVA did not bind directly with them and the floral odor compound β-ionone and the bitter tasting berberine chloride and coumarin showed good affinity.

2. Materials and methods

2.1. Insect rearing

The *D. suzukii* colonies were initially collected from a cherry orchard in Shiyan, Hubei, China and reared on artificial diets based on banana under laboratory conditions of the constant temperature of 24 ± 1 °C, the relative humidity (HR) of 65 ± 5% and the photoperiod of 16 h light: 8 h dark (Dalton et al., 2011).

2.2. Cloning and sequence analysis of DsOBP69a and DsOBP76a

Ten male and female adults of *D. suzukii* were frozen in liquid nitrogen followed by total RNA extraction using the RNAiso Plus reagent (TaKaRa, Beijing, China). PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Beijing, China) was employed to synthesize the first-strand cDNA, following the manufacturer's protocol. Since the *D. suzukii* genome has been published (Chiu et al., 2013; Ometto et al., 2013), the coding sequences (CDS) of DsOBP69a and DsOBP76a (accession number: XM_017079398.1 and XM_017075568.1) were amplified. Signal peptides were predicted using SignalP-5.0 server (Armenteros et al., 2019). The sequences encoding mature DsOBP69a and DsOBP76a were amplified using the following primers: GGAATTCCATATG CAGGACATTAACCCCATGATT (DsOBP69a forward, *Nde*I underlined); CCGCTCGAGTCCAAGTAGCGATACCATCTG (DsOBP69a reverse, *Xho*I underlined); GGAATTCCATATGATGACGATGGACCAGTTCTTA (DsOBP76a forward, *Nde*I underlined) and CCGCTCGAGAGGCCACA-TAAACTTTCCTTC (DsOBP76a reverse, *Xho*I underlined).

The PCR amplifications were performed using PrimeSTAR HS DNA polymerase (TaKaRa, Beijing, China). The PCR program included an initial denaturation at 94 °C for 3 min; followed by 30 cycles of 98 °C for 10 s, 60 °C for 5 s, and 72 °C for 1 min; then 72 °C for 10min. PCR products were detected on a 1% agarose gel and sequenced by Tsingke Biological Technology (Beijing, China).

The OBP putative orthologs from other insects were obtained from the National Center for Biotechnology Information (NCBI) (Table s1). Sequence alignment and phylogenetic tree generation were performed using the ClustalW and maximum likelihood methods in the software MEGA 5 (Tamura et al., 2011).

2.3. Bacterial expression and protein purification of DsOBP69a and DsOBP76a

Using NdeI and XhoI as restriction sites, DsOBP69a and DsOBP76a were ligated into vector pET-30a (+) and transferred to *Escherichia coli* BL21 (DE3) cells. The cells were cultured in LB/kanamycin (50 μg/mL) for 4–6 h (OD600 = 0.4–0.6) at 37 °C and 180 rpm. Recombinant protein expression was induced with adding IPTG (1 mmol L^{-1}) at 28 °C and 180 rpm for 8 h. All bacterial cultures were harvested by centrifugation at 12000 xg for 1 min. Each cell pellet was resuspended using bacterial lysate solution (50 mmol/L Tris-HCl, 2 mmol/L ethylenediaminetetraacetic acid, 100 mol/L NaCl, 0.5% Triton X-100 and 1 mg/mL lysozyme). Then cells were disrupted by ultrasonication (active for 3 s, stopped for 5 s, 30 min in total). DsOBP69a and DsOBP76a were mainly detected in the inclusion bodies, then dissolved with 8 M urea, and added into high affinity Ni-NTA resin (Genscript, China). Bound proteins were eluted with 50 mM Tris containing 20 mM, 100 mM, 250 mM and 500 mM imidazole, and detected by SDS-PAGE. Eluted proteins were refolded by the method of cystine/cysteine (Prestwich, 1993). Dialysis was carried out for 24 h with 50 mM Tris-HCl (pH 7.4) at 4 °C, during which time the dialysate was changed 3 times. Protein concentrations were measured using the bicinchoninic acid (BCA) Protein Assay Kit (ThermoFisher Scientific-Life Technologies, Carlsbad, CA, USA), following the manufacturer's protocol.

