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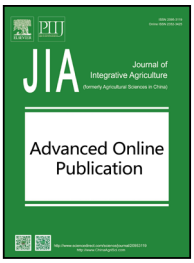

RESEARCH ARTICLE

Identification and functional characterization of a novel odorant receptor in pea aphid *Acyrthosiphon pisum*

HUANG Tian-yu[1, 3*], ZHANG Rui-bin[1, 2*], YANG Lu-lu[1], CAO Song[1], Frederic FRANCIS[3], WANG Bing[1], WANG Gui-rong[1, 2]

[1] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*

[2] *Guangdong Laboratory for Lingnan Modern Agriculture (Shenzhen Branch), Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, P.R.China*

[3] *Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Gembloux 5030, Belgium*

Abstract

Pea aphid, *Acyrthosiphon pisum*, is a serious pest of many different leguminous plants, and it mainly relies on its odorant receptors (Ors) to discriminate among host species. However, less is known about the role that Ors play in the host plant location. In this study, we identified a novel conserved odorant receptor clade by phylogenetic analysis, and conducted the functional analysis of ApisOr23 in *A. pisum*. The results showed that the homologous Ors from *A. pisum*, *Aphis glycines* and *Aphis gossypii* share 94.28% identity in amino acid sequences. Moreover, conserved motifs were analyzed using the annotated homologous Or23 from eight aphid species, providing further proof of the high conservation level of the Or23 clade. According to the tissue expression pattern analysis, *ApisOr23* was mainly expressed in the antennae. Further functional study using a heterologous *Xenopus* expression system revealed that ApisOr23 was tuned to five plant volatiles, namely *trans*-2-hexen-1-al, *cis*-2-hexen-1-ol, 1-heptanol, 4´-ethylacetophenone, and hexyl acetate. Among them, *trans*-2-hexen-1-al, which is one of the main volatile organic compounds released from legume plants, activated the highest response of ApisOr23. Our findings suggest that the conserved Or23 clade in most aphid species might play an important role in host plant detection.

Keywords: *Acyrthosiphon pisum*, odorant receptor, phylogenetic analysis, two-electrode voltage clamp, *trans*-2-hexen-1-al

1. Introduction

Insects rely on their chemosensory organs to detect and decipher a variety of chemical cues in natural environment (Hansson and Stensmyr 2011). The insect olfactory system plays a vital role in many critical behaviors related to host plant location, natural enemy avoidance, mate interactions and oviposition site selection (Leal 2013; Liu *et al.* 2013; Wada-Katsumata *et al.* 2018; Chen L *et al.* 2020). In the process of odor reception, odorants enter into the antennal

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HUANG Tian-yu, E-mail: huangty_caas@hotmail.com; ZHANG Rui-bin, E-mail: zhangruibin1990@126.com; Correspondence WANG Gui-rong, E-mail: wangguirong@caas.cn; WANG Bing, E-mail: bwang@ippcaas.cn; Frederic FRANCIS, E-mail: frederic.francis@uliege.be
* These authors contributed equally to this study.

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sensillum lymph through pores, where they are bound and transported by odorant binding proteins (OBPs), and are subsequently released to odorant receptors (Ors) which are expressed on the dendritic membranes of olfactory sensory neurons (OSNs) (Suh *et al.* 2014). Insect Ors are critical elements in the process of chemical signal transmission, as they convert the chemical signal into electrical signal which are transmitted by the central nervous system and subsequently induce a series of corresponding behavioral responses (Hallem *et al.* 2004).

One efficient way to understand how the Or repertoires of insects contribute to their adaptation to a particular environmental cue is to identify the specific ligands of these Ors. Therefore, the functions of Ors in insects have been intensively studied by using different expression systems, including many *in vitro* systems that are performed in *Xenopus* oocytes (Wang *et al.* 2011), HEK293 cells (Forstner *et al.* 2009) or Bm5 cells (Tsitoura *et al.* 2010). Other *in vivo* systems are conducted by transgenic *Drosophila* techniques with the "empty neuron" system (Dobritsa *et al.* 2003) or the *Or67d*^{GAL4} knock-in system (Kurtovic *et al.* 2007; Wang *et al.* 2016), RNA interference (RNAi) (Pan *et al.* 2020), or the clustered regularly interspaced short palindromic repeat/CRISPR-associated nuclease 9 (CRISPR/Cas9) system (Chang *et al.* 2017).

