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# An odorant receptor and glomerulus responding to farnesene in *Helicoverpa assulta* (Lepidoptera: Noctuidae)

Han Wu[a,b], Rui-Ting Li[a,c], Jun-Feng Dong[a,d], Nan-Ji Jiang[a,c], Ling-Qiao Huang[a], Chen-Zhu Wang[a,c,*]

[a] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
[b] Guangdong Engineering Research Center for Pesticide and Fertilizer, Guangdong Provincial Bioengineering Institute (Guangzhou Sugarcane Industry Research Institute), Guangzhou, China
[c] College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China
[d] Forestry College, Henan University of Science and Technology, Luoyang, China

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ABSTRACT

Terpenoids emitted from herbivore-damaged plants were found to play an important role in regulating tritrophic interactions. How herbivores and their natural enemies perceive terpenoids has not been thoroughly elucidated to date. Using *in vivo* calcium imaging, we found in this study that farnesene activates one glomerulus in the antennal lobe of female *Helicoverpa assulta*. The response induced by a mixture of farnesene isomers is stronger than that elicited by E-β-farnesene alone. In the *Xenopus* oocyte expression system, HassOR23/ORco is narrowly tuned to farnesene isomers and compounds with similar structures. Finally, the behavioral studies showed that the farnesene isomers have an inhibitory effect on oviposition of female *H. assulta*, but have an attractive effect on host searching of *Campoletis chlorideae,* the key endoparasitoid of *H. assulta* larvae. These results demonstrate that farnesene isomers are encoded by a labeled-line mode in the olfactory system of female *H. assulta*, suggesting that farnesene as a chemical signal from plants has important behavioral relevance and evolutionary implications in the tritrophic context.

## 1. Introduction

For insects, olfaction plays a vital role in finding mates, host plants, and oviposition sites, as well as avoiding predators. The olfactory system of insects can be subdivided into two subsystems: one is involved in detecting and processing information regarding most environmental odorants, and the other is dedicated to information regarding social signals such as sex pheromone (Christensen and Hildebrand, 2002; Galizia and Rössler, 2010). Extensive studies have been conducted to reveal the insect behavioral, physiological and molecular mechanisms of social chemical cues. However, what we know concerning olfactory perception of environmental odorants in insects is relatively limited.

Odorant molecules are mainly discriminated by odorant receptors (ORs) in the dendritic membrane of olfactory sensory neurons (OSNs) housed in olfactory sensilla on the antennae, from which odor information is relayed to the primary olfactory center - antennal lobe (AL) (Hansson and Anton, 2000; Hildebrand and Shepherd, 1997). In the AL, the OSNs expressing the same OR converge onto a single glomerulus (Gao et al., 2000; Vosshall et al., 2000). It is generally assumed that the main encoding strategy of odor quality is a combinatorial receptor code scheme, in which odorants are discriminated by different sets of ORs (Hallem and Carlson, 2006; Hallem et al., 2006; Kaupp, 2010). Most environmental odorants, such as plant volatiles, can activate multiple ORs as well as glomeruli and they are encoded by an across-fiber pattern (Bisch-Knaden et al., 2018; Haverkamp et al., 2018; Knaden et al., 2012). However, some odorants are coded by labeled-lines in the olfactory system of insects. For example, sex pheromone components usually activate the ORs on one type of OSNs and one glomerulus (Kurtovic et al., 2007; Wu et al., 2013). In the *Drosophila melanogaster*, certain odorants associated with oviposition sites also activate a single specialized OR and the activation of these type ORs can elicit innate behaviors (Dweck et al., 2013; Stensmyr et al., 2012).

In response to insect herbivory, plants usually release volatile organic compounds, which may attract the natural enemies of the herbivores and act as indirect defenses (Dicke and Baldwin, 2010). Terpenoids make up a large proportion of these herbivore-induced plant volatiles (HIPVs) and play an important role in regulating the

* Corresponding author. Institute of Zoology, Chinese Academy of Sciences, Beijing, China.
*E-mail address:* czwang@ioz.ac.cn (C.-Z. Wang).

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**Table 1**
The shared terpene volatiles in response to herbivore attack among different plant species.

| Plant | E-β-Ocimene | (E,E)-α-Farnesene | E-β-Farnesene | Linalool | Reference |
|---|---|---|---|---|---|
| Tobacco (Solanaceae) | ✓ | ✓ | ✓ | ✓ | De Moraes et al. (1998) |
| Corn (Gramineae) | ✓ | ✓ | ✓ | ✓ | Turlings et al. (1990) |
| Cotton (Malvaceae) | ✓ | ✓ | ✓ | ✓ | Paré and Tumlinson (1997) |
| Lima bean (Fabaceae) | ✓ | | ✓ | ✓ | Takabayashi et al. (1994) |
| Apple (Rosaceae) | ✓ | ✓ | | | Takabayashi et al. (1991) |
| Cucumber (Cucurbitaceae) | ✓ | ✓ | | | Takabayashi et al. (1991) |
| Potato (Solanaceae) | ✓ | ✓ | ✓ | ✓ | Bolter et al. (1997) |

oviposition of conspecific females and attracting the natural enemies of herbivores (De Moraes et al., 2001; Dicke and van Loon, 2000; Kessler and Baldwin, 2001). For example, linalool, E-β-ocimene, (E, E)-α-farnesene, and E-β-farnesene are commonly shared volatiles in damaged plants from different families (Table 1). However, the chemoreception and processing mechanism of insects to these terpenoids are still unclear.