2.4. Ligand binding assays of DsOBP69a and DsOBP76a

All ligands were diluted to 1 mmol L^{-1} with chromatographic grade methanol. The protein was diluted with 50 mM Tris-HCl (pH 7.4) to a final concentration of 2 μmol L^{-1}. An aliquot of 2 mL of the diluted protein was placed into the cuvette and titrated with bis-ANS. Protein was detected and affinity was probed by a fluorescence spectrophotometer. The excitation wavelength was set to 295 nm and the scanning range was 300–550 nm. The binding curve was linearized according to the Scatchard plot, assuming that the protein was 100% active, and its stoichiometric ratio with the ligand was 1:1; and the binding dissociation constant $K_{bis\text{-}ANS}$ of DsOBP69a and the fluorescent probe bis-ANS was determined. The affinity of the protein for the ligand was determined by fluorescence competition binding assays. Proteins with a final concentration of 2 μmol L^{-1} and bis-ANS were added to the cuvette and titrated with the selected ligand compound at concentrations ranging from 2 to 16 μmol. The dissociation constant Ki was calculated as: $Ki = [IC_{50}]/(1+[\text{bis-ANS}]/K_{bis\text{-}ANS})$, where $[IC_{50}]$ is the concentration of the ligand when the fluorescence intensity is reduced by half and [bis-ANS] is the free bis-ANS concentration.

2.5. Homology modeling

The protein sequences of DsOBP69a and DsOBP76a, from which signal peptides were removed, were used for matching suitable templates and predicting the 3D structures of the proteins in SWISS-MODEL (Biasini

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DmOBP69a : VEINPTIIKQVRKLRMRCLNQTGASVDVIDKS-VKNRILPTDPEIKCFLYCMFDMFGLIDSQN----IMHL :  66
DsOBP69a : QDINPMIIRQVKKLRTRCLNQTGASVEIMDHS-VKNRMLPNDPLVKCFLHCMFDMFGLIDSQN----IMHL :  66
DmOBP76a : -MTMEQFLTSLDMIRSGCAPKFKLKTEDLDRLRVGDFNFPPSQDLMCYTRCVSLMAGTVNKKGEFNAPKAL :  70
DsOBP76a : -MTMDQFLSSLDMIRSGCAPKFKLNIEDLDRLRVGDFSFPPSQDLMCYTRCVSLMAGTVNKKGEFNAAKAL :  70

DmOBP69a : EALLEVLPEEIHKTINGLVSSCG--TQKGKDGCDTAYETVKCYIAVN-GKFIWEEIIVLLG : 124
DsOBP69a : EALVEVLPEELHNTIIGLVGACG--TKKGKDGCETAYETVKCYIDVN-GIFIWKQMVSLLG : 124
DmOBP76a : AQLPHLVPPEMMEMSRKSVEACRDTHKQFKESCERVYQTAKCFSENADGQFMWP------- : 124
DsOBP76a : ASLPHLVPTEMMEASKRSVEACRDAHKAFKESCERVFQTAKCLAENGEGKFMWP------- : 124
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Figure 1. Sequence alignment of the OBPs. Cysteines are highlighted in black.

et al., 2014). The rationality of protein structure was evaluated using a Ramachandran plot generated by ProCheck (Laskowski et al., 1993). The secondary structure of the protein was predicted using the ESPript 3.0 program (Robert and Gouet, 2014).