Aphids, which constitute a major family of Hemiptera, feed exclusively on plants by inserting their stylet into the sieve elements to suck sap (Moreno *et al.* 2011). Among 5 000 aphid species, many are agricultural pests and can not only feed on the phloem of plants, but also transmit plant viruses (Hodge and Powell 2010). *Acyrthosiphon pisum* is the first aphid species with sequenced genome, and its genome was re-sequenced recently (International Aphid Genomics 2010; Li *et al.* 2019); it also serves as a model for studying molecular aspects related to various biological features, such as wing dimorphism (Li *et al.* 2020; Shang *et al.* 2020), sex chromosome evolution (Jaquiery *et al.* 2018), horizontal gene transfer (Moran and Jarvik 2010), symbiont association (Hansen and Moran 2011; Manzano-Mari *et al.* 2020), and host plant adaption (Jaquiery *et al.* 2012). However, few studies have examined the chemosensory mechanisms of aphids. Currently, most studies in this area have focused on the identification and expression profiling of aphid chemoreceptors and OBPs (Robertson *et al.* 2019; Wang *et al.* 2019), but only a few Ors of pea aphid have received complete functional characterization. Our previous studies have shown that ApisOr5 is the receptor of the main alarm pheromone compound (*E*-β-farnesene) and ApisOr4 is broadly tuned to eight plant volatiles (Francis *et al.* 2005; Zhang *et al.* 2017; Zhang *et al.* 2019). Several studies also demonstrated the importance of chemical reception in aphids (Vandermoten *et al.* 2012), such as aphid–plant interactions (Sobhy *et al.* 2017) and particularly the host plant selection (Dardouri *et al.* 2019; de Oliveira *et al.* 2020). Therefore, uncovering the mechanism of odorant reception in aphids will contribute to the development of new ways to control the aphids.

In this study, we focused on a highly conserved Or clade (ApisOr23) identified from the phylogenetic tree of three aphid species. We cloned the *ApisOr23* gene from *A. pisum* antennae and further analyzed the conserved protein motifs of the Or23 clade among eight aphid species. Then, we analyzed tissue expression patterns by semi-quantitative RT-PCR. Moreover, a functional analysis was performed using the *Xenopus* oocyte system, in order to find chemicals able to stimulate ApisOr23. Our results shed light on the molecular mechanisms of those host plant detection in *A. pisum*, and will contribute to the discovery of novel pea aphid attractants or repellents.

2. Materials and methods

2.1. Insect rearing

The pea aphid *A.pisum* was fed on potted broad bean plants (*Vicia faba* L.) at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. Clonal rearing was maintained under constant environmental conditions, 21±2°C and 70±5% relatively humidity with a 16 h light: 8 h dark cycle.

2.2. RNA extraction and cDNA synthesis

Different pea aphid tissues, including 600 antennae, 300 heads without antennae, 360 legs and 5 bodies, were collected and immediately frozen in liquid nitrogen and stored at –80°C before RNA extraction. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and was exposed to DNase I (Thermo Scientific, USA) to remove genomic DNA. Reverse transcription was performed using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). We selected the cDNA sample from antennae as the template for *ApisOr23* cloning, and the cDNA samples of the four tissues mentioned above were used for semi-quantitative RT-PCR.