*Helicoverpa assulta* is an oligophagous moth species that uses several Solanaceae species as its host plants, including tobacco and hot pepper. *Campoletis chlorideae* is a key larval endoparasitoid of *H. assulta* and its closely related species *Helicoverpa armigera*. In this study, we first find that farnesene isomers only activate a single glomerulus in the AL of female *H. assulta* by *in vivo* optical imaging. Next, we identify that HassOR23 is specially tuned to farnesene isomers by using *Xenopus* oocyte expression system and two-electrode voltage-clamp recording. Finally, we indicate that farnesene is an oviposition inhibitor to *H. assulta* and an attractant to *C. chlorideae*. Such a labeled-line system coding for farnesene iosmers in *H. assulta* seems to have important ecological and evolutionary significances, and may also be used to develop sustainable pest management methods.

## 2. Materials and methods

### 2.1. Insects

*H. assulta* and *H. armigera* were collected from tobacco field in Zhengzhou, Henan Province, China, and reared in the laboratory of Institute of Zoology, Chinese Academy of Sciences, Beijing. The larvae were fed with an artificial diet, mainly constituted by wheat germ, yeast and chilli for *H. assulta*, wheat germ, yeast and tomato paste for *H. armigera*. The colony of *C. chlorideae* was maintained on the second and third instar larvae of *H. armigera* and *H. assulta*. 10% and 30% honey solutions were used as diets for *Helicoverpa* adults and wasp adults, respectively. The successive generations were maintained in an environmental chamber at 26 ± 1 °C with a relative humidity 60–75% on a 16L: 8D photoperiod. Adult moths and wasps used in the physiological and behavioral experiments were 2–4 days old.

### 2.2. Chemicals

Odorants used in the experiments were purchased from sources as listed in Table S1. The purity of most compounds exceeded 97%. Some compounds are isomers mixtures for the pure compounds were unavailable. In addition to (E, E)-α-farnesene (49%), the farnesene isomers obtained from Sigma-Aldrich also contained E-β-farnesene (26%), Z-β-farnesene (18%), and (Z, E)-α-farnesene (7%) (Lu et al., 2012). In the calcium imaging and behavioral tests, a series of dilutions of the terpenoid in redistilled paraffin oil (Analytical grade, Fluka) were stored in 2 mL glass vials (Agilent, Technologies, USA) at −20 °C. In voltage-clamp recordings, the compounds tested were diluted with Ringer's buffer.

### 2.3. In vivo calcium imaging

Optical recording was performed as described previously (Wu et al., 2013, 2015). Briefly, female moths were restrained in plastic tubes and fixed with dental wax. After exposing the brain, a calcium-sensitive dye, CaGR-1-AM (Molecular Probes, Eugene, OR, USA) was bath applied to stain the antennal lobes. The dye was firstly dissolved in 20% Pluronic-127 in dimethyl sulfoxide and later diluted with Ringer solution to a final concentration of 30 μmol/L. The moth was placed in the dark for 1 h at 12 °C, and was thoroughly rinsed with Ringer solution. Imaging data were collected using a Till-Photonics imaging system (Till Photonics, Germany). Monochromatic light was 475 nm, dichroic: 500 nm, and emission LP, 515 nm. A sequence of 40 frames was acquired with a sampling rate of 4 Hz and exposure time was 200 ms. Stimulation was set at frame 12 and lasted for 500 ms. The final size of the image presentation was 320 × 240 pixels by binning 2 × 2 on chip. For the false color images, the relative calcium change of each frame was calculated as relative changes in fluorescence (ΔF/F) by MATLAB software. 100 μg (10 μL of 10 μg/μL solutions) of the terpene compounds were used as stimuli, applied in random order. Paraffin oil was used as the control.

### 2.4. Cloning of HassOR23 and qRT-PCR analysis

In the OR repertoire of *Spodoptera exigua*, SexiOR3 was found to be specially tuned to E-β-farnesene (Liu et al., 2014). We used *SexiOR3* as the subject sequence (GenBank accession number: JF747606), and ran Blastn to *H. assulta* antennal transcriptome in our laboratory. We found a candidate gene formerly named as *HassOR23* has the highest sequence identity to *SexiOR3*. Total RNA was respectively isolated from the antennae of 50 pairs of female and male adults using an RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. M-MLV Reverse Transcriptase (Promoga, Wisconsin, WI, USA) was used to synthesis cDNA. PCR was performed using gene specific primers with Kozak consensus sequence and a restriction enzyme cutting site based on the mRNA sequences of *HassOR23*. The primer sequences are listed in Table S2. The PCR program included initial denaturation 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s; and 72 °C for 8 min (Jiang et al., 2014).

qRT-PCR was performed to compare the relative expression level of *HassOR23* in female and male antennae of *H. assulta* adults. Specific primers were designed for qPCR analysis (Table S2). All reactions were performed in triplicate in a total volume of 20 μL containing 10 μL SYBR Premix Ex TaqII (TaKaRa, Otsu, Japan) and 0.4 mM of each primer under the following conditions: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 34 s, and 72 °C for 30 s, 1 cycle 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. Expression levels of *HassOR23* were calculated using the $2^{-\Delta\Delta Ct}$ method, with 18S gene transcript as an internal control for sample normalization. The experiment was repeated three times using three independent RNA samples.