3. Results

3.1. Gene cloning and sequence analysis

The CDS of DsOBP69a and DsOBP76a were successfully cloned, consistent with the sequences in NCBI (XM_017079398.1 and XM_017075568.1). According to SignalP-5.0 server (Armenteros et al., 2019), the N-terminus of the DsOBP69a protein contains a signal peptide consisting of 24 residues. The N-terminus of the DsOBP76a protein contains a signal peptide consisting of 29 residues. Both DsOBP69a and DsOBP76a, having six conserved cysteines, belong to the classic OBP family (Figure 1). The phylogenetic tree analysis indicated that Dipteran OBP69a and OBP76a were each clustered into one branch, with branch support value of 0.9. DsOBP69a and DmOBP69a have the closest relationship, with branch support value of 0.76. Similarly, DsOBP76a and DbLush have the closest relationship, with branch support value of 0.97 (Figure 2).

3.2. Binding characteristics of DsOBP69a and DsOBP76a

The DsOBP69a and DsOBP76a sequences lacking the signal peptides were successfully ligated into pET30a and transferred to *E. coli* BL21 (DE3) cells. Both DsOBP69a and DsOBP76a proteins were expressed in inclusion bodies, and after renaturation, the pure proteins of interest were obtained (Figure 3 and s1).

1-NPN is a commonly used fluorescent probe, but it can react with cVA to produce fluorescence in the absence of protein addition (Figure s2), thus affecting subsequent experiments. Therefore, we chose bis-ANS as the fluorescent probe for this experiment (Figure 4). The dissociation constant of DsOBP69a and bis-ANS is 0.8292 μM, and the linear equation of the Scatchard plot is y = -4.357x+8.183 (R^2 = 0.9867). The dissociation constant of DsOBP76a and bis-ANS is 0.8116 μM, and the linear equation of the Scatchard plot is y = -4.509x+8.497