2.3. Identification of homologous ApisOr23 in different aphids

The *Or23* genes in five aphid genomes (*Rhopalosiphum maidis* (Chen *et al.* 2019), *Sitobion miscanthi* (Jiang *et al.* 2019), *Diuraphis noxia* (Legeai *et al.* 2010), *Myzus cerasi* (Legeai *et al.* 2010) and *Myzus persicae* (Mathers *et al.* 2017)) were annotated using *ApisOr23* (Smadja *et al.*

2009) for the query in TBLASTN searches of the genome assembly (with a cutoff 10^{-5}), and the genes obtained were named as *RmaiOr23*, *SmisOr23*, *DnoxOr23*, *McerOr23*, and *MperOr23*, respectively. Gene models were checked manually. The amino acid sequences of these genes are listed in Appendix A.

2.4. Sequence and phylogenetic analysis

The transmembrane domains of ApisOr23, AglyOr14 and AgosOr23 were predicted by TMHMM version 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The alignment of the amino acid sequences was generated by DNAMAN version 8 (Lynnon LLC, San Ramon, CA, USA), and carried out using the Ors sequences from three aphid species (*A. pisum*, *Aphis glycines*, and *Aphis gossypii*) (Cao *et al.* 2014; Robertson *et al.* 2019). AgosOr18, AgosOr31, AgosOr40 and AgosOr44 were excluded because of their depressed annotation quality. The alignment was generated by Mafft version 7.0 (Katoh and Standley 2013) with default settings, and trimmed by TrimAl version 1.4 with the "gappyout" option (Capella-Gutierrez *et al.* 2009). The motifs that were conserved among the aphids were identified by the MEME Program (Bailey *et al.* 2009) with a maximum number of motifs of ten, and decorated by TBtools Program (Chen C *et al.* 2020). The phylogenetic analysis was conducted by MEGA7 (Kumar *et al.* 2016) using the neighbor-joining method, and node support was assessed using a bootstrap procedure of 1 000 replicates. The resultant tree was constructed by Evolview version 2 (He *et al.* 2016).

2.5. Molecular cloning

The open reading frame (ORF) of *ApisOr23* was cloned using a coding sequence identified from the first version of the *A. pisum* genome (International Aphid Genomics 2010). The 25 μL PCR reaction system contained 0.25 μL PrimeSTAR HS DNA polymerase (2.5 units μL^{-1}), 1 μg μL^{-1} cDNA template, 5 μL 5× PrimeSTAR buffer (Mg^{2+} Plus), 2 μL dNTP mixture (2.5 mmol L^{-1} of each), and 10 μmol L^{-1} of each primer. The PCR was performed according to the following conditions: 94°C for 5 min; 40 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; and 72°C for 10 min. The PCR products were ligated into the cloning vector pEASY-Blunt (TransGen Biotech, Beijing, China) and verified by DNA sequencing. The ORF of *ApisOr23* was ligated into the pT7TS expression vector using specific primers with restriction enzyme cutting sites (Appendix B).

2.6. Tissue expression pattern of *ApisOr23* gene

The expression pattern of *ApisOr23* was detected by semi-quantitative RT-PCR. The first cDNA strand was synthesized from the RNA of each tissue, namely antennae (A), heads without antennae (H), legs (L) and bodies (B). The succinate dehydrogenase B (*ApisSDHB*) gene (GenBank accession number: NM_001162436) (Yang *et al.* 2014) was selected as a reference. The specific primers used in RT-PCR are listed in Appendix B. The RT-PCR reactions were performed using EasyTaq SuperMix (TransGene, Strasbourg, France) under conditions of 95°C for 3 min; 28 cycles of 94°C for 30 s, 55–60°C for 30 s, 72°C for 30 s; and 72°C for 10 min. The experiment was biologically repeated three times.

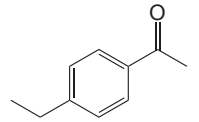
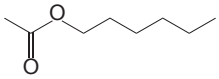
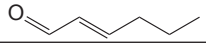
2.7. Chemical compounds

The 57 representative compounds used in this study are listed in Table 1 and Appendix C. These compounds include common host plant volatiles and aphid alarm pheromones.