### 2.5. Functional characterization of HassOR23

To identify candidate ligands of HassOR23, *HassOR23* and

*HassORco* were co-expressed in *Xenopus* oocytes, and the currents of the oocytes responding to odorants were recorded using a two-electrode voltage-clamp (Jiang et al., 2014). In brief, the open reading frame sequences of *HassOR23* and *HassORco* were separately cloned into pGEM-T easy vector (Promega, Madison, WI, USA), and subcloned into pCS2 + vector. The pCS2 + vectors were linearized using NotI (Takara, Dalian, China), and cRNAs were synthesized from the linearized pCS2 + vectors with mMESSAGE mMACHINE SP6 (Ambion, Austin, TX, USA). cRNAs were dissolved in RNase-free water and stored at −80 °C. Mature healthy oocytes were treated with 2 mg/mL of collagenase type I (Sigma-Aldrich) in $Ca^{2+}$-free saline solution (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.6) for 1–2 h at room temperature to get separated. Each oocyte was microinjected with 23.6 nL (50 ng) of *HassOR2*3 cRNA and 23.6 nL (50 ng) of *HassORco* cRNA. Injected oocytes were incubated for 3–5 days at 16 °C in the bath solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH = 7.5) supplemented with 100 mg/mL gentamycin and 550 mg/mL sodium pyruvate to express the proteins. Odorant-induced currents were recorded from the incubated *Xenopus* oocytes using a two-electrode voltage-clamp setup. Intracellular glass electrodes were filled with 3 M KCl and had resistances of 0.2–2.0 MΩ. Signals were amplified with an OC-725C amplifier (Warner Instruments, Hamden, CT, USA) at a holding potential of −80 mV, low-pass filtered at 50 Hz and digitized at 1 kHz. Data acquisition and analysis were carried out with Digidata 1322A and pCLAMP software (Axon Instruments Inc., Foster City, CA, USA). Dose-response data were analyzed using GraphPad Prism 6. For the panel of odorants, see Table S1.

### 2.6. Effect of farnesene isomers on the oviposition of female H. assulta

(E, E)-α-farnesene and E-β-farnesene usually co-occur and mix together in the HIPVs. Therefore, in the behavioral assay, we investigated the effect of farnesene on *H. assulta* and *C. chlorideae* by using the farnesene isomers rather than single E-β-farnesene. Oviposition preference of female *H. assulta* was performed in screened cages (1 m × 1 m × 1 m) as described in Sun et al. (2018). In each cage, four potted tobacco plants with 8–10 leaves were respectively placed at the four corners. In Group 1 (G1), two plants treated with farnesene isomers were put in one diagonal, and two plants with paraffin oil were put in the other diagonal and used as control. Farnesene isomers were dissolved in paraffin oil and then dropped on a filter paper, which was pinned on one leaf of the tobacco plant. The dosage of farnesene isomers used in the experiment was 100 μg. In Group 2 (G2), two tobacco plants treated with paraffin oil and two plants without any treatment as control were placed in each cage. Five mated female moths were released in each cage at dusk, and egg numbers on each plant were counted at 8:00 in the next morning. Six and five replications were run for G1 and G2, respectively. The oviposition index (OI) was calculated as (T − C)/(T + C). In G1, T is the number of eggs on tobacco with farnesene isomers, and C is the number of eggs on tobacco with paraffin oil. In G2, T is the number of eggs on tobacco with paraffin oil, and C is the number of eggs on tobacco without anything.

### 2.7. Effect of farnesene isomers on searching behaviors of C. chlorideae

A Y-tube olfactometer was used to investigate the behavior response of the endoparasitoid *C. chlorideae* towards farnesene isomers (Du et al., 1998). By using air pressure, a continued air through distilled water, activated charcoal and odor source flowed to two separated arms of the olfactometer. The speed of air through each arm was 900 mL/min, which was measured by a flow meter. One odorant source was farnesene isomers, and the other was paraffin oil. Mated female wasps were individually introduced into the olfactometer for 5 min. The observation ended when the wasp moved more than 5 cm into either arm. The behavior of 56 wasps who made a successful choice was recorded. The dosage used for farnesene isomers and paraffin oil was 100 μg.

### 2.8. Data analyses

In calcium imaging, data were acquired by the software Till-vision (Till photonics, Germany) and further analyzed by software ImageJ (NIH, USA) and custom-made programs in MATLAB (The Math Works, Inc). Imaging and electrophysiological data were analyzed with a one-way ANOVA for analysis of variance, and the least significant difference (LSD) test was used for means multiple comparisons ($P < 0.05$). In the oviposition test of *H. assulta*, the deviation of the OI against zero was tested by a *t*-test ($P < 0.01$). Behavioral data of *C. chlorideae* were subjected to a chi-square test of independence with Yates' continuity correction ($P < 0.01$).