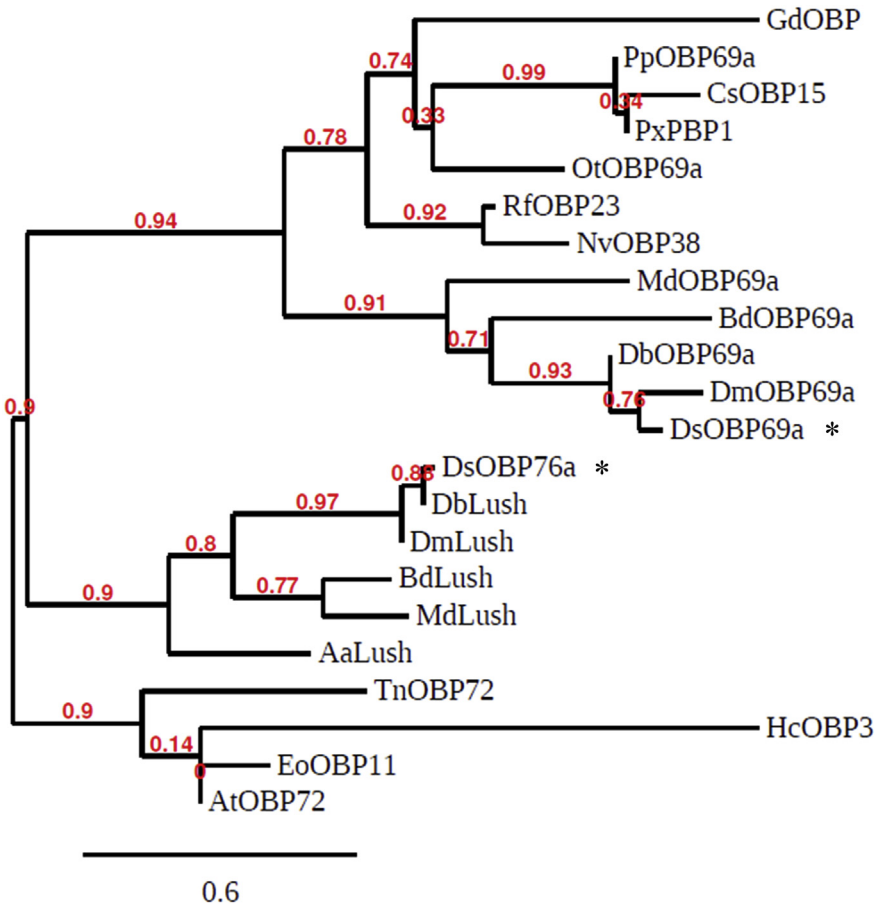

Figure 2. Phylogenetic tree of DsOBP69a, DsOBP76a and their putative orthologues from other insects. DsOBP69 and DsOBP76a is marked with an asterisk. Ds, *Drosophila suzukii*; Ot, *Onthophagus taurus*; Rf, *Rhynchophorus ferrugineus*; Gd, *Galeruca daurica*; Nv, *Nezara viridula*; Pp, *Papilio polytes*; Cs, *Carposina sasakii*; Px, *Papilio xuthus*; Db, *Drosophila biarmipes*; Dm, *Drosophila melanogaster*; Md, *Musca domestica*; Bd, *Bactrocera dorsalis*; Aa, *Aedes aegypti*; Tn, *Trichoplusia ni*; Eo, *Ectropis obliqua*; At, *Amyelois transitella*; Hc, *Hycleus cichorii*. The number on the branch indicates branch support value.

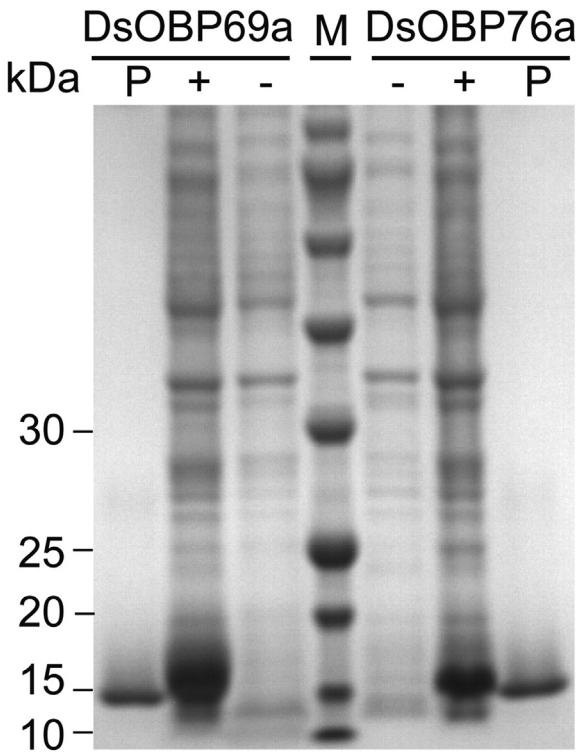

Figure 3. SDS-PAGE analysis of expressed and purified DsOBP69a and DsOBP76a. M: molecular weight markers; -: bacterial sample before induction with IPTG; +: samples after induction; P: purified protein.

($R^2 = 0.9733$). Bis-ANS binds well to both DsOBP69a and DsOBP76a, so we chose bis-ANS for subsequent fluorescence competition binding experiments.

The ligand was gradually added to the 2 μM protein and bis-ANS mixture, and the change in fluorescence intensity was recorded to calculate the IC_{50} and the dissociation constant K_d (Table s3). Of the 26 potential ligands selected, only five were able to bind to DsOBP69a (Figure 5A). β-Ionone has the strongest binding ability with DsOBP69a, and the K_d is 4.03 μM. Two kinds of bitter substances, berberine chloride and coumarin, can also bind to DsOBP69a with K_d values of 7.21 and 20.37 μM, respectively. *Drosophila* pheromone (Z)- 7-tricosene can also bind to DsOBP69a with a K_d of 19.34 μM. Naringenin can also be combined with DsOBP69a with a K_d of 23.76. The binding ability of DsOBP76a to the ligands is similar to that of DsOBP69a, with the exception of (Z)- 7-tricosene (Figure 5B). β-Ionone has the strongest binding to DsOBP76a, and its K_d is 3.37 μM. Two kinds of bitter substances, berberine chloride and coumarin, can also bind to DsOBP76a with K_d values of 7.64 and 7.76 μM, respectively. The K_d of naringenin and DsOBP76a is 24.10 μM. The cVA and other selected ligand compounds associated with OBP69a and OBP76a, which are more thoroughly studied in *Drosophila*, do not bind to either DsOBP69a or DsOBP76a.

