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Olfactory proteins of *Endoclita signifer* larvae and their roles in host recognition

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Abstract

Background: *Endoclita signifer* causes severe damage to eucalyptus plantations, and the larvae transfer to and damage eucalyptus accurately in mixed forests, suggesting that the larval olfactory system contributes to host selection. The olfactory proteins in the head and tegument of *E. signifer* larvae were previously identified. To identify the relationship between olfactory protein expression in the larval head the larvae head and the developmental expression dynamics, and its functions in further recognition of plant volatiles, the head transcriptomes of two instar larvae and the expression profiles of olfactory proteins in the instars after exposure to volatiles were studied.

Results: Eight odorant-binding proteins, six chemosensory proteins, three odorant receptors, three gustatory receptors, and 18 ionotropic receptors were identified. Half of the olfactory proteins were the most highly expressed in the young (5th) larval head, and EsigGOBP2, EsigGOBP4, EsigGOBP5, EsigCSP1, EsigCSP3, EsigGR1 and EsigGR3 were highly expressed and showed a specific expression pattern. In addition, after exposure to o-cymene, α-phellandrene, n-butyl ether, and 4-ethylacetophenone, EsigGR3 was downregulated significantly, and exposure to n-butyl ether caused EsigGR1 to be downregulated significantly.

Conclusions: Seven specific olfactory proteins may be important genes in larval olfactory recognition. Furthermore, based on the receptors that were downregulated after exposure to volatiles and the previous electrophysiological activity in the third larvae, we speculated that the ligand of EsigGR1 was n-butyl ether, and the ligands of the newly identified EsigGR3 are all electrophysiologically active compounds, which demonstrated host recognition in the third larvae of *E. signifer*. These results provide a way to find key plant volatiles recognized by the key olfactory proteins as new targets for pest control.

Keywords: Transcriptome, Plant volatile, Larvae, Expression

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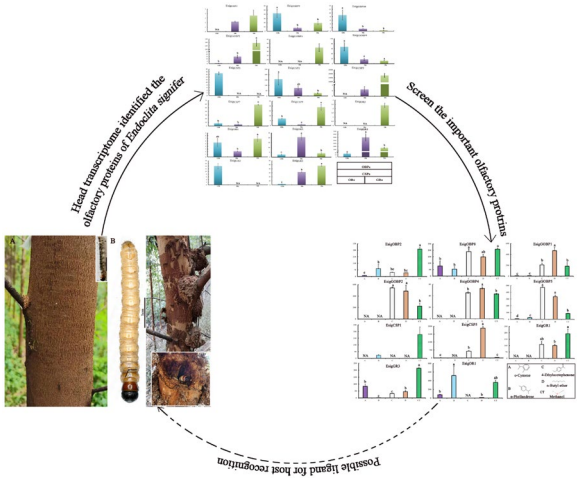
Graphical Abstract



Background

The ghost moth *Endoclita signifer* Walker (Lepidoptera, Hepialidae) is the primary wood-boring pest of eucalyptus, which was first paid attention to in China in 2007 and has caused great economic losses and ecological impacts in southern China, especially in Guangxi and Guangdong [1]. *E. signifer* is widely distributed in Japan and Korea in eastern Asia and from central, southern and southwestern China to India, Thailand and Myanmar in southern Asia [2]. In China, *E. signifer* is a native pest, and its host plants include 30 families, 40 genera and 51 species [3]. Before eucalyptus was planted in large areas in Guangxi and Guangdong, *E. signifer* feeding on trees was not damaging large areas of forest and no one treated it as pest. After eucalyptus plantations were established almost everywhere in Guangxi, a large area of damage to *E. signifer* was found in 2007. Currently, all of the plantations in Guangxi, except for 17.1% of counties, are infested [3].

In Guangxi, the *E. signifer* occurs in one generation a year, rarely two in a year. The adults (Fig. 1A) emerge during the middle of March to April, followed by mating and oviposition. The larvae hatch in one month and then live in the soil. Interestingly, the larvae move from the soil to a standing tree after the third instar from July to August (Fig. 1B). The larvae feed on bark, bore into the interior of the wood, and weave packages with wood bits and silk to cover the entrance to the wormholes, constructing homes (Fig. 1C–E). The larvae live in their homes from July or August to January of the following year, and pupariation occurs in February.

Female oviposition is dispersed; however, the larvae can specifically damage eight species of eucalyptus in mixed forests accurately, so we hypothesized larval olfactory cues contribute to host selection. *E. signifer* is a native polyphagous insect pest, but it universally and severely damaged eucalyptus after it was planted in southern China in 2007, which is a typical example of native insect adaptation to exotic hosts [4]. Olfactory proteins in the head and tegument of the *E. signifer* larval transcriptome were previously identified, and 39 olfactory proteins were found to be expressed in the head, with EsigGR1 and EsigCSP3 as the key olfactory proteins [5], establishing a basis for studying the dynamic changes in *E. signifer* olfactory proteins and their relationship with larval host selection.

The study of olfaction in insects has focused mainly on adults, especially on sex-linked behaviors such as sex pheromone detection and host and predator volatile selection [6–8]. For example, the first transcriptome for identifying olfactory proteins of *Helicoverpa assulta* included 131 putative chemosensory genes comprising 64 odorant receptors (ORs), 19 ionotropic receptors (IRs), 29 odorant-binding proteins (OBPs), 17 chemosensory proteins (CSPs), and two sensory neuron membrane proteins (SNMPs) in male and female antennal transcriptome analysis [9]. Whole transcriptome PacBio sequencing identified 27 OBPs, 24 CSPs, four pheromone-binding proteins (PBPs), 68 ORs, and two SNMPs expressed in the antennae of male and female *H. assulta* [10].

In addition, the functions of many olfactory proteins in insects have been explored using qRT-PCR, prokaryotic expression, immunofluorescence localization, fluorescence competitive binding, molecular docking, a *Xenopus* oocyte expression system, single-sensillum

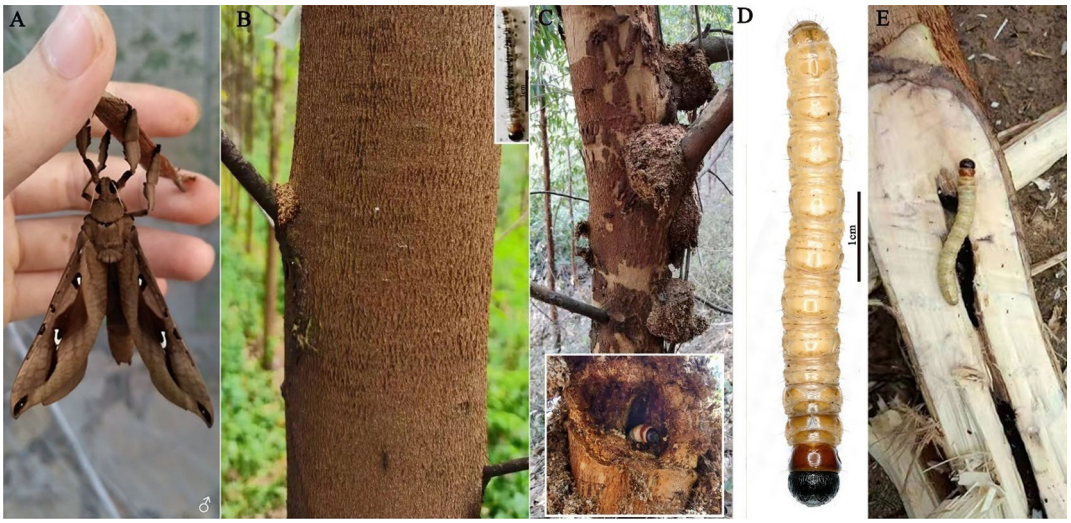


Fig. 1 Morphological characteristics of damage, adult and larva of *Endoclita signifer*. **A** Characteristics of male adults; **B** the 3rd larval package, white frame is the 3rd larvae; **C** damage characteristics of 12th larval package, white frame is entrance of 12th larval wormhole; **D** dorsal view of 12th larvae; **E** wormhole

recording, and behavioral studies. Fluorescence binding assays indicated that three *H. assulta* PBPs show selectivity for linear alcohols and aldehydes of different lengths, and PBP1 and PBP2 have optimal affinities to ligands containing 13–15 and 12–14 carbon atoms, respectively [11]. OBP10 might be a carrier of oviposition deterrents, favoring the spread of *H. assulta* eggs [12]. For olfactory receptors, nonanal is the main ligand of OR67, as demonstrated with an in vitro *Xenopus* oocyte expression system and single-sensillum recording [13]. HassOR40/ORco is expressed in the B neurons of short trichodea sensilla, and the active tobacco volatile ligand nerolidol attracts both sexes in adult *H. assulta* [14]. HassOR23/ORco is narrowly tuned to farnesene isomers in the *Xenopus* oocyte expression system; farnesene inhibits *H. assulta* and attracts its endoparasitoid [15]. HassOR31 has much higher expression in the ovipositor than in the antennae or other tissues, and the *Xenopus* oocyte model system, electrophysiological responses, and oviposition preference experiments suggest that HassOR31 expression in the *H. assulta* ovipositor helps females to determine precise egg-laying sites in host plants [16]. Overall, olfactory proteins, including odorant-binding proteins and odorant receptors, interact with plant volatiles.