## 3. Results

### 3.1. Farnesene isomers and E-β-farnesene activate the same single glomerulus

E-β-ocimene, linalool, (E, E)-α-farnesene and E-β-farnesene are common compounds in the HIPVs of tobacco (Table 1). The results of *in vivo* calcium imaging experiments indicated that linalool and E-β-ocimene activated multi-glomeruli, while farnesene isomers and E-β-farnesene activated a single glomerulus in the antennal lobe of female *H. assulta* (Fig. 1). Further analysis of superimposed images showed the glomerulus activated by farnesene isomers and the glomerulus by E-β-farnesene were the same (Fig. 1B). Although the AL atlas of the female *H. assulta* is available (Berg et al., 2002), it is difficult to identify the name of the glomerulus because it is in the middle of the AL without clear landmarks (Fig. 1). In addition, the front view angles of the AL had variances across different individuals, but the results are repeatable among different samples (Fig. S1).

The dose responses of farnesene isomers and E-β-farnesene showed that the activities induced became stronger with increasing dosages (Fig. 1C and D). The minimum concentration of farnesene isomers that evoked a significant response was 10 times lower than that of E-β-farnesene, indicating that the female *H. assulta* was more sensitive to farnesene isomers than to single E-β-farnesene (Fig. 1D).

### 3.2. HassOR23 is narrowly tuned to farnesene and its derivatives

Based on the published cDNA sequence of SexiOR3 tuned to E-β-farnesene in *S. exigua* (Liu et al., 2014), we assembled and obtained a full length of cDNA sequence of *HassOR23*, an ortholog of *SexiOR3* from *H. assulta* antennal transcriptome (GenBank accession number: MH252530). The nucleotide sequence was further verified by PCR cloning and sequencing. The ORF of *HassOR23* contains 1242 nucleotides encoding 413 amino acids. A BLASTX analysis showed that the amino acid sequence of HassOR23 had an 81% identity with that of SexiOR3. Further qRT-PCR showed that *HassOR23* was expressed in antennae of both adult sexes with a female bias (Fig. 2A).

To identify candidate ligands of HassOR23, the cRNAs of *HassOR23* and *ORco* in *H. assulta* were co-expressed in *Xenopus* oocytes, and responses to odorants were recorded using a two-electrode voltage-clamp (Jiang et al., 2014). In total, we tested 51 chemicals at concentration of $10^{-4}$ M, including host plant volatiles and sex pheromone related components of *H. assulta* (listed in Table S1). The results showed that the oocytes expressing *HassOR23/ORco* robustly responded to E-β-farnesene, farnesene isomers and farnesol, with much lower response to geraniol and citral (Fig. 2B and C). No responses were detected when we stimulated the oocytes with other compounds. We conclude that HassOR23 is a highly specialized odorant receptor that is narrowly tuned to farnesene and its structural analogs.

For female adults of *H. assulta* were more sensitive to farnesene isomers (containing about half of (E, E)-α-farnesene) than to single E-β-farnesene in calcium imaging, we also compared the sensitivity of the oocytes expressing HassOR23 to the two reagents of farnesene. The