3.3. Homology modeling

According to SWISS-MODEL (Biasini et al., 2014), DsOBP69a has the highest degree of matching with *Ceratitis capitata* OBP22 (ID: 6hhe.1.A), with a Global Model Quality Estimation (GMQE) value of 0.71, and a sequence similarity of 36.44%. The similarity between CcapObp22 and *D. melanogaster* OBP69a was 37% [40]. DsOBP76a has the highest degree of matching with *D. melanogaster* OBP76a (ID: 3b86.1.A), with a GMQE value of 0.96 and sequence similarity of 83.87%. The evaluation of the DsOBP69a model showed that 92.9% of the amino acid residues fell in the optimal region and 7.1% of them fell in the allowable region (Figure s3 A). The evaluation of the DsOBP76a model showed that 92.8% of the amino acid residues fell in the optimal region and 7.2% of them fell in the allowable region (Figure s3 B). The 3D structural model predicted for DsOBP69a consists of seven alpha helices (Figure 6): Gln10-Thr22 (α1), Val26-Asn35 (α2), Pro42-Met54 (α3), Ala68-Val72 (α4), Glu75-Cys88 (α5), Gly96- Val110 (α6), and Gly112-Gln118 (α7) (Figure 6A). The 3D structural model predicted for DsOBP76a consists of six alpha helices: Met3-Ser15 (α1), Ile25-Arg32 (α2), Gln42-Ala55 (α3), Ala66-His75 (α4), Thr79-Cys92 (α5), and Ser102- Glu115 (α6) (Figure 6B).

4. Discussion

Insects need to find a suitable host to complete a normal life history and breeding population. Its host selection relies on smell and taste (Knolhoff and Heckel, 2014). OBPs play an important role in the host

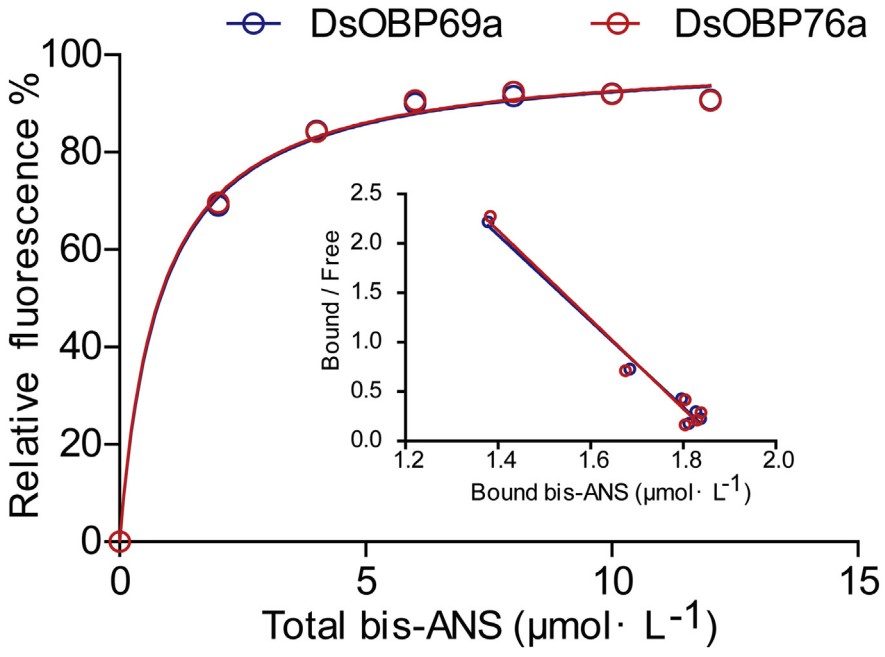

Figure 4. Binding curve and Scatchard plot of bis-ANS to recombinant DsOBP69a and DsOBP76a.

