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Detecting Host-Plant Volatiles with Odorant Receptors from *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae)

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ABSTRACT: *Grapholita molesta* is a global pest of stone and pome fruits. The sensitive olfactory system plays a crucial role in regulating key behavioral activities of insects and *G. molesta* relies heavily on general odorant receptors (ORs) to detect host-plant volatiles. In this study, three general OR genes from *G. molesta* (*GmolOR12*, *GmolOR20*, and *GmolOR21*) were identified. Quantitative polymerase chain reaction revealed that *GmolORs* expression was considerably higher in adults and adult antennae than in any other life stages and body parts, respectively. Moreover, the expression of *GmolORs* was significantly higher in the antennae of females than in those of males, with a peak in the antennae of 3-days-old adult females. GmolOR20 and GmolOR21 displayed no responses to any of the odorant compounds tested in the *Xenopus* oocyte system. GmolOR12 was tuned mainly to 5 of the 47 odorant components tested (including decanol, heptanal, octanal, nonanal, and decanal), and the response to aldehydes among the 5 components was the highest. Additionally, they all elicited female and male antennae electroantennogram responses, and the aldehydes elicited the highest response among the 5 components. These results suggested that *GmolOR12* in the *G. molesta* olfactory system plays an important role in sensing aldehydes and that *GmolOR12* is involved in sensing host-plant volatiles. These findings provide insight into the possibility of using host-plant volatiles for the control of *G. molesta*.

KEYWORDS: *Grapholita molesta, oriental fruit moth, odorant receptor, plant volatile, heptanal*

INTRODUCTION

Grapholita molesta Busck (Lepidoptera: Tortricidae), known as the oriental fruit moth, is a common, economically important, multivoltine pest that causes serious damage to pome and stone fruits worldwide.[1,2] Its larvae cause damage by not only feeding on young twigs but also directly feeding on the pulp.[3] The adults can move between host plants, switching between different species or cultivars, such as peaches, nectarines, apples, and pears.[4,5] Changes in the components of host-plant volatiles are the primary reason that *G. molesta* switch among host plants.[6] *G. molesta* responds to a variety of host-plant volatiles, including alcohols, aldehydes, esters, terpenes, benzonitriles, and alkanes.[6,7] Green-leaf volatiles and aromatics attract female *G. molesta*,[8] whereas host-plant volatiles mixed with sex pheromones can enhance attractiveness to male *G. molesta*.[9] Therefore, host-plant volatiles may be used to develop new *G. molesta* control methods.

The olfactory system plays a vital role in the survival and reproduction of insects, and this olfactory sensitivity enables insects to detect and discriminate a variety of chemical compounds.[10,11] Odorant molecules move through pore tubules in the plasma membranes of olfactory sensilla on the antennae, and they are transferred by odorant binding proteins (OBPs) and chemosensory proteins (CSPs) onto odorant receptors (ORs) located on the dendritic membrane of olfactory receptor neurons (ORNs), where ORs are activated.[12−14] Odorant molecules are promptly inactivated by ligand uptake or degradation by odorant degrading enzymes (ODEs).[15,16] Among these proteins, insects rely primarily on ORs for the perception of odorant molecules.[17,18]

Insect OR membrane topology has an intracellular N-terminus and extracellular C-terminus, which is opposite that in mammalian ORs.[11,19] Insect ORs can be classified into two types: a diverse conventional ligand-binding OR and a highly conserved nonconventional odorant receptor coreceptor (Orco).[20,21] Diverse conventional ligand-binding ORs are usually divided into three groups according to their functions: general ORs, pheromone receptors (PRs), and gustatory receptors (GRs).[14] Generally, ORs are more abundantly expressed in female antennae, and they function to perceive host-plant volatiles.[22,23] Numerous ORs in insects have been studied by heterologous expression in the *Xenopus* oocyte system, HEK293 cells, *Drosophila* ORNs, and RNA interference (RNAi).[24−27] The *Xenopus* oocyte system has been used to study general OR genes in lepidopteran insects, including *Bombyx mori*,[22] *Spodoptera litura*,[28] *Spodoptera exigua*,[23] *Helicoverpa armigera*,[29] *Helicoverpa assulta*,[30] and *Mythimna separata*.[31] These functional studies of general OR genes have helped develop effective pest traps and attractants.[29,30]

Several olfactory-related genes have been previously studied in *G. molesta*;[32−36] however, the functions of ORs are unclear.