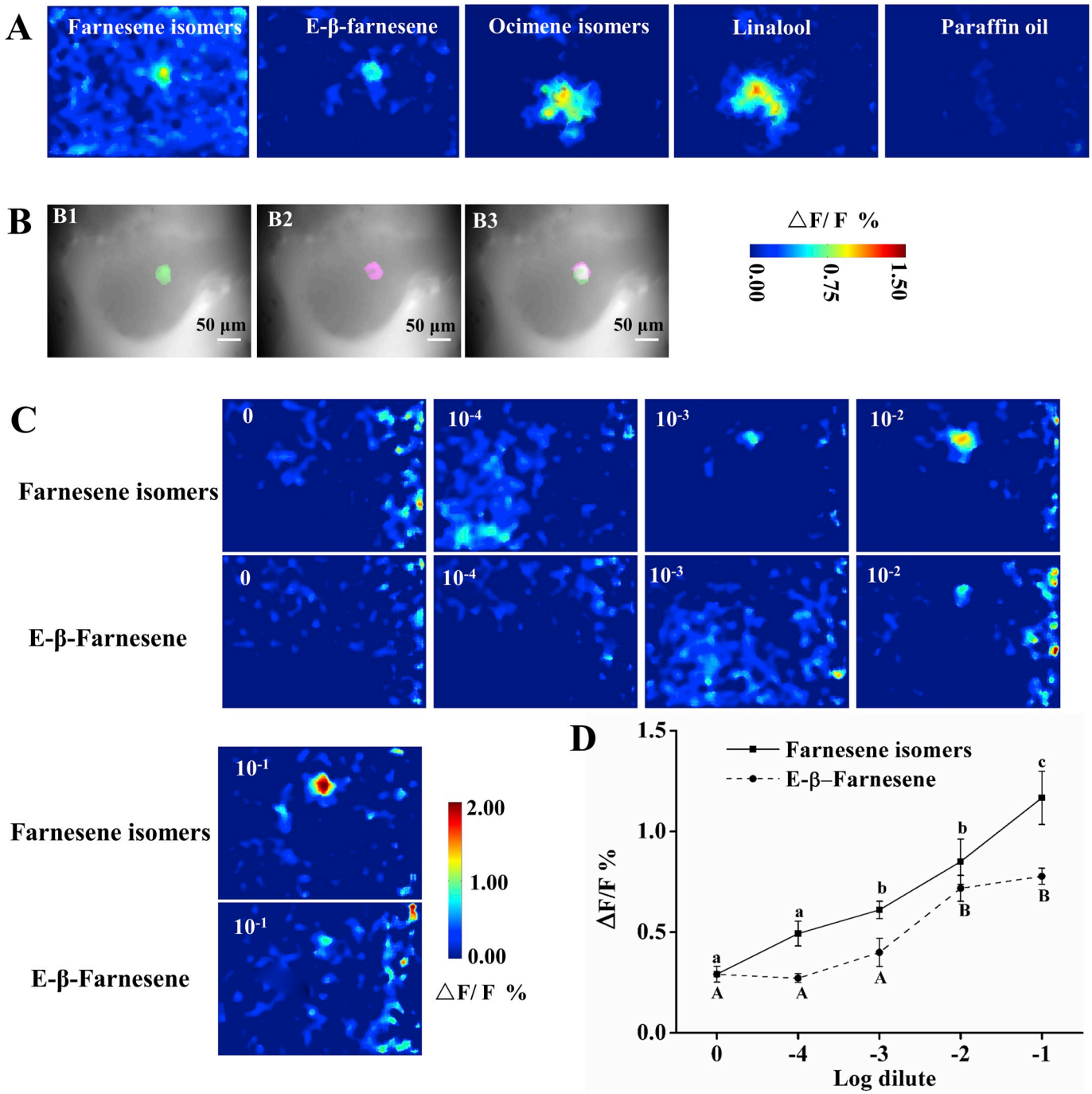


**Fig. 1.** Farnesene isomers activate a single glomerulus in the antennal lobe of female *Helicoverpa assulta*. (A) False color-coded images show the spatial representation of four terpene compounds (farnesene isomers, E-β-farnesene, ocimene isomers, linalool) in the antennal lobes (ALs) of female *H. assulta*. (B) Activated patterns of farnesene isomers and E-β-farnesene in the ALs of female *H. assulta*. B1 and B2 show the activated patterns (exceeding 50% of the maximum activity) of farnesene isomers (green) and E-β-farnesene (pink) superimposed on the gray-scale images of AL, respectively, and B3 is an overlap of B1 and B2 showing the relative positions of the activated glomeruli. (C) False color-coded images show the dose responses of farnesene isomers and E-β-farnesene. (D) Dose-response curve of farnesene isomers and E-β-farnesene. Error bars represent SEM (n = 7). Different letters indicate the significance (One-way ANOVA, $P < 0.05$).

farnesene isomers induced remarkable responses from the concentration of $3.3 \times 10^{-7}$ M (Fig. 2D and F). However, the fitted dose response curves showed that E-β-farnesene had a lower $EC_{50}$ than farnesene isomers (Fig. 2E and G).

### 3.3. Farnesene inhibits the oviposition of H. assulta female

Farnesene usually occurs in nature by the form of a mixture with other plant volatiles from host plants. Therefore, we investigated the effect of farnesene isomers on oviposition of *H. assulta* in the presence of host plant tobacco (Fig. 3A and B). The results of the behavioral experiment showed that the oviposition of female *H. assulta* was remarkably reduced when farnesene isomers were presented ($P = 0.001$, Fig. 3C), while the solvent paraffin oil had no effect on the oviposition of *H. assulta* ($P = 0.523$, Fig. 3C). We also compared the total numbers of eggs laid by female *H. assulta* on plants in the cages of G1 (presence of farnesene isomers) and G2 (absence of farnesene isomers) (Fig. 3D). The total number of eggs laid on the plants in G1 was significantly