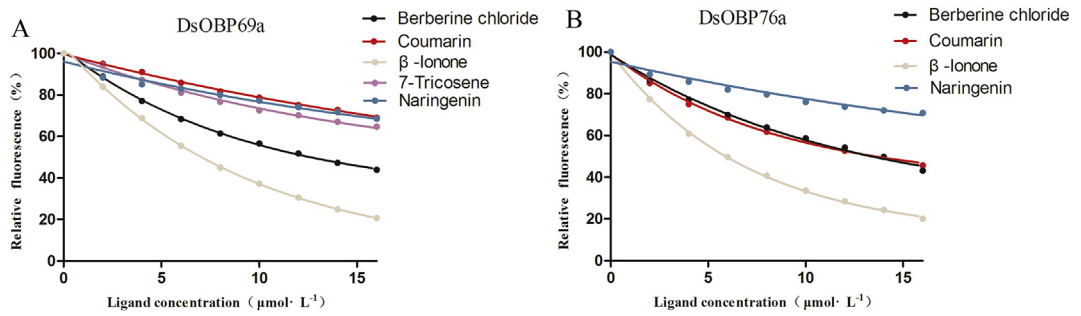

Figure 5. OBP69a and OBP76a binding affinity for potential ligands. (A) Fluorescent competitive binding curves of DsOBP69a bound to berberine chloride, coumarin, β-ionone, 7-tricosene and naringenin. (B) Fluorescent competitive binding curves of DsOBP76a bound to berberine chloride, coumarin, β-ionone, and naringenin.