Transcriptome analyses of larvae have focused on pesticides or ecological adaptability, exploring the molecular evidence and differences based on physiological and biochemical reactions, such as the exposure of *Spodoptera exigua* to Cry1Ca protein [17] and *Apis mellifera* to carbendazim [18] and the desiccation tolerance of *Polypedilum vanderplanki* [19]. Regarding larval olfaction, six novel OBPs and CSPs were identified in the transcriptomes of *H. assulta* larval antennae and mouthparts, respectively, and four novel OBPs and seven novel CSPs were identified in the same transcriptomes of *Helicoverpa armigera* [20]. Tissue-specific profiles of *H. armigera* showed that six OBPs and four CSPs were specific to larval tissue, while 15 OBPs and 13 CSPs were expressed in both larvae and adults, and the remainder were adult-specific [20]. SexiOBP13 was highly expressed in the larval head but not in other larval parts and was not detected in any adult tissue; SexiOBP13 showed high binding affinity to the sex pheromone component of S. *exigua* Z9, E12–14: OAc. This is supported by behavioral tests, indicating that SexiOBP13 plays a role in female sex pheromone reception in S. *exigua* larvae [21]. Immunohistochemistry demonstrated that anti-MscuOBP8 binds specifically to MscuOBP8 and showed that MscuOBP8 is expressed in *Melipona scutellaris* larvae in the mandibular region, supporting the hypothesis of olfactory function in immature stages [22]. Single-sensillum recordings revealed that larval antennal sensilla of the moth *Heliothis virescens* respond to specific sex pheromone components; the pheromone receptors HR6 and HR13, SNMP1, and pheromone-binding protein 1 (PBP1) and PBP2 were expressed in larval antennae sensilla or cells, indicating the responsiveness of larval sensilla to female-emitted sex pheromones [23]. All of

the above indicate that olfactory proteins can be identified in larval tissues and that they function to detect plant volatiles or sex pheromones.

Based on larval olfactory protein reactions with plant volatiles, their specific lifestyle and the previously identified olfactory proteins [5] of *E. signifer* larvae, this study examined the transcriptomes of the heads of different instar *E. signifer* larvae and determined the expression profiles of *E. signifer* larvae olfactory proteins during larval development. In addition, we identified olfactory protein functions in host selection in young larvae. The larval stage is the longest period in insects, and its olfactory system is simple. Exploring the olfactory proteins in larvae, especially the olfactory proteins that recognize plant volatiles, can provide new insight into pest control.

Materials and methods

Insect and tissue collection

Eighteen larvae of the 5th and 12th instars and nine larvae of the ninth instar of *E. signifer* were collected from a damaged eucalyptus plantation by cutting trees from December 2019 to January 2020 and September to November 2020 at the Gaofeng forest station (N22.907°, E108.266°), Guangxi, China. Larval samples were collected and stored at − 80 °C.

cDNA library construction and Illumina sequencing

Total head RNAs of nine of the 5th- and 12th-instar larvae were extracted using TRIzol reagent (Ambion) and the RNeasy Plus Mini Kit (No. 74134; Qiagen, Hilden, Germany), and the quantity was detected by a NanoDrop 8000 (Thermo Fisher Scientific, Waltham, MA, USA). Three RNA samples from the fifth larvae heads and three RNA samples from the 12th larvae heads were used to construct one cDNA library of the 5th and 12th instar heads, respectively. cDNA library construction and Illumina sequencing of the samples were performed at MajorBio Corporation (Shanghai, China). All cDNA library preparation methods, such as mRNA sample purification, fragmentation, synthesis of first-strand cDNA, end repair, and PCR amplification, were performed according to Zhang [5]. The cDNA library was sequenced on the HiSeq2500 platform.

Assembly, functional annotation and chemosensory gene identification

All raw reads acquisition and clean read assembly were performed according to Zhang [5]. The clean reads were used in TransRate (http://hibberdlab.com/transrate/) and CD-HIT (http://weizhongli-lab.org/cd-hit/) to evaluate the sequences and remove redundant and similar sequences. Then, BUSCO (Benchmarking Universal Single-Copy Orthologs, http://busco.ezlab.org) was used to assess the assembly integrity of the transcriptome by single-copy direct homologous genes. The annotation of unigenes was performed using NCBI BLASTx searches in the Nr protein database, with an E-value threshold of $1e^{-5}$. GO annotation was performed by the Blast2GO pipeline. The longest ORF for each unigene was determined by the NCBI ORF Finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Expression levels were expressed in terms of FPKM values (fragments per kilobase per million reads) [24], which were calculated by RSEM (RNA-Seq by Expectation–Maximization) (Version: 1.3.1) with default parameters [25]. Based on the EPKM results, we used DESeq2 (version: 1.38.0, threshold value $\left|\text{Log2FC}\right| \geq 1$ & padjust < 0.05) to analyze the genetic variations between groups and to identify differentially expressed genes. Chemosensory gene (OBP, CSP, OR, GR, IR, and SNMP) identification was performed using BLASTx and manually checked by tBLASTn as described in Zhang [5]. The nucleic acid sequences encoded by all chemosensory genes that were identified from the *E. signifer* larval head transcriptomes are listed in Additional file 1.

Sequence and phylogenetic analysis

Amino acid sequence alignment was performed using the Muscle method implemented in the Mega v6.0 software package [26]. The phylogenetic tree was constructed using the neighbor-joining (NJ) method [27] with a P-distance model and a pairwise deletion of gaps performed in the Mega v6.0 software package. The reliability of the tree structure and node support was evaluated by bootstrap analysis with 1000 replicates. The phylogenetic trees were colored and arranged in FigTree (Version 1.4.2). Considering that *E. signifer* is a primitive Lepidoptera moth, the phylogenetic analyses of the OBPs were based on Lepidoptera PBPs and OBPs of Coleoptera *Dastarcus helophoroides* [28], Diptera *Chrysomya megacephala* [29], Lepidoptera *Plutella xylostella* [30], *S. exigua* [31, 32], *H. armigera* [20], and *E. signifer*. The gene names and GenBank numbers of *P. xylostella*, *H. armigera* and Lepidoptera PBPs are listed in Additional file 2, and the other gene sequences are listed in the reference articles.

Expression analysis of different instars and volatile exposure

The total RNA of nine larval heads of the 5th, 9th and 12th stage was extracted following the methods described above for expression analysis of different instars. Four volatiles with gas chromatography–mass spectrometry (GC–MS) and gas chromatography-electroantennographic detection (GC-EAD) active substances were selected for exposure of the third larvae as described

Table 1 Number and length of unigenes

Quality index	The 12th larval head			The 5th larval head		
	Duplication 1	Duplication 2	Duplication 3	Duplication 1	Duplication 2	Duplication 3
Raw reads	50, 635, 098	45,975,844	49,019,202	50,644,410	50,847,054	54,178,160
Clean reads	49,654,820	45,050,796	48,011,990	50,008,250	50,046,390	53,312,984
Q20 (%)	98.17	98.17	98.19	97.83	97.92	97.84
Q30 (%)	94.6	94.61	94.67	93.65	93.95	93.77
GC content (%)	52.24	51.96	52.21	45.74	46.24	46.32
Total transcripts number	62,499					
Total unigenes number	48,699					
Largest unigenes length (bp)	63,226					
Average unigenes length (bp)	915					
N50 of unigenes	1666					
BUSCO of unigenes	C:94.00% [S:91.60%; D: 2.40%]					

by [32]. Thirty-six 3rd *E. signifer* larvae were placed in a 50 mL jar covered with silver paper, and a glass pipe containing a piece of Whatman filter paper soaked with 50 µL of the odorant diluted to 10 g/L in methanol was added to the jar. Controls with nine 3rd *E. signifer* larvae were exposed to methanol only. All larval heads were dissected after 24 h of exposure, and RNA was extracted as described above. Three independent replicates for each treatment (nine larvae) were carried out.