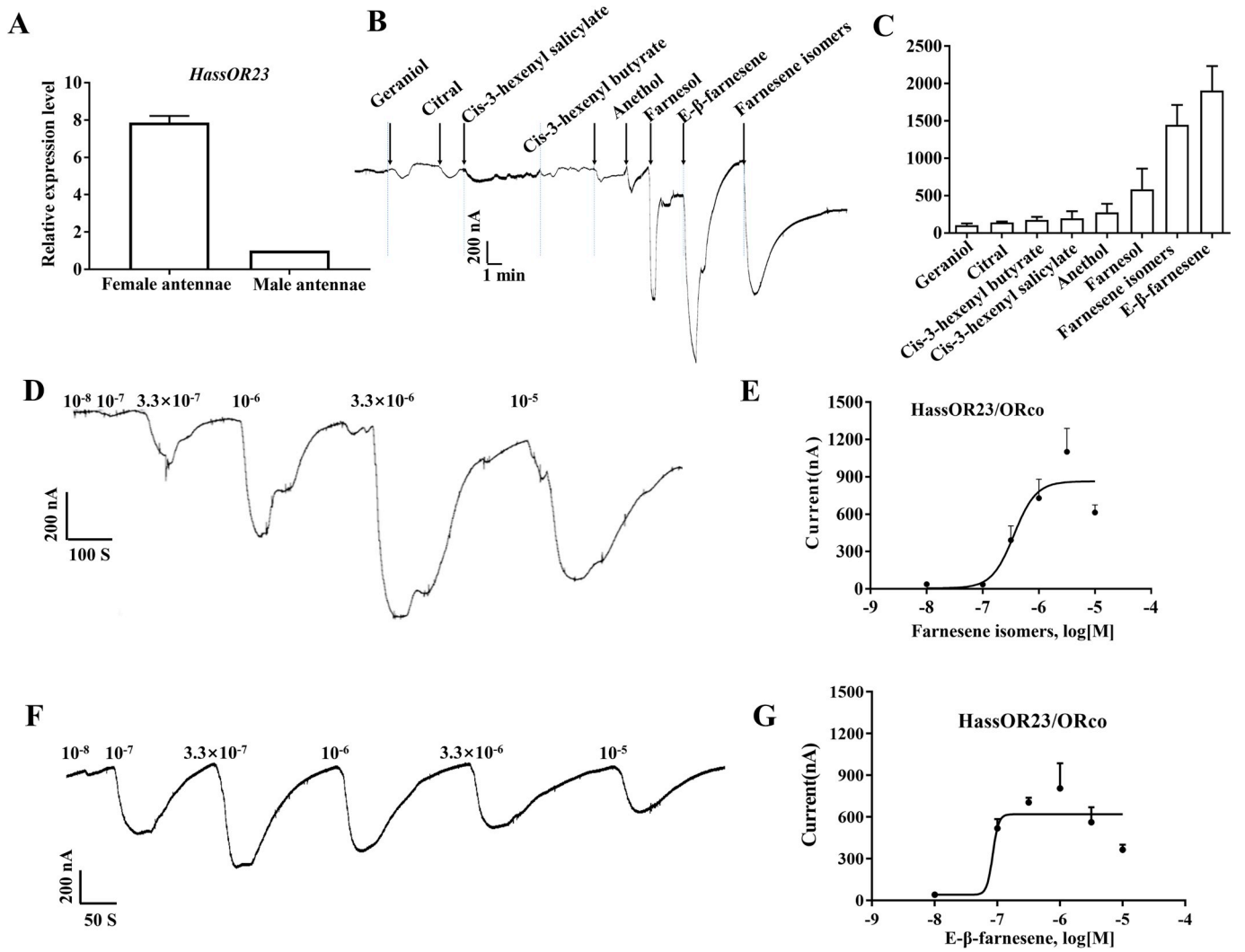


**Fig. 2.** HassOR23 is narrowly tuned to farnesene and related compounds with similar structure. (A) The expression level of *HassOR23* in female and male antennae relative to the housekeeper gene 18S (n = 3). (B–G) Two-electrode voltage-clamp recordings of *Xenopus* oocytes with co-expressed HassOR23/ORco in response to farnesene and other odorants: (B) The inward current responses to odorants; (C) The response profile (n = 3–6). (D) The inward current responses to a range of farnesene isomers concentrations; (E) The dose–response curve to farnesene isomers (n = 5), $EC_{50}$ = 3.611 × $10^{-7}$ M; (F) The inward current responses to a range of E-β-farnesene concentrations; (G) The dose–response curve to E-β-farnesene (n = 5), $EC_{50}$ = 8.456 × $10^{-8}$ M. Error bars represent SEM.

lower than that on the plants in G2 ($P$ = 0.009, Fig. 3D), indicating that farnesene isomers play an inhibitory role on *H. assulta* oviposition.

### 3.4. Farnesene attracts the parasitoid C. chlorideae

Knowing the HIPVs play an important role in regulating tritrophic interactions, we further address how farnesene isomers affect the searching behavior of *C. chlorideae,* the parasitoid of *H. assulta* (Fig. 4A). In the Y-tube olfactometer choice tests (Fig. 4B), $\chi^2$ test showed that the parasitoids were strongly attracted by the farnesene isomers ($P$ = 0, Fig. 4C).

## 4. Discussion

### 4.1. Labeled-line coding for farnesene isomers in female H. assulta

In moths, the ALs are used to process information about pheromones and plant odorants. In addition to the male specific macroglomerular complex (MGC) and large female glomeruli (LFGs), there are still many ordinary glomeruli in ALs of both sexes (Berg et al., 2002; Rospars and Hildebrand, 1992; Skiri et al., 2005). Optical imaging and electrophysiology combined with neurons staining have demonstrated that the MGC in males is used to process pheromone-related compounds and the LFG in females is involved in processing the oviposition-related compounds (Hansson et al., 2003; Reisenman et al., 2004, 2005; Stranden et al., 2003; Zhao and Berg, 2010). The ordinary glomeruli are mainly responsible for receiving input information about plant volatiles (Galizia et al., 2000; Masante-Roca et al., 2002; Skiri et al., 2004; Varela et al., 2011).