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In the present study, three general ORs were identified, and their expression levels were evaluated. Furthermore, the function of *GmolORs* in *G. molesta* was characterized by host-plant volatile recognition. These findings provide insights into the molecular mechanism of olfactory recognition in *G. molesta* and a strategy for pest control.

MATERIALS AND METHODS

Insects Rearing and Tissue Collection. *G. molesta* populations were reared in the laboratory at 25 ± 1 °C, 70 ± 10% relative humidity with a photoperiod of 15:9 h (L:D) in the laboratory. Larvae were maintained on an artificial diet, and adults were fed with 5% honey solution.[37] Different developmental stages (eggs, first through fifth larvae, pupae, and both female and male adults), various tissues (antennae, heads without antennae, thoraxes, abdomens, legs, and wings), and antennae of female adults at different ages (1-, 3-, 5-, and 7-days-old) were collected, frozen in liquid nitrogen immediately, and then stored at −80 °C for future use.

RNA Extraction and cDNA Synthesis. Total RNA was extracted using RNAiso Plus (TaKaRa, Dalian, China) and treated with DNase I (Thermo Scientific, USA) to remove genomic DNA. Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) in a 20 μL reaction mixture containing 1 μg of total RNA. The concentration and quality of RNA were verified using a SimpliNano spectrophotometer (GE Healthcare, UK) and 1.0% agarose gel electrophoresis.

Molecular Cloning. Three full *GmolORs* open reading frame (ORF) was cloned by a complete coding sequence from a previous antennal transcriptome of female adults of *G. molesta*.[38] The PCR primers containing the putative start and stop codons are listed in Table S1 (Supporting Information). All primers were synthesized by Sangon Biotech (Sangon, Shanghai, China). A 20 μL PCR reaction system included 10 μL of 2×Es Taq MasterMix (ComWin, Beijing, China), 1 μL of each primer (10 μM), 1 μL of cDNA, and 7 μL of RNase-free water. The PCR was carried out under the following conditions: 94 °C/5 min; 40 cycles of 94 °C/30 s, 55 °C/30 s, 72 °C/30 s; 72 °C/10 min. The PCR products were purified by a Universal DAN Purification Kit (TianGen, Beijing, China), ligated into the pMD-19T cloning vector (TaKaRa, Dalian, China) and transformed into DH5α competent cells (TianGen, Beijing, China). Positive clones were sequenced by Sangon Biotech (Sangon, Shanghai, China).

Quantitative Real-Time PCR (qPCR). Sex- and tissue-biased expression profiles were detected by qPCR. The reference genes were the *β-actin* (GenBank No.: KF022227.1) and elongation factor 1-alpha (*EF1-α*) (GenBank No.: KT363835.1). The specific primers are listed in Table S1. The qPCR reaction system and conditions were the same as those used in a former study.[27] Melting curves were generated with measurements taken every 0.5 °C in the temperature range 60−95 °C. The specific primers were verified by melting curve analysis, and the amplification efficiencies were calculated by the standard curve with a 5-fold cDNA dilution series. Experiment was biologically repeated three times and each was analyzed in triplicate. Relative expression levels of GmolORs were calculated using the $2^{-\Delta\Delta Ct}$ method.[39]

Receptor Expression in *Xenopus* Oocytes and Electrophysiological Recordings. The specific primers with restriction enzyme cutting sites were designed to subclone ORF of GmolORs (Table S1) and ligated into expression vector pT7Ts using T4 DNA ligase (TaKaRa, Dalian, China). The plasmid DNA was extracted using TIANprep Mini Plasmid Kit (TianGen, Beijing, China), *Sma*I (TaKaRa, Dalian, China) was used to linearize the plasmid DNA, with the phenol and chloroform method performed, and then cRNA was synthesized by using mMESSAGE mMACHINE T7 Kit (Ambion, Austin, TX, USA). Mature healthy *Xenopus* oocytes (stage V−VII) were treated as detailed in a previous study.[21] Oocytes were microinjected with 27.6 ng of GmolORs cRNA and 27.6 ng of GmolOR2 cRNA. After injection, oocytes were cultured for 4−7 days at 18 °C. Currents induced by odorants were recorded using an OC-725C oocyte clamp (Warner Instruments, Hamden, CT, USA) at a holding potential of −80 mV. Data acquisition and analysis were accomplished with Digidata 1440 A and Pclamp10.0 software (Axon Instruments Inc., Union City, CA, USA). The 47 tested compounds are listed in Table S2 and prepared as a 1 mol/L in dimethyl sulfoxide and stored at −20 °C. Every GmolOR/OR2 coexpressed in *Xenopus* oocytes were tested, at least 50 cells. Experiments were performed in three biological replicates. Before the experiments, stock solutions were diluted in 1× Ringer's buffer to the concentration of 1×10^{-4} mol/L.