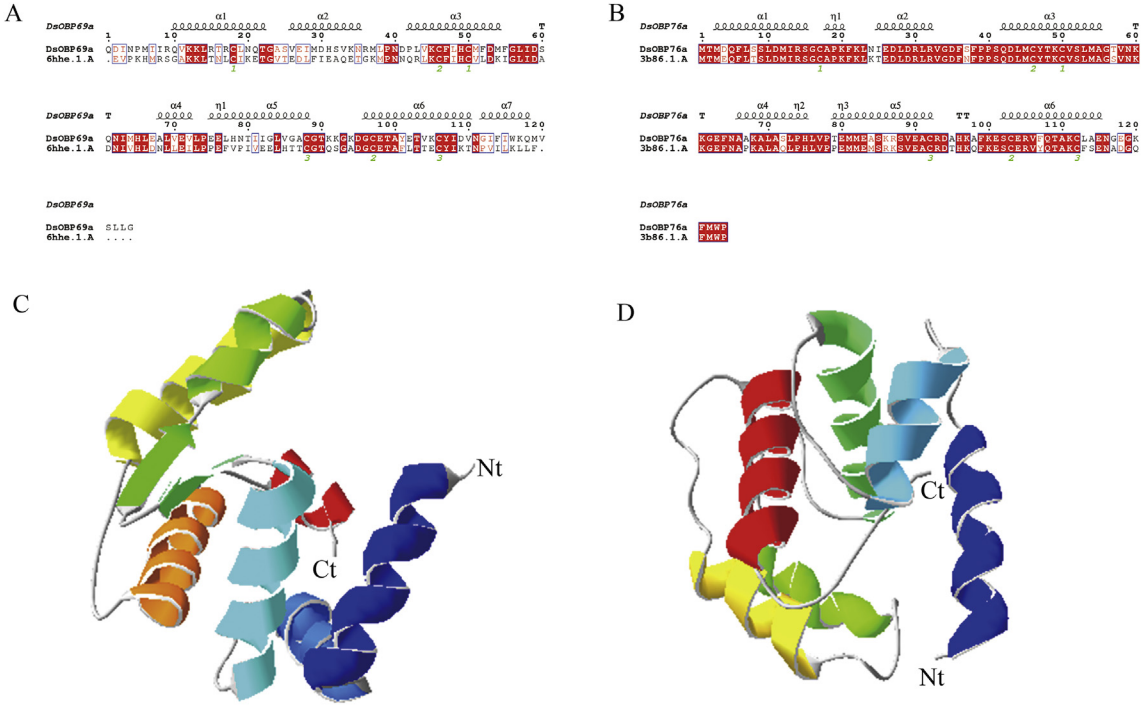

Figure 6. Secondary and 3D structures of DsOBP69a and DsOBP76a. (A) Sequence alignment of DsOBP69a with *Ceratitis capitata* OBP22 (ID: 6hhe.1.A). (B) Sequence alignment of DsOBP76a with *D. melanogaster* OBP76a (ID: 3b86.1.A). (C) The predicted 3D structure of DsOBP69a. (D) The predicted 3D structure of DsOBP76a.

selection of *Drosophila*. For example, OBP57d and OBP57e of *D. sechellia* affect the recognition of the plant-derived poisons caproic acid and caprylic acid produced by host *Morinda citrifolia*, which in turn affects host selection behavior (Matsuo et al., 2007). In this study, we identified OBP69a and OBP76a in *D. suzukii*, whose function may have changed compared to *D. melanogaster*.

RNA-seq analysis detected expression of *Obp69a* and *Obp76a* genes in *D. suzukii* third antennal segments (Crava et al., 2019). In *D. melanogaster*, the expression of OBP69a was significantly increased after females were exposed to males; whereas for isolated males, the expression of OBP69a were significantly higher than that in males when females were also in the population. In contrast, no significant changes were observed for OBP76a in the expression level. In addition, cVA treatment did not impact the expression levels of OBP69a in male and female (Bentzur et al., 2018). However, the atrophied ejaculation bulb of the *D. suzukii* male does not produce cVA (Dekker et al., 2015). Some *Drosophila* species do not produce but still detect cVA (Khallaf et al. 2020). Electrophysiological recording revealed that the *D. melanogaster* and *D. suzukii* females both can able to detect cVA by olfactory neurons in antennal trichoid sensilla (Khallaf et al., 2020). cVA bind to the DmOBP69a (Bentzur et al., 2018) and DmOBP76a (Billeter and Levine, 2015). The amino acid sequence of OBP69a and OBP76a between *D. melanogaster* and *D. suzukii* are highly consistent. They are theoretically orthologous genes and have similar functions. However, in this study, fluorescence competition binding assays showed that cVA had not affinity with the DsOBP69a and DsOBP76a. Their difference may be related to the protein structure, which is worthy of further study. Moreover, we speculate that there are other OBPs to promote cVA transport in the *D. suzukii*, which will be our future work.

(Z)-7-Tricosene among eight tested *Drosophila* pheromones was the only one detected by the DsOBP69a, although electrophysiological recording revealed that the *D. suzukii* females do not detect (Z)-7-Tricosene via single sensillum recordings (Khallaf et al., 2020). On the contrary, all tested pheromones were not affinity with the DsOBP76a. This suggests that there may be other unknown sex pheromones or other substances that regulate the expression of the DsOBP76a. So far, many *D. melanogaster* pheromones are still unknown. (Z)-7-Tricosene, the male-specific sex pheromone, was detected by the *D. melanogaster* (Khallaf et al., 2020). The currently known *Drosophila* sex pheromones are cVA produced by males and CHCs produced by oenocytes, but males still show strong courtship for females without oenocytes (Billeter and Levine, 2015), indicating that females produce sex pheromones other than CHCs, and further research is clearly needed.