In this study, we find farnesene isomers at a series of concentrations activate a single ordinary glomerulus in the AL of female *H. assulta* (Fig. 1). It is generally assumed that plant volatiles are coded in a cross-fiber manner (i.e., a single odorant can simultaneously activate multiple glomeruli) in the AL of insects (Christensen and Hildebrand, 2002; Galizia and Rössler, 2010), which is different from that pheromone components are coded in a labeled-line system. However, the labeled-line coding manner is also found for a few non-pheromonal compounds in *Drosophila* species; for example, the volatile of harmful microbes –geosmin activates only a single class of sensory neurons that target the DA2 glomerulus in *D. melanogaster* (Stensmyr et al., 2012). It has been hypothesized that the narrowly tuned peripheral neurons project to narrowly tuned neurons in the glomeruli toward higher brain areas and

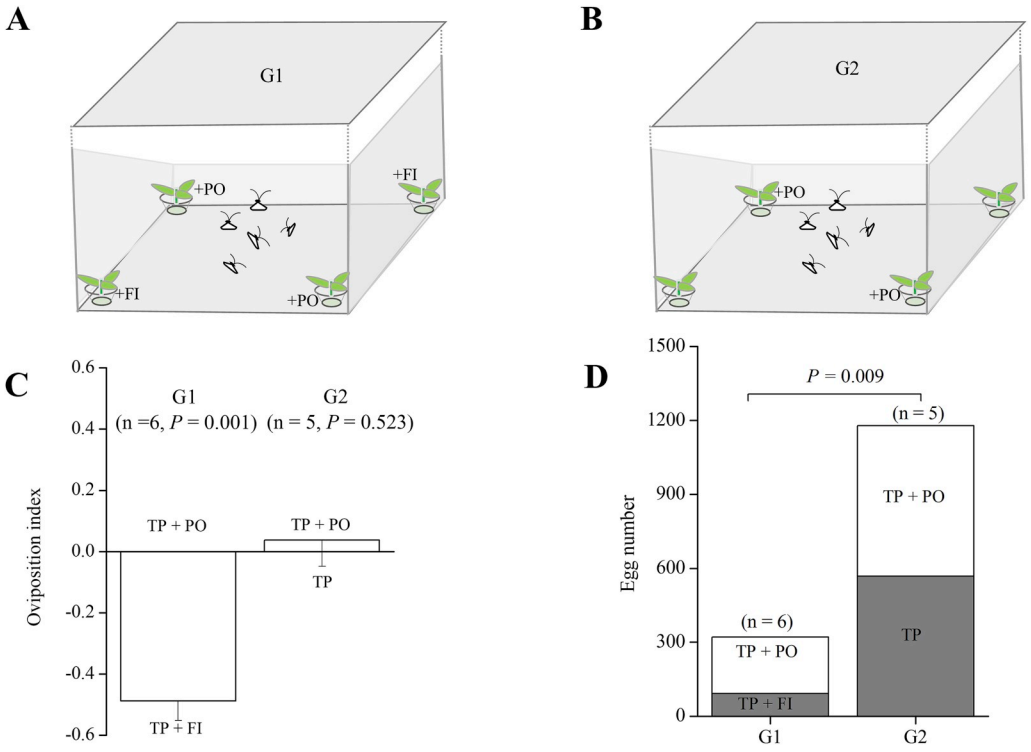


**Fig. 3.** Farnesene isomers have an inhibitory effect on oviposition of female *H. assulta* in tobacco plants (TP). (A) Schematic drawing of the oviposition assay. Group 1 (G1), the oviposition choice test between tobacco with paraffin oil (PO) and tobacco with farnesene isomers (FI). (B) Group 2 (G2), the oviposition choice test between tobacco only and tobacco with paraffin oil. (C) The oviposition index in each group. Deviation of the oviposition index against zero was tested with a *t*-test ($P < 0.01$). Error bars represent SEM. (D) The total number of eggs laid by female moths in G1 and G2. The mean egg number was tested with a *t*-test ($P < 0.01$). Error bars represent SEM.

that dedicated circuits can ensure a tight connection between stimuli and behavior (Schlief and Wilson, 2007).

HassOR23 is significantly different from pheromone receptors of *H. assulta* according to the phylogenetic analysis (Fig. S2), but narrowly tuned to farnesene isomers and some structurally similar compounds (Fig. 2). Based on the ligand types, ORs are broadly classified into general odorant receptors and pheromone receptors (Touhara and Vosshall, 2009). Pheromone receptors in moth species have a narrow tuned range (Jiang et al., 2014; Liu et al., 2013; Wang et al., 2011; Wanner et al., 2010), while general odorant receptors usually have a broader tuned range (Di et al., 2017; Yan et al., 2015). However, some previous studies and our results suggest that the general odorant receptors could have a narrowly tuned range as pheromone receptors (Jordan et al., 2009; Tanaka et al., 2009; Zhang et al., 2013). For example, BmOR56 in *Bombyx mori* and SexiOR3 in *S. exigua* were found to be dedicated to *cis*-jasmone and E-β-farnesene, respectively (Liu et al., 2014; Tanaka et al., 2009). Narrowly tuned receptors have been suggested to be specialist channels that carry information regarding odorants of high biological relevance (Tanaka et al., 2009; Wilson and Mainen, 2006).