Electroantennogram Assay (EAG). EAGs were used to record the antennal responses of *G. molesta* to five volatiles from its host plant (Table S2). All chemicals were diluted with liquid paraffin to the final concentration of 20 mg/mL. Liquid paraffin and (*Z*)-3-hexenyl acetate were used as a negative and reference control, respectively. Antennae were stimulated with solutions randomly. Both ends of female and male antennae were removed and blocked with Spectra 360 Electrode Gel (Parker Laboratories, Fairfield, NJ, USA). Filter paper strips (0.6 cm × 4.5 cm) were loaded with 15 μL of solution and inserted into a 1.5 mL micropipet tip. During EAG recording, the continuous air flow was delivered by a Syntech stimulus controller (CS55 model, Syntech, Germany) at a constant flow of 50 cm/s, and the time of the stimuli flow was 0.5 s. The EAG signals were recorded and analyzed using the GC-EAD software.

Sequence and Data Analyses. Searching for orthologs of GmolORs was performed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The transmembrane domain (TM) of GmolORs was predicted using TMHMM Server v 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/), and the molecular weights (MW) and isoelectric points (PI) of GmolORs were determined using the Compute pI/Mw tool (https://web.expasy.org/compute_pi/). The specific primers were designed using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/#PRIMER_SEQUENCE_INPUT). The sequences were aligned and compared using DNAMAN 6.0 and ClustalX 2.1 software. The phylogenetic tree was constructed using the neighbor-joining method in MEGA 6 software. Values indicated at the nodes are bootstrap values based on 1000 replicates. Data were analyzed using SPSS 22 and dose-response data were analyzed using GraphPad Prism 6.

RESULTS

Gene Cloning and Sequence Analysis of GmolORs. The ORFs of *GmolOR12*, *GmolOR20*, and *GmolOR21* (GenBank Nos.: MK910370, MH898864, and MH898865, respectively) consisted of 1200, 1284, and 1131 base pairs (bp), which encoded 399, 427, and 376 amino acids (AAs), respectively. The molecular weights of the proteins were 45.98, 49.83, and 42.98 kDa, respectively, and their theoretical isoelectric points were 7.95, 8.57, and 5.00, respectively. The three GmolORs had 7, 7, and 5 transmembrane domains, respectively, with an intracellular N-terminus and extracellular C-terminus (Table 1). Amino acid sequence alignment illustrated that these three GmolORs were highly divergent, with identities of 24.21% (Figure 1).

The phylogenetic tree illustrated that the ORs of lepidopteran insects were clustered into three different groups (Figure 2). GmolORs were divided into group 1 (GmolOR21), group 2 (GmolOR12), and group 3 (GmolOR20), whereas the insect Orcos were clustered into one group

Table 1. Characteristic of GmolORs from *G. molesta*

name	ORF (bp)	no. of AAs	MW (kDa)	PI	TMs
GmolOR12	1200	399	45.98	7.95	7
GmolOR20	1284	427	49.83	8.57	7
GmolOR21	1131	376	42.98	5.00	5

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OR12-ORF   ...........MRNKVEARQEIRAT.LNLCMFCM.QCIGVSFEKATTTGRYTRQKLVFAVSVCSIVYHVFSEIVYIGLTLSNSPRVEDVVPLFHTFGYG     86
OR20-ORF   MKTYKRKESSTLPLVPATDVQEFKPFRETYKIITFNMIVGMLYPTPDTAACRTVGILAILVVMTPVCFAALLDMWTSWVRGDIINIIRHCTVMGPFLGA.     99
OR21-ORF   ..........MFGSILELSDDFFAYNLKYLFLVG.LWPNDPWAKAHPN...TYKMFEYTTHVLSIIFLIISGIGTYKIKDDVVLLMTNLDKCLVAYNF.     84