NanoDrop2008 and agarose gel electrophoresis were used to examine the density and quality of the RNA. cDNA was synthesized with the TransScript One-Step gDNA Removal and Synthesis Super Mix (No. O10306; Trans, Beijing, China). Primers of the newly identified genes were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) (Additional file 3), and the previously designed gene primers and those for the reference genes were the same as those used by Zhang [5]. PCR analysis was conducted using a Roche LIGHT CYCLE 480II (USA). Genious 2X SYBR Green Fast qPCR Mix (No ROX) (No. RK21205; ABclonal, Wuhan, China) was used for the PCR under a three-step amplification. Each PCR was conducted in a 20 µL reaction mixture containing 10 µL of Genious 2X SYBR Green Fast qPCR Mix (No ROX), 0.8 µL of each primer (10 mM), 2 µL of sample cDNA (2.5 ng of RNA), and 7.2 µL of dH2O (sterile distilled water). The qRT-PCR cycling parameters were as follows: 95 °C for 180 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, and 65 °C to 95 °C in increments of 0.5 °C for 5 s to generate the melting curves. Each qRT-PCR for each instar and exposure was performed in three biological replicates and three technical replicates. Negative controls without either template were included in each experiment. Roche LIGHT CYCLE 480II was used to normalize the expression based on ΔΔCq values, with EsigCSP9 in ninth larval heads and EsigGR3 in.alpha.-phellandrene as control samples, and the $2^{-\Delta\Delta Ct}$ method was used [33]. Before comparative analyses, we examined the normal distribution and equal variances test, and all logarithm data followed a normal distribution with equal variances. The comparative analyses for every gene among the three stages were assessed by a one-way nested analysis of variance (ANOVA), followed by Tukey's honestly significance difference (HSD) tests implemented in SPSS Statistics 18.0. Values are presented as the means ± SE.

Results

Transcriptome sequencing and sequence assembly

In total, on average, we generated 50 million raw reads from each cDNA library of the *E. signifer* larvae. The average percentages of reads with q20 and q30quality scores were 98.02% and 94.21%, respectively. After trimming the adapters, removing low-quality raw sequences using Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic), and blending the head sequences, followed by splicing and assembly, we obtained 62,499 transcripts, with an N50 of 1666 bp, average length of 915 bp, and maximal length of 63,226 bp (Table 1; Fig. 2A). BUSCO analysis showed that the completion

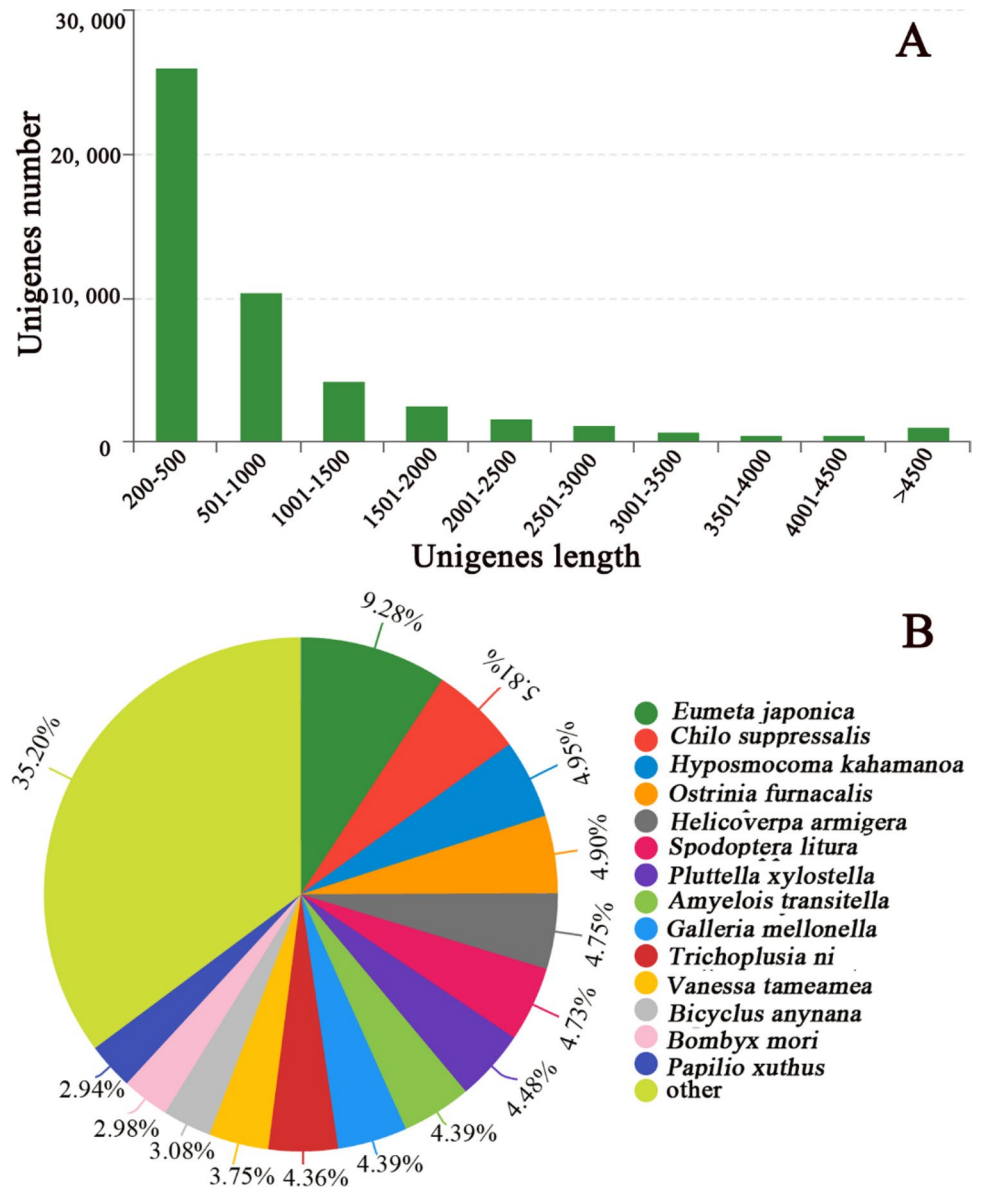


Fig. 2 Length distribution of unigenes and BLASTx unigenes with other species in the *E. signifer* transcriptome. **A** Length distribution of unigenes; **B** BLASTx analysis of identified unigenes with known homologs from other species

rate was 94.00%, the single copy rate was 91.60%, and the duplicate rate was 2.40% (Table 1).

Homology analysis and Gene Ontology annotation

For 23.00% of the transcripts, we obtained matches to entries in the Nr protein database by BLASTx with an E-value cutoff value of $1e^{-5}$. We observed the most sequence matches to *Eumeta japonica* (9.28%), followed by *Chilo suppressalis* (5.81%), *Hyposmocoma kahamanoa* (4.95%) and so on (Fig. 2B). We used Gene Ontology (GO) annotations to classify the 11,566 transcripts into functional groups using BLAST2GO, which had a P value calculated by a hypergeometric distribution test, and the E-value was less than 1×10^{-5}. In the *E. signifer* transcriptome, molecular functions accounted for most of the GO annotations (37.60%), followed by cellular component (33.37%) and biology process (28.94%). In the molecular function category, the terms binding, catalytic activity, and transporter activity were the most highly represented. In the biological process category, the terms cellular process, metabolic process, and biological regulation were the most frequent. Membrane part, cell part, and organelle were the most abundant cellular component terms (Fig. 3A). In addition, 48,699 unigenes were

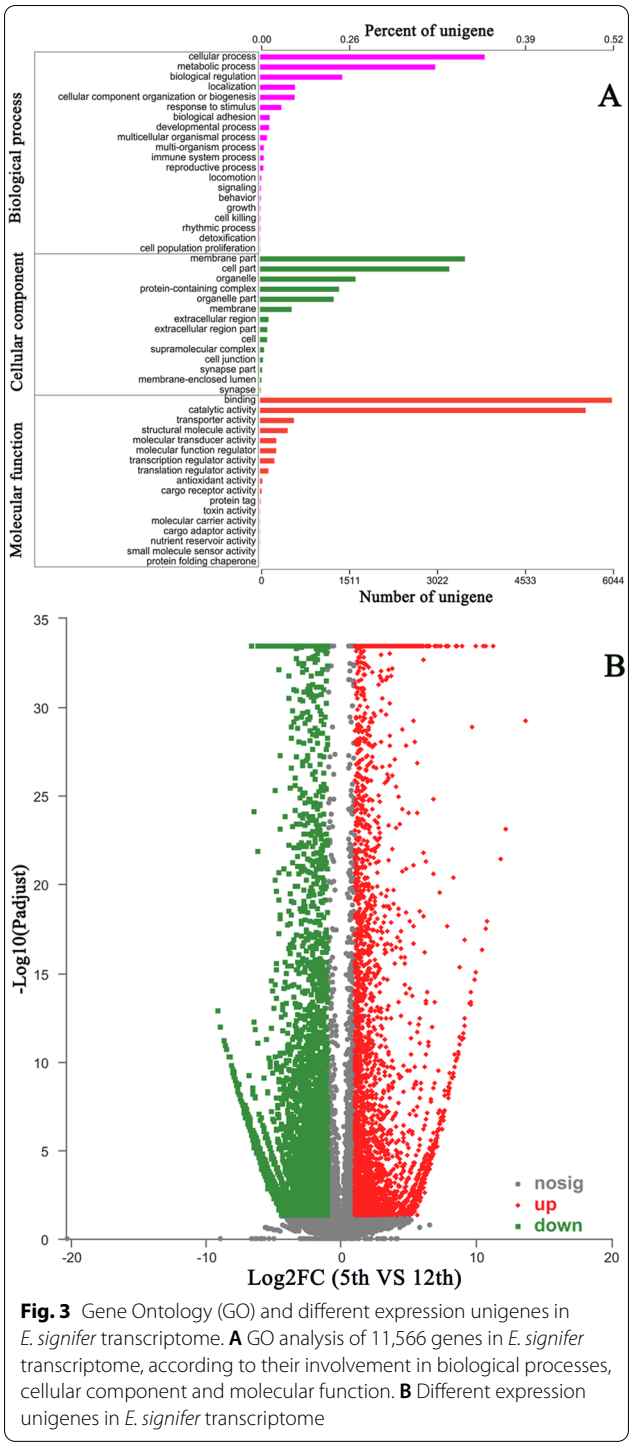


Fig. 3 Gene Ontology (GO) and different expression unigenes in *E. signifer* transcriptome. **A** GO analysis of 11,566 genes in *E. signifer* transcriptome, according to their involvement in biological processes, cellular component and molecular function. **B** Different expression unigenes in *E. signifer* transcriptome

assembled in the *E. signifer* larvae head transcriptome, of which 10,338 were differentially expressed (Fig. 3B).