The calcium imaging results in this study show that the responses of the farnesene isomers are stronger than that of single E-β-farnesene at the same dosage (Fig. 1C and D), implying that other active components may be present in the farnesene isomers. A previous study demonstrated that there was one type of odorant receptor neuron responding to both (E, E)-α-farnesene and E-β-farnesene by GC-SSR (Stranden et al., 2003), which suggests that (E, E)-α-farnesene could be another active component besides E-β-farnesene. In contrast, the oocytes expressing *HassOR23/ORco* showed a higher sensitivity to E-β-farnesene than to farnesene isomers in the voltage clamping experiment (Fig. 2E and G). One possible reason is that the *Xenopus* system is a heterologous expression system, and not the native cells in which the OR is normally expressed.

### 4.2. Behavioral implications of farnesene isomers

Farnesene isomers, especially (E, E)-α-farnesene and E-β-farnesene, are important and common sesquiterpenes in the emitted blends of a range of plant species under herbivore attack (Bolter et al., 1997; De Moraes et al., 1998, 2001; Kessler and Baldwin, 2001; Paré and Tumlinson, 1999; Turlings et al., 1990). HIPVs have an influence on the oviposition preference of conspecific adult females (Allmann et al., 2013; De Moraes et al., 2001; Hatano et al., 2015; Zakir et al., 2013). Field and laboratory experiments showed that the oviposition of *Heliothis virescens* female were inhibited by the HIPVs from tobacco, in which (E,E)-α-farnesene and E-β-farnesene are included (De Moraes

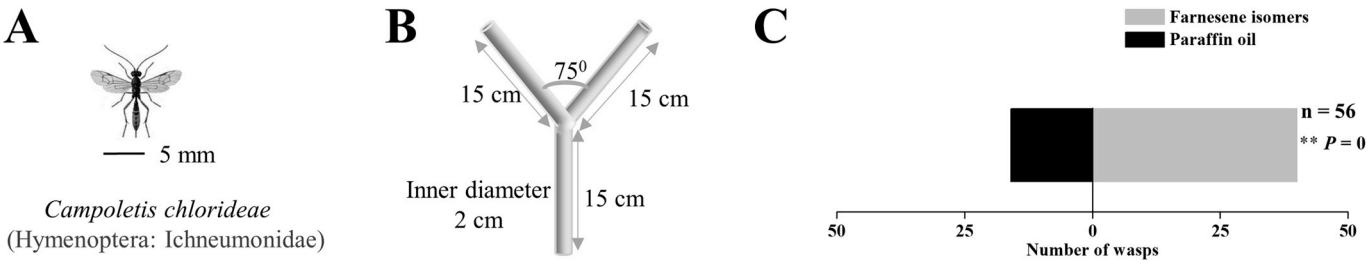


**Fig. 4.** Farnesene isomers have a remarkable attraction to mated females of *Campoletis chlorideae* . (A) Schematic drawing of *C. chlorideae* female, one of the key natural enemies for *H. assulta*. (B) Schematic drawing of the Y-tube olfactometer used in the experiment. (C) Preference of wasps between paraffin oil and farnesene isomers in Y-tube choice tests. Significant difference is indicated by asterisk ($\chi^2$ test; $P < 0.01$).

et al., 2001; Kessler and Baldwin, 2001). Our study shows that farnesene isomers significantly inhibit the oviposition of female *H. assulta* (Fig. 3 C, D). However, *Ostrinia nubilalis* female prefer to oviposit on the plants emitting farnesene and farnesol (Binder et al., 1995). *B. mori* larvae are also attracted to the mulberry HIPVs induced by conspecific feeding, in which α-farnesene makes up a large proportion (Mooney et al., 2009).

Farnesene isomers also have important significance to the third tropical level. Farnesene isomers are attractive for egg parasitoids and predatory insects (Francis et al., 2004; Mumm and Hilker, 2005; Verheggen et al., 2008). Single E-β-farnesene is attractive to the Braconidae parasitoids, *Aphidius uzbekistanicus* (Micha and Wyss, 1996), *Aphidius ervi* (Du et al., 1998) and *Microplitis mediator* (Zhang et al., 2011). Our study shows that farnesene isomers have a remarkable attraction to mated females of *C. chlorideae* (Fig. 4), suggesting that farnesene isomers would be an important volatile signal for host-seeking of this ichneumon parasitoid. The transgenic plants emitting high quantities of farnesene isomers would potentially have the function of recruiting natural enemies of herbivores as well as pest resistance (Schnee et al., 2006; Verma et al., 2015; Wang et al., 2015).

This study demonstrates that farnesene isomers are coded by a labeled line manner in the olfactory system of female *H. assulta*. The odorant receptor HassOR23 is selectively tuned and one glomerulus in ALs of female *H. assulta* is specifically responding to this group of chemicals. Sesquiterpenes have important significance in the context of tritrophic interactions. To explore the potential application, the push-pull strategy could be implemented by volatiles emitted from transgenic plants and could facilitate the ecological control and sustainable development of agriculture. In the future, we will also test whether farnesene isomers have a similar behavioral regulation and olfactory coding mechanism in other herbivores.

## Author contributions

Designed the experiments: HW and CZW; Performed the experiments: HW, RTL, JFD, NJJ and LQH; Wrote the manuscript: HW and CZW; Conceived the project: CZW.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2018.11.006.

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