OR12-ORF   ALSIAKVFALWSKKEVFAEHLEELSGIWP.MEPLDEDARIIKQKSVTALRLVHQWYFSVNVG.GVLFYNVTPICIYLYQVWQGQDAQVGFVWVSWYEFDK    184
OR20-ORF   ...IFKMMLFFYSRKEAWAIIQKIDADHARYNTIPEEHKEIARRHIQNTQYYSEKCWSITVATCVLTFPLTAVVLTLYNSVFKEEPIKYMIHDIDKEFSP    196
OR21-ORF   ...VAKVVIFVYKRKQIEILISEI.........IDSGDEITEERKKIMLMVI...VIVTGMS.TSIVGAFSALALYHNELSVEA.......WMPFDEMES    161

OR12-ORF   HKP.LNHVFVYIFEVFAGQTCVWIMICTDLL..FSGLASHIAMLLRLFHKRLEVLAETEKTQDEYYQEIVANIKLHQRFIRYCNDLEEAFTIVNLFNVVF    281
OR20-ORF   REDRFSSPYFEVMFFYMSICSLFYIISFTGFDAFFGITINHACMKMEFACKTMADAMLEVDRGSRHRRMLDVISEQNDLFGMVELIQETFAIWLGFIVIA    296
OR21-ORF   TMN.LLTASQLLAITFV.VPVIWRAVAMQGI..VCSLIMYLCDQLIELQDRIRSLEFTTMTERAVREEFKDIVIKHVRFMRYTQVMNKIFEEYFLIQNLA    257

OR12-ORF   SSVNICCVVFVIVLL...EPFVAVSNKLFLGSALIQIGMLCWYADDIFHSNADVALAVYNSGWYRTD.PRSRRALIFLIRRACKFVAFTPMKFTNLSLVT    377
OR20-ORF   TMLQICNCMYQIIEG....YGIDPRYLVFIVGTIAHIYLPCRYAAKLQASALEVSTHLYCCGWERVNDERARKMIIFMIARACIFMKITPFNMFFFDMDL    392
OR21-ORF   VTLELCLNALMATMIGFEQKTLLATFFAFLCVALMNAYIYCYLGNEMIIQSENIALAAMESSWISWP.LDLQKDLVILLRVSCKGLFLSPGGMVAMSIQT    356

OR12-ORF   YSSILTR.SYSYFALLYTMYNDS...........                                                                    399
OR20-ORF   FVSVSSRTGKNIYTLEKKNTMRKLLYRMYTLWWS                                                                     426
OR21-ORF   YSQTLYN.GYSIFAVLNDVVA.............                                                                     376
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Figure 1. Amino acid sequence alignment of GmolORs.

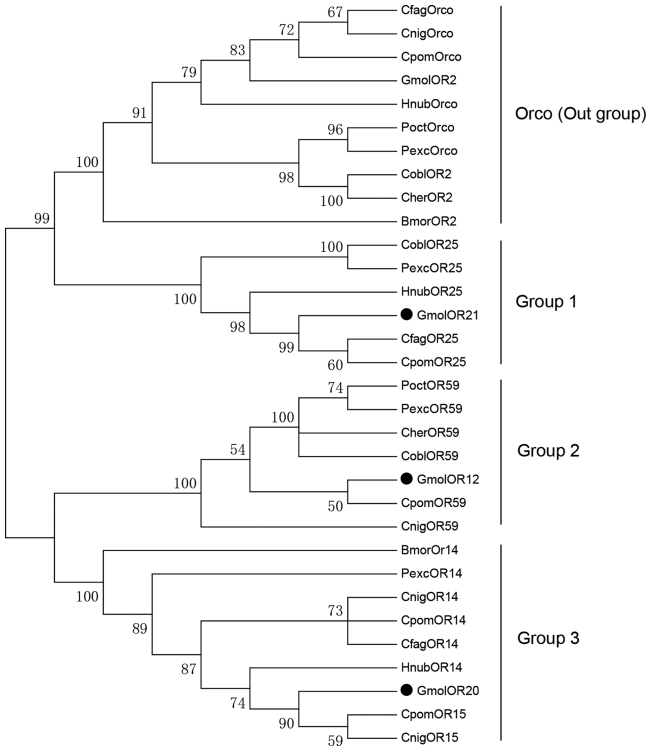

Figure 2. Phylogenetic tree of GmolORs of *G. molesta* and general ORs of several lepidopteran insects. The Orcos are defined as an out group. The accession numbers of amino acid sequences for the genes used in the tree are listed in Table S3.

separated from the ORs. Sequence blasting revealed that GmolOR12 was 90.23% identical to CpomOR59 from *Cydia pomonella*; GmolOR20 and GmolOR21 shared the highest identity of 86.73% with CpomOR15 from *C. pomonella* and 90.69% with CfagOR25 from *Cydia fagiglandana*, respectively.