Olfactory proteins

We identified eight transcripts encoding putative OBPs in *E. signifer,* of which three were general odorant-binding proteins (GOBPs) and five were identified in the head, thorax and abdomen cuticula transcriptome (Table 2 labeled with underline) [5]. In addition, EsigOBP9 and EsigOBP11 were more highly expressed in the 5th head, and the opposite was true according to the FPKM of unigenes (Table 2). We identified six transcripts encoding putative chemosensory proteins CSPs, three of which were previously identified (labeled with underline), and all were more highly expressed in the 12th head (Table 2). Three ORs were identified, and EsigOR5 was more highly expressed in the 5th head (Table 2). We identified three transcripts encoding putative gustatory receptor GRs, among which EsigGR1 was identified previously and was more highly expressed in the 12th head, while the others were more highly expressed in the 5th head (Table 2). We identified 18 ionotropic receptors IRs, among which EsigIR1, EsigIR93a-1, EsigIR11, EsigIR75p-6, EsigIR93a-4, EsigIR93a-5, and EsigIR12 were more highly expressed in the 12th head, while the others were the reverse. EsigIR1, EsigIR40a-1, EsigIR8,3 EsigIR93a-1, EsigIR25a and EsigIR6 were identified previously (labeled with underline) (Additional file 4).

Phylogenetic analysis of OBPs and CSPs

In the phylogenetic tree of OBPs (Fig. 4), the PBP and GOBP clades labeled with red included EsigGOBP1, EsigGOBP7, EsigOBP10, PxylGOBP1, PxylGOBP2, HarmGOBP2 and all Lepidoptera PBPs. The PBP clade with a 100% support rate is labeled with a yellow circle, and the GOBP clade with a 100% support rate is labeled with a red circle. Interestingly, the support rate between the PBP clade and GOBP clade was 95%.

Expression of olfactory proteins (except IRs) among the three instars

We characterized the expression profiles of the identified olfactory proteins in the transcriptomes of *E. signifer* 5th-, 9th-, and 12th-instar larval heads. Except for EsigOBP4, EsigOBP11, and EsigCSP8, all of the OBPs, CSPs, ORs, and GRs were expressed in at least one head (Fig. 5). Two, EsigCSP1 and EsigGR2, were not expressed in the 5th larval heads; EsigGOBP4, EsigCSP1, EsigOR3 and EsigGR2 were not expressed in the 9th larval heads; and EsigOBP3, EsigGOBP4, EsigCSP3, and EsigOR3 were not expressed in the 12th larval heads (Fig. 5). However, EsigGOBP2, EsigGOBP5, EsigCSP3, and EsigGR1 were expressed the highest among all of the olfactory proteins (Fig. 5). Among all of the olfactory proteins, nine (52.94%) were expressed the most in the 5th larval heads, among which EsigGOBP2, EsigCSP7, EsigCSP9, and EsigGR3 had significantly different expression patterns ($p < 0.05$);

Table 2 Best blastx hits for putative chemosensory proteins of *Endoclita signifer*

Name	Nr description	Species	Acc. NO	12th FPKM	5th FPKM	12th VS 5th
EsigOBP3	Odorant binding protein LOC100307012 precursor	*Bombyx mori*	NP_001159621.1	28.07	11.08	Up
EsigOBP4	Odorant binding protein LOC100307012 precursor	*Bombyx mori*	NP_001159621.1	11.52	2.43	Up
EsigOBP9	General odorant-binding protein 83a-like	*Plutella xylostella*	XP_011554700.1	1.76	1.82	Down
EsigOBP10	General odorant-binding protein 1	*Athetis dissimilis*	ALJ93806.1	122.19	22.23	Up
EsigOBP11	Odorant binding protein	*Conogethes punctiferalis*	APG32543.1	0.67	1.43	Down
EsigGOBP2	General odorant-binding protein 56d-like	*Hyposmocoma kahamanoa*	XP_026319368.1	2996.59	1094.38	Up
EsigGOBP4	General odorant-binding protein 19d-like	*Papilio xuthus*	XP_013173035.1	36.45	8.73	Up
EsigGOBP5	General odorant-binding protein 19d	*Eumeta japonica*	GBP31818.1	65.30	7.13	Up
EsigCSP1	Chemosensory protein 10, partial	*Carposina sasakii*	AYD42214.1	3.77	0.77	Up
EsigCSP2	Chemosensory protein 24	*Cnaphalocrocis medinalis*	ALT31606.1	16.38	4.00	Up
EsigCSP3	Chemosensory protein 5	*Agrotis ipsilon*	AGR39575.1	19.63	1.31	Up
EsigCSP7	Chemosensory protein	*Cnaphalocrocis medinalis*	AIX97837.1	3117.16	1258.55	Up
EsigCSP8	Chemosensory protein	*Eogystia hippophaecolus*	AOG12893.1	1.06	1.22	Up
EsigCSP9	Chemosensory protein 1	*Athetis dissimilis*	AND82443.1	107.79	3.05	Up
EsigOR3	Putative odorant receptor 85e	*Hyposmocoma kahamanoa*	XP_026314611.1	1.36	0.20	Up
EsigOR4	Odorant receptor Or1-like	*Anoplophora glabripennis*	XP_023310030.1	3.06	0.00	Up
EsigOR5	Putative odorant receptor OR40	*Cydia pomonella*	AFC91741.2	0.29	1.53	Down
EsigGR1	Gustatory receptor	*Eogystia hippophaecolus*	AOG12970.1	192.70	114.83	Up
EsigGR2	Putative gustatory receptor 10	*Conopomorpha sinensis*	AXY83426.1	0.48	1.77	Down
EsigGR3	Putative gustatory receptor GR55, partial	*Hedya nubiferana*	AST36215.1	0.39	1.78	Down

Olfactory proteins with underline had been identified before [5]

six (35.29%) olfactory proteins were expressed the highest in the 12th larval heads, among which EsigCSP2, EsigOBP9 and EsigOBP10 were significantly different ($p < 0.05$); and EsigOR5 and EsigGR1 (11.77%) were the most highly expressed in the 9th larval heads and were significantly different ($p < 0.05$) (Fig. 5). For the different kinds of olfactory proteins, 50.00% of the EsigOBPs, 50.00% of the olfactory receptors 12th: the oldest 12th instar larval head; 9th: the ninth instar larval head; 5th: the fifth instar larval head. 18S was used as the reference gene to normalize target gene expression. The standard errors are represented by the error bars, different lowercase letters (a, b, c) above the bars denote significant differences at $p < 0.05$ and 60% of the EsigCSPs were expressed the highest in the 5th larvae; 50.00% of the EsigOBPs, 40% of the EsigCSPs and 16.70% of the olfactory receptors were expressed the highest in the 12th larvae; and only 33.33% of the olfactory receptors were the highest expressed among all olfactory proteins in the 9th larvae (Fig. 5). Furthermore, only 50% of the olfactory receptors and 20.00% of the EsigCSPs were expressed at their lowest levels in the 12th and 9th larvae, respectively (Fig. 5). For the development tendency of olfactory proteins in instars, EsigOBP3, EsigGOBP2, EsigCSP3 and EsigGR3 expression decreased with larval instar, while that of EsigOBP10, EsigGOBP5, and EsigCSP2 increased. What's more, EsigGR3 was expressed the most in the 5th instar heads, followed by the 9th and 12th instar heads in turn, and all of which differed significantly from the others (Fig. 5).