β-ionone is a floral odor substance that attracts *D. melanogaster* and has a strong affinity with OBP28a (Gonzalez et al., 2019). Ab4 and pb2 sensilla are the most sensitive to β-ionone, and OBP28a mutants cause the responses of ab4 and pb2 sensors to β-ionone to be reduced, but they did not completely disappear, indicating that ab4 and pb2 sensors can also detect β-ionone by means other than OBP28a (Gonzalez et al., 2019). Similarly, in our experiments, β-ionone bind to DsOBP69a and DsOBP76a and have the highest affinity compared to all the ligands tested. Strawberry leaves have an attractive effect on *D. suzukii*, and strawberry leaves also volatilize β-ionone, but the effect of β-ionone on *D. suzukii* is still unclear (Keesey et al., 2015). Therefore, we guessed that *D. suzukii* detect β-ionone emitted by strawberry leaves through DsOBP69a and DsOBP76a, and find their host strawberries. However, behavioral research of DsOBP69a and DsOBP76a is needed for further verification, which is very important.

The DsOBP69a and DsOBP76a proteins not only play a crucial role in olfactory, but also play an important role in taste. This is consistent with the researches that OBP have an important role in non-olfactory, such as taste (Jeong et al., 2013; Rihani et al., 2019). In this study, berberine chloride and coumarin have a good affinity with the DsOBP69a and DsOBP76a proteins. These two bitter substances also have a high affinity with the DsOBP59h proteins (Li et al., 2019). However, other bitter substances, including denatonium benzoate and *N*-Phenylthiourea, were not affinity with the DsOBP59h, DsOBP69a and DsOBP76a proteins (Li et al., 2019). In insects, identifying bitter substances can help them avoid plant toxins and unfavorable spawning points. OBP49a can suppress the appetence for sweet-tasting compounds through the perception of bitter stimuli. The deletion of OBP49a reduced the inhibition of sucrose-induced action potential by bitter chemicals (Jeong et al., 2013). The reduction of expression of individual OBP genes induced either an increase or a decrease of sucrose intake in the presence of bitter compounds (Swarup et al., 2013). The roles of DsOBP69a and DsOBP76a in the taste of *D. suzukii* remain to be further studied. The *D. melanogaster* OBP19b protein, a taste-expressed OBP, not only is detected some bitter compounds, such as berberine, but also plays a crucial role in the detection of indispensable nutrients, such as L-phenylalanine (Rihani et al., 2019).

DsOBP69a has the highest matching degree with *C. capitata* OBP22 (ID: 6hhe.1.A), while CcapObp22 has a close genetic relationship with *D. melanogaster* OBP69a, with a sequence similarity of 37%. CcapObp22 can interact with male pheromone (E,E)-α-farnesene. Using CcapObp22 (ID: 6hhe.1.A) as a template, we constructed a 3D structural model of the DsOBP69a protein, which is composed of seven alpha helices like CcapObp22. After cVA binds to OBP76a in *D. melanogaster*, the conformation of F121 changes. The change of the F121 residue will affect the binding activity of OBP76a to cVA. In addition, the binding of OBP76a to cVA will lead to the fracture of the salt bridge between D118 and K87. Substituting residue D118 with alanine in *D. melanogaster* OBP76a can directly activate T1 neurons without cVA (Laughlin et al., 2008). The DsOBP76a protein sequence of *D. suzukii* was highly matched with *D. melanogaster* OBP76a. The GMQE value was 0.96 and the sequence similarity was 83.87%. The sequence of the DsOBP76a protein indicated that F121 was still conserved, but D118 and K87 became E and R, respectively. Therefore, the binding capacity of the protein may have changed. In our study, cVA did not bind to DsOBP76a. Whether the changes in residues result in cVA not binding to DsOBP76a remains to be proven.

In this study, the OBP69a and OBP76a proteins of *D. suzuki* were identified and expressed, and their affinities for *Drosophila* pheromones, plant volatiles, bitter agents and other substances were tested. The results indicated that they may act on both olfactory and taste systems. Between the fruit fly and the fruit industry, a deeper understanding of its chemosensory system will help to provide new ideas for the ecological control of these problematic insects.

Declarations

Author contribution statement

Fengqi Li: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Haixa Zhan, Du Li: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Youssef Dewer: Analyzed and interpreted the data; Wrote the paper.

Changying Niu: Conceived and designed the experiments.

Chen Luo: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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