Sex- and Tissue-Biased Expression Profiles of GmolORs. *GmolOR12*, *GmolOR20*, and *GmolOR21* were expressed in all stages (eggs, larvae, pupae, and adults), and they were highly expressed in adults. Moreover, *GmolORs* expression was markedly higher in adult females than in adult males ($P < 0.05$) (Figure 3A). Three genes were expressed mainly in the antennae of both adult females and males. In other tissues (heads without antennae, thoraxes, abdomens, legs, and wings), they were very weakly expression or not expressed. Three genes in the antennae of females presented significantly higher expression levels than those in males ($P < 0.01$) (Figure 3B). In addition, these three genes were expressed in the antennae of adult females of different ages, with a peak in the antennae of 3-day-old adult females ($P < 0.05$) (Figure 3C).

GmolOR12 Mainly Tunes to Odorants in the *Xenopus* Oocytes Expression System. The coexpression of GmolORs/OR2 in oocytes were tested against 47 chemicals at a concentration of 10^{-4} mol/L, comprising four sex pheromones and 43 host-plant volatiles (Table S2). The results indicated that GmolOR20 and GmolOR21 failed to respond to any of the 47 odorants tested in this study (Figure 4A, upper). GmolOR12 responded to alcohols (decanol C10) and aldehydes (heptanal C7, octanal C8, nonanal C9, and decanal C10) (Figure 4A, lower). The highest response of GmolOR12/OR2 was to nonanal (amplitude of 701.08 nA), whereas the lowest response was to decanol (amplitude of 191.45 nA); in addition, nonanal was significantly higher than decanol ($P < 0.05$) (Figure 4B). Furthermore, heptanal, a volatile compound principally found in host plants, was selected as a stimulus to explore the dose−response of GmolOR12. At a concentration of 10^{-7} mol/L, heptanal elicited the responses of GmolOR12/OR2 coexpression (Figure 4C,D).

***G. molesta* EAG Responses to Five Host-Plant Volatiles.** The EAG responses to five host-plant volatiles (Table S2) that were found to be active in the *Xenopus* system were tested. All five volatiles elicited antennae EAG responses. Evidently, heptanal and nonanal produced the strongest EAG responses in females, whereas in males, the EAG responses of heptanal, nonanal, and octanal were significantly higher than those of other odorants ($P < 0.05$). Additionally, decanol elicited the weakest response in both sexes (Figure 5).

DISCUSSION

The detection of host-plant volatiles is important for guiding both male and female adults to food sources and oviposition sites.[40] Previous studies indicated that ORs are located on the ORNs and that they enable the entire olfaction process.[41]