Expression pattern of olfactory proteins after volatile exposure

After exposure to n-butyl ether, the expression of EsigCSP5, EsigGOBP1, EsigGOBP2, EsigGOBP5 and EsigGOBP4 were significantly upregulated ($p < 0.05$) while EsigCSP5, EsigGOBP2 and EsigGOBP5 were upregulated significantly by exposure to 4-ethylacetophenone ($p < 0.05$) (Fig. 6). However, after exposure to o-cymene, EsigOBP2, EsigOBP8, EsigGOBP1, EsigGOBP5, EsigCSP5 and EsigGR3 were significantly downregulated ($p < 0.05$). EsigOBP2, EsigOBP8, EsigGOBP1, EsigGOBP5, and EsigGR3 were downregulated significantly by α-phellandrene ($p < 0.05$). After exposure to n-butyl ether, the expression of EsigOBP2, EsigGR1 and EsigGR3 was downregulated significantly ($p < 0.05$), and exposure to 4-ethylacetophenone caused the downregulation of EsigOBP2, EsigGOBP1, and EsigGR3 ($p < 0.05$) (Fig. 6). After exposure to other volatiles, the expression of the studied olfactory proteins did not change.

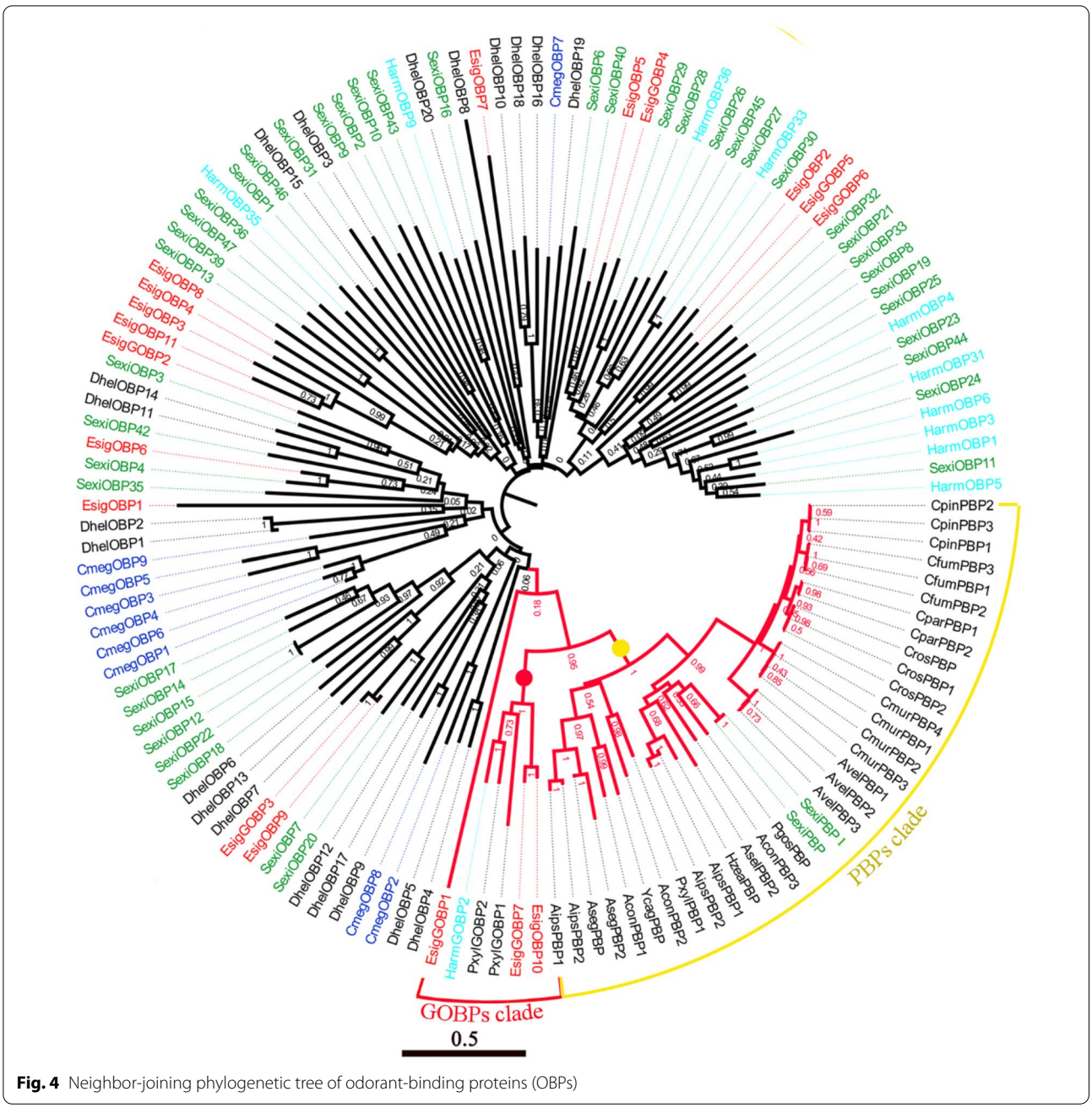
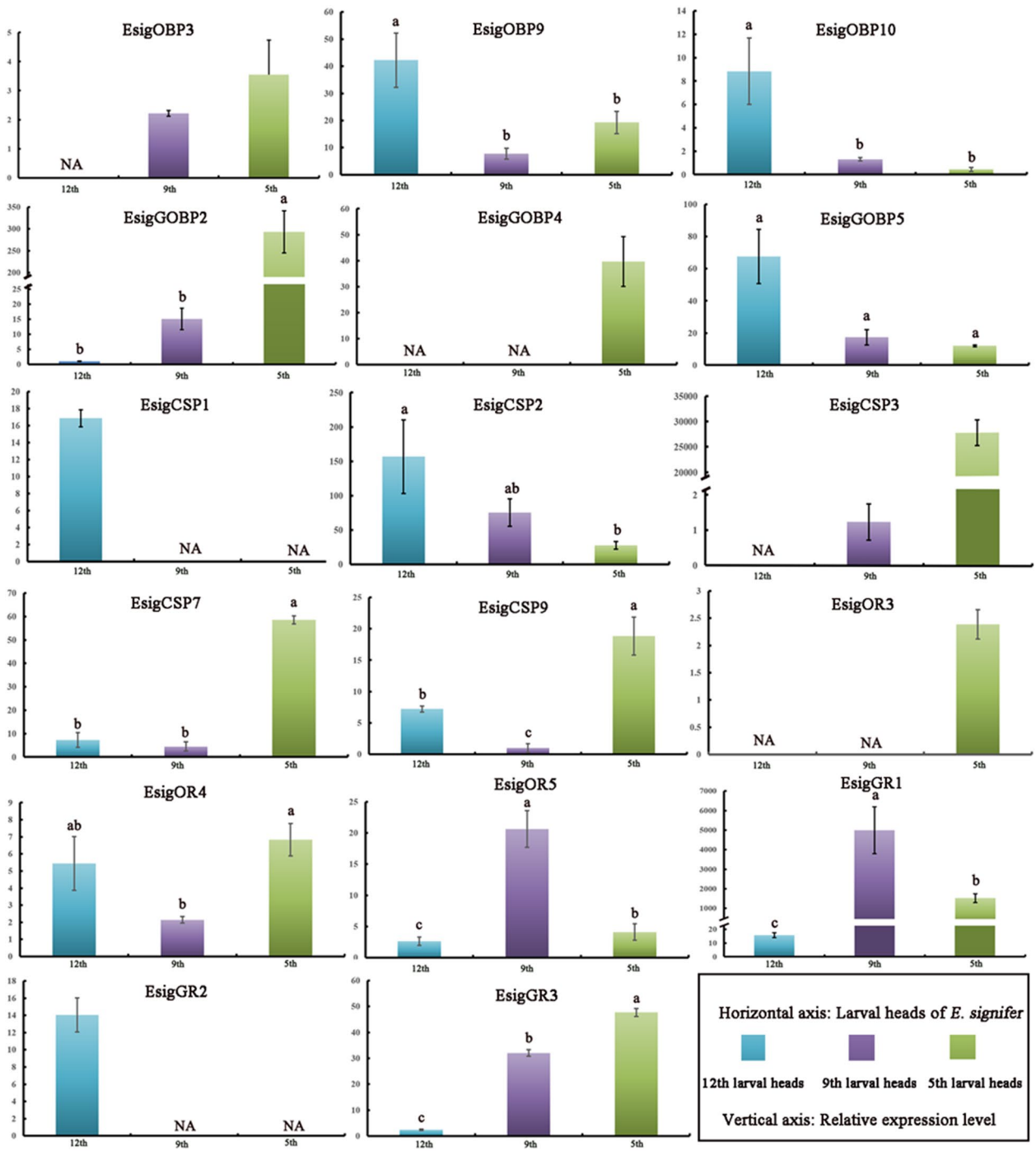


Fig. 4 Neighbor-joining phylogenetic tree of odorant-binding proteins (OBPs)

Discussion

Eight transcripts encoding putative OBPs, six CSPs, three ORs, three GRs and 18 IRs, were identified in the *E. signifer* head transcriptome, among which there were two new OBPs, three new CSPs, two new GRs, and 12 new IRs compared with the previous head and thorax and abdomen cuticula transcriptome [5], although the number of identified olfactory genes in the two *E. signifer* transcriptomes was almost the same. However, in other larval transcriptomes, there were fewer than 20 OBPs, 11 CSPs, 9 ORs, 11 IRs, 7 GRs and 4 SNMPs in the newly hatched *D. helophoroides* larval transcriptome [28] and 13 CSPs in the *Chilo auricilius* larval transcriptome [34]. In larvae and other life stage transcriptomes, there were much fewer than the 127 olfactory genes in the adult antennae and caterpillar antennae and maxillary palps transcriptome [35]; 25 ORs, 26 OBPs, 19 IRs, 23 GRs and 11 SNMPs in the *Chlorops oryzae* larvae, pupae and adult transcriptomes [36]; 58 ORs, 20 GRs and 21 IRs in the *Cydia pomonella* adult antennae and neonate head



12th: the oldest twelfth instar larval head; 9th: the ninth instar larval head; 5th: the fifth instar larval head. 18S was used as the reference gene to normalize target gene expression. The standard errors are represented by the error bars, different lowercase letters (a, b, c) above the bars denote significant differences at $p<0.05$.

Fig. 5 Expression profile of *E. signifer* olfactory proteins in three larval instars. The NJ phylogenetic analysis of OBPs of *E. signifer* (*EsigOBP*, red) was performed with reference OBPs of *D. helophoroides* (black), *C. megacephala* (blue), *S. exigua* (green), *H. armigera* (purple indigo) and PBPs of Lepidoptera. Red branch was OBPs/GOBPs clade. The stability of the nodes was assessed by bootstrap analysis with 1,000 replications. The scale bar represents 0.5 substitutions per site

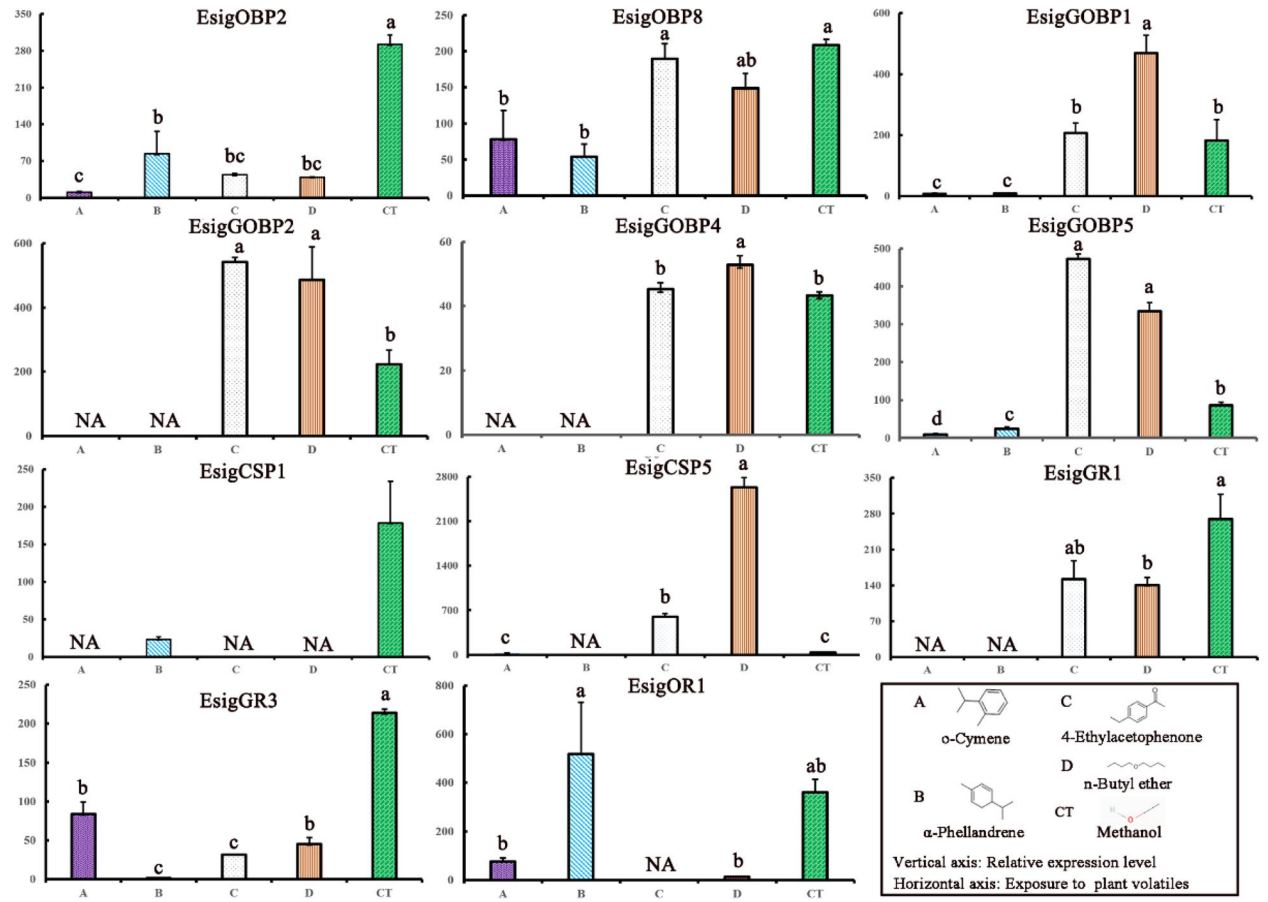


Fig. 6 Expression profile of *E. signifer* olfactory proteins in the 3rd larvae by exposure in four GC-EAD-active volatiles. **A** o-Cymene; **B** α-phellandrene; **C** 4-ethylacetophenone; **D** n-butyl ether; CT: methanol; NA: no expression. The standard errors are represented by the error bars, different lowercase letters (a, b, c) above the bars denote significant differences at $p < 0.05$

transcriptomes [37]; 57 OBPs, CSPs, 47 ORs, 6 GRs and 17 IRs in the *Spodoptera littoralis* adult antennae, larval antennae and maxillary palps transcriptomes [35]; and 34 OBPs, 20 CSPs, 10 ORs, six GRs and six IRs and three SNMPs in the eggs, 1st to 5th instar larvae, pupae, female and male adult *S. exigua* transcriptomes [31]. The reasons for the differences are that the primitive Lepidoptera moths, such as Hepialidae, have rarely been studied, with fewer data in the Nr database, and a small number of olfactory proteins have also been found in other larvae [20].

More importantly, the simplicity of larval olfactory systems, their long lifetime and their ease of feeding make larvae an excellent model to study olfactory signal transduction and coding pathways [38] and to provide details of the molecular mechanisms of larval olfaction, such as in *Helicoverpa/Heliothis* [39] and *S. littoralis* [40]. According to the phylogenetic tree of OBPs supporting EsigGOBP7 as the PBP of *E. signifer* [5], we found that EsigGOBP7 and EsigOBP10 were in a PBP/GOBP clade with 98% support, and both were in a GOBP clade with a 100% support rate; however, the sister group, the PBP clade, had a 100% support rate, which indicated that EsigOBP10 and EsigGOBP7 were the GOBPs of *E. signifer.*

α-Pinene treatment regulated four CSPs in *C. auricilius* larvae, and CSP8 had good binding affinity with α-pinene in vitro [34]. SexiOBP13 may play a role in female sex pheromone reception in S. *exigua* larvae [21]. Many larval binding proteins function in the recognition of volatiles or pheromones. Except for EsigOBP4 and EsigCSP8, all of the olfactory proteins studied were expressed in larval heads of *E. signifer*. In comparison, one larva-specific OBP was found in *S. littoralis* [35] larvae and 10 in *Lymantria dispar* larvae [41], suggesting that the expression of OBPs in larvae is common in Lepidoptera. Furthermore, *S. exigua* OBP2 [31] showed predominantly larval head-biased expression, and 14 *S. exigua* OBPs were expressed in larval heads but not in adult antennae [32], indicating the existence of larval head-specific

OBPs in insects. Two *P. xylostella* GOBPs were abundantly expressed in the three major sensilla basiconica of the larval antenna [30]. Among all olfactory proteins and instars, first, half of the olfactory proteins, including 50.00% of the EsigOBPs, 50.00% of the olfactory receptors and 60% of the EsigCSPs, had the highest expression in the 5th *E. signifer* larvae; second, 35.29% of the olfactory proteins, including 50.00% of the EsigOBPs, 40% of the EsigCSPs and 16.70% of the olfactory receptors, were expressed the highest in 12th *E. signifer* larvae; and finally, only two olfactory receptors were expressed the highest in middle (9th) larvae, indicating the olfactory proteins were expressed the highest in young (5th) larval head, followed by old (12th) and only a few in the middle (9th) stage larvae, which is in accordance with the need of young instar to select a host; however, the high expression in older instars needs to be researched further. Furthermore, EsigGOBP2, EsigGOBP5 and EsigCSP3 were expressed the highest among all olfactory proteins in the three larval head stages and were also previously reported to be the most strongly expressed in the 5th stage head of *E. signifer* larvae [5]. EsigGR1 exhibited the highest expression in the 5th larval tissues [5] and the highest expression among all olfactory proteins in the three larval head stages. *C. megacephala* OBP Cmeg33593_c0 was upregulated with increasing larval instar [29], which is consistent with the expression pattern of EsigOBP10, EsigGOBP5, and EsigCSP2 increased with increasing larval instar. However, the expression pattern of EsigOBP3, EsigGOBP2, EsigCSP3, and EsigGR3 were inversely, especially EsigGR3, with obvious tendencies and significant differences. EsigGOBP4 was the specific OBP of the 5th *E. signifer* instar larvae, and EsigCSP1 was the specific CSP in the 12th-instar larvae.