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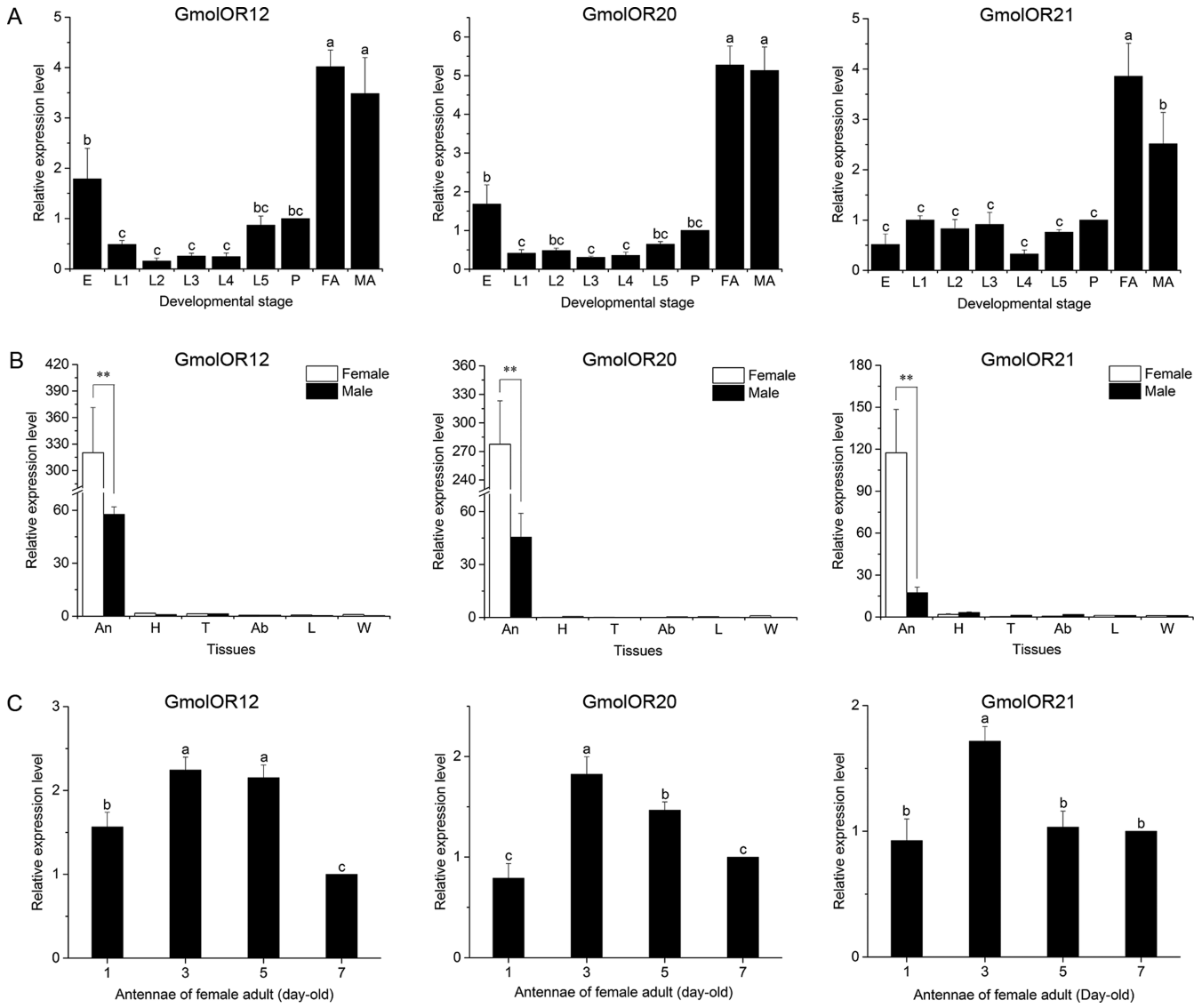

Figure 3. Sex- and tissue-specific expression profiles of *GmolORs*. (A) Relative expression level of *GmolORs* in different developmental stages of *G. molesta*. E, eggs; L1−L5, first through fifth instar larvae, respectively; P, pupae; FA, female adults; MA, male adults. (B) Relative expression level of *GmolORs* in different tissues of adult *G. molesta*. An, antennae; H, heads (with antennae removed); T, thoraxes; Ab, abdomens; L, legs; W, wings. (C) Relative expression level of *GmolORs* in antennae of *G. molesta* female adults of different ages (days old). Data were mean ± standard error (SE). Different lowercase letters indicate significant differences among different developmental stages and antennae of adults of different ages ($P < 0.05$, Tukeys test). The double asterisk indicates extremely significant difference between female and male ($P < 0.01$, independent samples T-test).

Here, GmolOR12 and GmolOR20 have seven transmembrane domains, whereas GmolOR21 had five transmembrane domains; these features were consistent with those of insect ORs.[42] Previously, the diverse functional ORs were clustered into different branches.[28] In this study, three GmolORs were classified into groups 1−3, confirming that providing a foundation for analysis of the correspondence between diverse branches and OR functions. *GmolORs* were highly expressed in adults, especially in the antennae of females, implying that GmolORs play an important part in detecting host-plant volatiles.[25,31] Moreover, mating, oviposition, and peak flight activity of *G. molesta* females mainly occur in 3-days-old females after eclosion,[1,43] which coincided with the expression level of *GmolORs* in the antennae of adult females. These behavioral outcomes contribute to our understanding of the crucial role that GmolORs play in regulating female-specific behaviors, such as finding food sources and choosing oviposition sites.