Therefore, based on these expression patterns, EsigGOBP2, EsigGOBP4, EsigGOBP5, EsigCSP1, EsigCSP3, EsigGR1 and EsigGR3 may be the key olfactory proteins in *E. signifer* larvae, and might be pivotal in their host choices. Furthermore, with larval heads, a comparison between caterpillar antennae and maxillary palps revealed numerous organ-specific transcripts, suggesting the complementary involvement of these two organs in larval chemosensory detection [35]. Of note, while most of the genes examined were expressed in larval heads, over half of them were also detected in nonolfactory tissues, such as the egg and thorax [31]. Therefore, the expression and functions of *E. signifer* olfactory proteins in larval nonolfactory tissues should be explored. In *S. littoralis*, caterpillars express a smaller set of olfactory genes than adults, SlitOBP21 and SlitGOBP1 are adult-specific [35], and 7 of 10 OBPs and CSPs are expressed more in larvae than in adults, while 2 of 10 OBPs are expressed more in adults than larvae [29]. Whether the expression of olfactory proteins in *E. signifer* adults and larvae is the same as that in *S. littoralis* should be further explored. We did not identify PBPs and PRs in *E. signifer* larval heads, but four PBPs were expressed in S. *exigua* larval heads, and the expression of PBPs and pheromone receptors has been reported in the larvae of many lepidopterans [21, 23, 30, 42].

Several larval-enriched OR transcripts have been identified [37]. The *E. signifer* ORs and GRs were expressed differently among the three examined larval stages: EsigGR1 and EsigOR5 expression were the highest in 9th instar larval heads; EsigOR3, EsigOR4, and EsigGR3 were the highest in 5th instar heads; and EsigGR2 was the highest in 12th instar heads. In *E. signifer* larvae, EsigOR3 was 5th-instar specific, and EsigGR2 was 12th-instar specific. Similarly, the expression of 50 ORs has been reported in larval heads of *S. exigua* [32], adding sixteen ORs in *H. armigera* [38] and nine ORs in *C. pomonella* [37]. No larval-specific ORs were found in transcriptome data for larvae of *S. littoralis*, *Dendrolimus punctatus*, and *L. dispar* [35, 41, 43]. We identified 12 new IRs, in addition to the highly conserved subtype receptors of IRs, for example, IR8a and IR25a, which were also identified in the head and tegument transcriptome of *E. signifer* larvae. Interestingly, EsigGR1 expression was high in 5th- and 9th-instar larvae and lower in 12th-instar larvae; EsigGR1 may function in identifying the host among young larvae.

After exposure to four gas chromatography–mass spectrometry (GC–MS) and gas chromatography-electroantennographic detection (GC-EAD) active substances, the olfactory proteins showed different expression patterns. In the n-butyl ether treatment, 54.5% and 27.2% of genes were up- and downregulated, respectively. After 4-ethylacetophenone treatment, the same genes (27.2%) were up- and downregulated. This result is also supported by the fact that excitation of an OSN with its best ligand does not necessarily result in downregulation of gene transcription of the neuron's corresponding chemosensory receptor [44] in both *S. exigua* adults [45] and larvae [32], which was explained as a mechanism that mediates odor sensitization [46], a phenomenon that has also been observed in *S. littoralis* [47]. One study found that odorants induced a fast and reversible concentration-dependent decrease in the transcription of genes corresponding to activated receptors in intact mice [48]. Interestingly, after o-cymene and α-phellandrene treatment, 54.5% and 45.5% of genes were downregulated, without any upregulated genes, which was the same as in mice. Combined with the results of downregulated receptors and GC-EAD reactivity, we speculated that the ligand of EsigGR1 was n-butyl ether, and the ligands of EsigGR3 were o-cymene, α-phellandrene, n-butyl ether and 4-ethylacetophenone. Most importantly, changes in gene expression after

exposure to important plant volatiles can provide a way to find key plant volatiles for the key olfactory proteins among large amounts of plant volatiles. EsigGR3 was newly identified in the head transcriptome, and the 3rd larvae recognized all tested GC-EAD active compounds, which supported its role in host recognition in the 3rd larvae of *E. signifer.*

Conclusions

We identified 38 olfactory proteins in the heads of *E. signifer* larvae. Around half of the olfactory proteins were the most highly expressed in the young (5th) larval head. EsigGOBP2, EsigGOBP4, EsigGOBP5, EsigCSP1, EsigCSP3, EsigGR1 and EsigGR3 may be important proteins in larval olfactory recognition. In addition, based on the receptors downregulated after exposure to volatiles and the GC-EAD reactivity in the third larvae, we speculated that the ligand of EsigGR1 was n-butyl ether, and the ligands of EsigGR3 were all electrophysiologic active compounds. Important plant volatiles can be targeted for pest control. The simplicity of the *E. signifer* larval olfactory system, filtering important olfactory proteins, along with their long life and ease of feeding, make *E. signifer* larvae a suitable in vivo model for the study of olfactory signal transduction and coding pathways.

Abbreviations

CSPs: Chemosensory proteins; GO: Gene Ontology; GRs: Gustatory receptors; IRs: Ionotropic receptors; OBPs: Odorant binding proteins; ORFs: Open reading frame; ORs: Odorant receptors; ORco: Odorant coreceptor; HR: Pheromone receptor; SNMPs: Sensory neuron membrane proteins; qRT-PCR: Fluorescence quantitative real-time PCR; GC-EAD: Gas chromatography-electroantennographic detection; GC–MS: Gas chromatography–mass spectrometry.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00320-4.

Additional file 1. Nucleic acid sequences of all candidates chemosensory proteins identified in *Endoclita signifier* transcriptome.

Additional file 2. The protein names and gene accession numbers were used in phylogenetic trees.

Additional file 3. Primers were designed for fluorescence quantitative real-time PCR.

Additional file 4. Best blastx hits for ionotropic receptors (IRs) of *Endoclita signifier.*

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Author contributions

PH designed, conceived of the study, carried out the molecular genetic studies, performed the sequence alignment and experiments, and drafted the manuscript. ZQ collected almost all the samples and participated in some experiments. XC, YX and XS participated in all the experiments. ZY drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The raw reads for *E. signifer* generated during the current study are available in the NCBI SRA repository under the accession number PRJNA716094 [https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA716094]. The approximate complete CDS sequences of olfactory proteins are available in the GenBank under the accession number MW924374 to MW924381. Other supporting data are included within the supplementary files.

Declarations

Ethics approval and consent to participate

The ghost moth ***E. signifer*** is the forestry pest in China, and its collection was permitted by Guangxi forestry bureau. It is not included in the "List of Endangered and Protected Animals in China". All operations were performed according to ethical guidelines in order to minimize pain and discomfort to the insects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Yang X, Yang X, Xue D, Han H. The complete mitochondrial genome of *Endoclita signifer* (Lepidoptera, Hepialidae). Mitochondr DNA A DNA Mapp Seq Anal. 2016;27:4620–1.
2. Yang XH. Studies on the biological and ecological characteristics of *Endoclita signifer*. Beijing Forestry University; 2013.
3. Yang X, Luo Y, Wu Y, Zou D, Hu P, Wang J. Distribution and Damage of *Endoclita signifer* Walker, as an important wood borer pest insect on forest. For Pest Dis. 2021;40:34–40.
4. Yang XH. Biological ecology and control techniques of *Endoclita signifer*, an important pest of eucalyptus. Beijing: China Forestry Publishing House; 2017. p. 50–60.
5. Zhang X, Yang Z, Yang X, Ma H, Liu X, Hu P. Olfactory proteins and their expression profiles in the Eucalyptus pest *Endoclita signifer* larvae. Front Physiol. 2021;12: 682537. https://doi.org/10.3389/fphys.2021.682537.
6. García-Robledo C, Horvitz CC. Parent-offspring conflicts, "optimal bad motherhood" and the "mother knows best" principles in insect herbivores colonizing novel host plants. Ecol Evol. 2012;2:1446–57.
7. JD A, (Eds.) CR: Pheromone communications in Moths: evolution, behaviour and application. American Entomologist. 2016; https://doi.org/10.1093/ae/tmx062.
8. Haverkamp AHB, Knaden M. Combinatorial codes and labeled lines: how insects use olfactory cues to find and judge food, mates, and oviposition