GmolOR20 and GmolOR21 did not respond to any odorant tested in the *Xenopus* oocyte system, suggesting that they were not functionally expressed or that they may respond to other odorants that were not tested in the present study. However, here, GmolOR12 was mainly tuned to five host-plant components. Similar results have been reported for EposOR3 of *Epiphyas postvittana*, LmigOR3 of *Locusta migratoria*, GmolOR9 of *G. molesta*, and ApisOR4 of *Acyrthosiphon pisum*, and they broadly responded to several host-plant volatiles.[27,40,44,45] In addition to ORs that are broadly tuned and do not respond to any plant volatiles, some ORs are narrowly tuned odorants. For example, AlucOR40 of *Apolygus lucorum*, BmorOR56 of *B. mori*, and CchlOR62 of *Campoletis chlorideae* specifically tuned to (*Z*)-2-hexenol, *cis*-jasmone, and *cis*-jasmone, respectively.[22,46,47] These results indicate that

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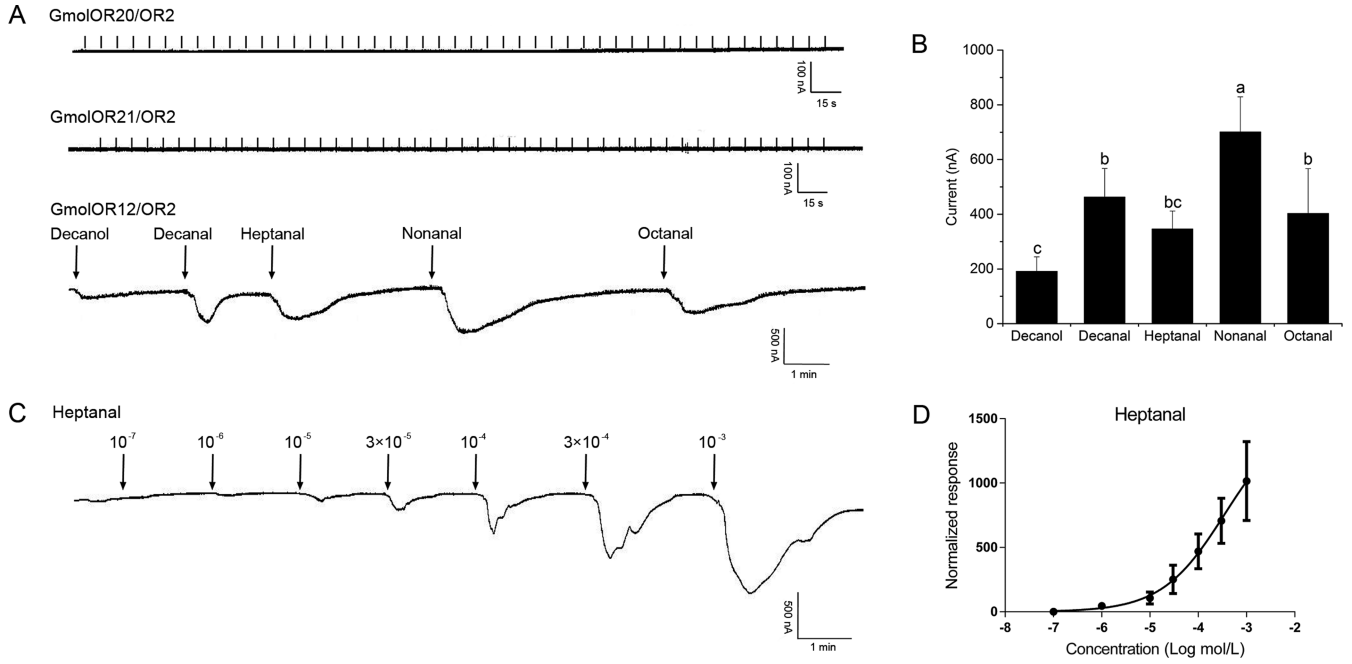

Figure 4. Responses of *Xenopus* oocytes with coexpressed GmolORs/OR2 to stimulation with odorant compounds. (A) GmolOR20/OR2 and GmolOR21/OR2 oocytes failed to respond to any of the tested odorants (upper). Inward current responses of GmolOR12/OR2 *Xenopus* oocytes in response to 10^{-4} mol/L solution of odorant compounds (lower). (B) Response profile of GmolOR12/OR2 *Xenopus* oocytes. The response value is shown as mean ± SE (n = 6). Different lowercase letters indicate significant differences among different odorants (P < 0.05, Tukey's test). (C) GmolOR12/OR2 *Xenopus* oocytes stimulated with a range of heptanal concentrations. (D) Dose−response curve of GmolOR12/OR2 *Xenopus* oocytes to heptanal. The response value was mean ± SE (n = 5).

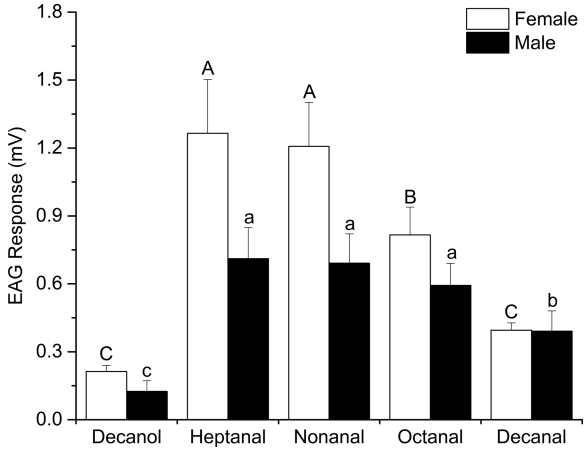

Figure 5. Electroantennogram responses of *G. molesta* to five odorants. Data represent mean ± SE (n = 10). Different capital letters and lowercase letters indicated significant differences (P < 0.05, Tukey's test) among females and males.

different OR genes have diverse functions in the same insect species.

Ligands of ORs have different shapes and structures with varying numbers of carbon atoms. GmolOR12 primarily responded to alcohols with C10 and aldehydes with C7−C10, with functional groups −OH and −CHO, respectively. Previous studies have reported that ligands of ORs have different forms and multiple sizes in other species; for instance, EposOR1 of *E. postvittana* responded to C8−C15 compounds (cyclic and acyclic forms).[40] LmigOR3 of *L. migratoria* was mainly responsive to ketones with C7−C10 and esters with C5−C8, with functional groups −CO− and −COO−, respectively.[44] GmolOR9 of *G. molesta* responded to plant volatiles of alcohols (C6−C10) and esters (C7−C9), with the −OH and −COO− functional groups, respectively.[27] The results of these studies support the idea that the length of the carbon−hydrogen chain affects the function of ligands.[48]

Aldehydes elicited higher responses in females and males by EAG, whereas the response of GmolOR12/OR2 to aldehydes was higher with the *Xenopus* oocyte system, suggesting that GmolOR12 plays a pivotal role in responding to aldehydes in the *G. molesta* olfactory system. Some of the odorants that elicit stronger responses from GmolOR12 include nonanal, octanal, and heptanal. In field experiments, nonanal from aspen trees was effective in inhibiting oviposition by female *Enarmonia formosana* on cherries,[49] whereas *Deraeocoris punctulatus* was attracted only to traps baited with octanal in cotton fields.[50] Additionally, heptanal increased the oviposition of *Phthorimaea operculella* at low concentrations (from 0.1875 to 3.0 mg/L) but repelled it at higher concentrations (from 12 to 24 mg/L).[51] Traps baited with sex pheromones and heptanal led to higher *H. armigera* catches in the field than traps baited with synthetic sex pheromones alone.[52] Accordingly, these odorants mixed with sex pheromone could be employed as attractants in fields to control *G. molesta*.

Overall, GmolORs exhibited functionally typical characteristics of general ORs, and GmolOR12 mainly responded to host-plant components including alcohols and aldehydes. These results are important for understanding the mechanisms underlying host-plant volatile perception and could provide potential targets to the design of OR-based pest control methods.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.9b07305.

Primers used in this study; odorants eliciting responses from GmolORs/Orco; all the ORs and their accession numbers used in phylogenetic analysis (PDF)

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Author Contributions

#L.C. and K.T. contributed equally to this work. X.L.X. and J.X.W. designed the experiment; L.H.C. and A.S.F. participated in collecting the biological samples and performed the experiment; L.H.C., K.T., W.L., and G.R.W. performed the *Xenopus* oocyte system; L.H.C., J.X.W., X.L.X., and W.N.C. prepared and revised the manuscript. All authors contributed to data analysis.

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Notes

The authors declare no competing financial interest.

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