sites in complex environments. Front Physiol. 2018;9:1–8. https://doi.org/10.3389/fphys.2018.00049.
9. Zhang J, Wang B, Dong S, Cao D, Dong J, Walker WB, Liu Y, Wang G. Antennal transcriptome analysis and comparison of chemosensory gene families in two closely related noctuidae moths, *Helicoverpa armigera* and *H. assulta*. PLoS ONE. 2015;10:e0117054.
10. Li H, Li W, Miao C, Wang G, Zhao M, Yuan G, Guo X. Identifcation of the differences in olfactory system between male and female oriental tobacco budworm *Helicoverpa assulta* between male and female oriental tobacco budworm *Helicoverpa assulta*. Res Square. 2020;107: e21829.
11. Guo H, Huang LQ, Pelosi P, Wang CZ. Three pheromone-binding proteins help segregation between two Helicoverpa species utilizing the same pheromone components. Insect Biochem Mol Biol. 2012;42:708–16.
12. Sun YL, Huang LQ, Pelosi P, Wang CZ. Expression in antennae and reproductive organs suggests a dual role of an odorant-binding protein in two sibling *Helicoverpa* species. PLoS ONE. 2012;7: e30040.
13. Wang C, Li G, Miao C, Zhao M, Wang B, Guo X. Nonanal modulates oviposition preference in female *Helicoverpa assulta* (Lepidoptera: Noctuidae) via the activation of peripheral neurons. Pest Manag Sci. 2020;76:3159–67.
14. Cui WC, Wang B, Guo MB, Liu Y, Jacquin-Joly E, Yan SC, Wang GR. A receptor-neuron correlate for the detection of attractive plant volatiles in *Helicoverpa assulta* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol. 2018;97:31–9.
15. Wu H, Li RT, Dong JF, Jiang NJ, Huang LQ, Wang CZ. An odorant receptor and glomerulus responding to farnesene in *Helicoverpa assulta* (Lepidoptera: Noctuidae). Insect Biochem Mol Biol. 2019;115: 103106.
16. Li RT, Huang LQ, Dong JF, Wang CZ. A moth odorant receptor highly expressed in the ovipositor is involved in detecting host-plant volatiles. Elife. 2020;9:e53706.
17. Ren X, Wang Y, Ma Y, Jiang W, Ma X, Hu H, Wang D, Ma Y. Midgut de novo transcriptome analysis and gene expression profiling of *Spodoptera exigua* larvae exposed with sublethal concentrations of Cry1Ca protein. 3 Biotech. 2020;10:1–13.
18. Wang K, Fan R-L, Ji W-N, Zhang W-W, Chen X-M, Wang S, Yin L, Gao F-C, Chen G-H, Ji T. Transcriptome analysis of newly emerged honeybees exposure to sublethal carbendazim during larval stage. Front Gene. 2018;9:426.
19. Ryabova A, Cornette R, Cherkasov A, Watanabe M, Okuda T, Shagimardanova E, Kikawada T, Gusev O. Combined metabolome and transcriptome analysis reveals key components of complete desiccation tolerance in an anhydrobiotic insect. P Natl A Sci. 2020;117:19209–20.
20. Chang H, Ai D, Zhang J, Dong S, Liu Y, Wang G. Candidate odorant binding proteins and chemosensory proteins in the larval chemosensory tissues of two closely related noctuidae moths, *Helicoverpa armigera* and *H. assulta*. PLoS ONE. 2017;12:e0179243.
21. Jin R, Liu N-Y, Liu Y, Dong S-L. A larval specific OBP able to bind the major female sex pheromone component in *Spodoptera exigua* (Hübner). J Integr Agricul. 2015;14:1356–66.
22. Carvalho WJ, Fujimura PT, Bonetti AM, Goulart LR, Cloonan K, da Silva NM, Araujo ECB, Ueira-Vieira C, Leal WS. Characterization of antennal sensilla, larvae morphology and olfactory genes of *Melipona scutellaris* stingless bee. PLoS ONE. 2017;12: e0174857.
23. Zielonka M, Gehrke P, Badeke E, Sachse S, Breer H, Krieger J. Larval sensilla of the moth *Heliothis virescens* respond to sex pheromone components. Insect Mol Biol. 2016;25:666–78.
24. Mortazavi A, Williams BA, Mccue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008;5:621–8.
25. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinform. 2011;12:93–9.
26. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9.
27. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
28. Li X, Dong G, Fang J, Liu H, Guo W, Yin H. Identification of putative olfactory genes in newly hatched larvae of a Coleopteran ectoparasitoid *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) by transcriptome analysis. Entomol Res. 2020;50:329–42.
29. Wang X, Xiong M, Lei C, Zhu F. The developmental transcriptome of the synanthropic fly *Chrysomya megacephala* and insights into olfactory proteins. BMC Genomics. 2015;16:20.
30. Zhu J, Ban L, Song LM, Liu Y, Pelosi P, Wang G. General odorant-binding proteins and sex pheromone guide larvae of *Plutella xylostella* to better food. Insect Biochem Mol Biol. 2016;72:10–9.
31. Liu NY, Zhang T, Ye ZF, Li F, Dong SL. Identification and characterization of candidate chemosensory gene families from *Spodoptera exigua* developmental transcriptomes. Int J Biol Sci. 2015;11:1036–48.
32. Llopis-Gimenez A, Carrasco-Oltra T, Jacquin-Joly E, Herrero S, Crava CM. Coupling transcriptomics and behaviour to unveil the olfactory system of *Spodoptera exigua* larvae. J Chem Ecol. 2020;46:1017–31.
33. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–8.
34. Yi X, Shi S, Wang P, Chen Y, Lu Q, Wang T, Zhou X, Zhong G. Characterizing potential repelling volatiles for "push-pull" strategy against stem borer: a case study in *Chilo auricilius*. BMC Genomics. 2019;20:751.
35. Poivet E, Gallot A, Montagne N, Glaser N, Legeai F, Jacquin-Joly E. A comparison of the olfactory gene repertoires of adults and larvae in the noctuid moth *Spodoptera littoralis*. PLoS ONE. 2013;8: e60263.
36. Qiu L, Tao S, He H, Ding W, Li Y. Transcriptomics reveal the molecular underpinnings of chemosensory proteins in *Chlorops oryzae*. BMC Genomics. 2018;19:890.
37. Walker WB 3rd, Gonzalez F, Garczynski SF, Witzgall P. The chemosensory receptors of codling moth Cydia pomonella-expression in larvae and adults. Sci Rep. 2016;6:23518.
38. Di C, Ning C, Huang L-Q, Wang C-Z. Design of larval chemical attractants based on odorant response spectra of odorant receptors in the cotton bollworm. Insect Biochem Mol Biol. 2017;84:48–62.
39. Tanaka K, Uda Y, Ono Y, Nakagawa T, Suwa M, Yamaoka R, Touhara K. Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Curr Biol. 2009;19:881–90.
40. De Fouchier A, Sun X, Caballero-Vidal G, Travaillard S, Jacquin-Joly E, Montagné N. Behavioral effect of plant volatiles binding to *Spodoptera littoralis* larval odorant receptors. Front Behav Neurosci. 2018;12:264.
41. McCormick AC, Heyer J, Sims JW, Mescher MC, De Moraes CM. Exploring the effects of plant odors, from tree species of differing host quality, on the response of *Lymantria dispar* males to female sex pheromones. J Chem Ecol. 2017;43:243–53.
42. Poivet E, Rharrabe K, Monsempes C, Glaser N, Rochat D, Renou M, Marion-Poll F, Jacquin-Joly E. The use of the sex pheromone as an evolutionary solution to food source selection in caterpillars. Nat Commun. 2012;3:1–7.
43. Zhang S-f, Liu H-h, Kong X-b, Wang H-b. Liu F, Zhang Z: Identification and expression profiling of chemosensory genes in *Dendrolimus punctatus* Walker. Front Physiol. 2017;8:471.
44. Koerte S, Keesey IW, Khallaf MA, Cortes Llorca L, Grosse-Wilde E, Hansson BS, Knaden M. Evaluation of the DREAM technique for a high-throughput deorphanization of chemosensory receptors in *Drosophila*. Front Mol Neurosci. 2018;11:366.
45. Wan X, Qian K, Du Y. Synthetic pheromones and plant volatiles alter the expression of chemosensory genes in *Spodoptera exigua*. Sci Rep. 2015;5:17320.
46. Dion E, Monteiro A, Nieberding CM. The role of learning on insect and spider sexual behaviors, sexual trait evolution, and speciation. Front Ecol Evol. 2019. https://doi.org/10.3389/fevo.2018.00225.
47. Anderson P, Sadek M, Larsson M, Hansson B, Thöming G. Larval host plant experience modulates both mate finding and oviposition choice in a moth. Anim Behav. 2013;85:1169–75.
48. Von der Weid B, Rossier D, Lindup M, Tuberosa J, Widmer A, Col JD, Kan C, Carleton A, Rodriguez I. Large-scale transcriptional profiling of chemosensory neurons identifies receptor-ligand pairs in vivo. Nat Neurosci. 2015;18:1455–63.

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