2.8. *Or* expression in *Xenopus* oocytes and electrophysiological recordings

The ORF of *ApisOr23* was subcloned into the pT7TS

Table 1 The information of odorants tuning to ApisOR23/Orco

Name	CAS numbers	Chemical formula	Structural formula
cis-2-Hexen-1-ol	928-94-9	$C_6H_{12}O$	
1-Heptanol	111-70-6	$C_7H_{16}O$	
4´-Ethylacetophenone	937-30-4	$C_{10}H_{12}O$	
Hexyl acetate	142-92-7	$C_8H_{16}O_2$	
trans-2-Hexen-1-al	6728-26-3	$C_6H_{10}O$	

vector based on the restriction enzyme digestion sites. The cRNA was synthesized by mMESSAGE mMACHINE T7 Kit (Ambion, Austin, TX, USA). Mature healthy oocytes were treated according to a previous study (Zhang *et al.* 2019). Oocytes were microinjected with 27.6 ng of *ApisOr23* cRNA and 27.6 ng *ApisOrco* cRNA (Zhang *et al.* 2019), then cultured for 4–7 days at 18°C. The cell currents induced by the odorants were recorded with a two-electrode voltage clamp (TEVC). Data acquisition and analysis were performed with Digidata 1440 A and Pclamp10.0 Software (Axon Instruments Inc., Union City, CA, USA). Each odorant used in this study (Appendix C) was prepared as a 1 mol L^{-1} stock solution in dimethyl sulphoxide (DMSO) and stored at −20°C. Before the experiments, stock solutions were diluted in 1× Ringer's buffer to a final concentration of 10^{-4} mol L^{-1}. Data were analyzed using software SAS 9.1, by the one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. Statistical significance was determined at the α=0.05 level.

3. Results

3.1. Phylogenetic and conserved motif analysis of the Or23 clade

Previous studies have shown that conserved Ors might ensure a number of crucial biological functions in aphids, such as alarm pheromone detection and plant volatile reception (Zhang *et al.* 2017; Zhang *et al.* 2019). Therefore, we selected *Or* genes from three aphid species, including *A. pisum*, *A. glycines* and *A. gossypii*, that were already annotated from previous genome studies (Cao *et al.* 2014; Robertson *et al.* 2019). The amino acid sequences of these Ors were used for phylogenetic analysis in order to discover the conserved Or clade. Intriguingly, ApisOr23, AgosOr23 and AglyOr14 were clustered together and showed a highly homologous relationship among these three aphids, indicating that this clade is relatively well-conserved (Fig. 1).

3.2. Gene cloning and sequence analysis

The sequence of *ApisOr23* was obtained from published data (Robertson *et al*. 2019). Specific primers were designed for cloning the full-length ORF of *ApisOr23* from antennal cDNA. The ORF of the *ApisOr23* gene was 1 242 bp, encoding 414 amino acids. The alignment of amino acid identity showed that ApisOr23 shared 94.28% sequence identity with its orthologous AglyOr14 and AgosOr23, and possessed seven transmembrane domains (Fig. 2).

To further confirm whether this clade is conserved among aphid species, we annotated the corresponding orthologous genes from five other aphids (*R. maidis*, *S. miscanthi*, *D. noxia*, *M. cerasi* and *M. persicae*). The amino acid sequences of the eight homologous *Or23* genes from all eight aphids mentioned above were included in the conserved motif analysis by MEME Program (the full sequences are listed in Appendix A). A total of ten conserved motifs were predicted by the MEME Program (Appendix D). These Ors shared a highly conserved motif pattern, as each gene included all ten motifs, and the motifs were in almost the same order (motif order: 7-6-4-1-5-10-3-8-2-9) and locations (Fig. 3). Furthermore, seven of the ten motifs possessed extremely high conservation, with *P*-values less than 10^{-190}. Such highly conserved amino acid sequence patterns indicated that these Or23s might tune to the same ligand spectrum, as the motifs covered almost all the receptor sequences, and consequently demonstrated the conservation of most of the functional amino acid sites.

3.3. Tissue expression pattern of *ApisOr23*

In order to investigate the expression pattern of *ApisOr23*, we selected *SDHB* as the reference gene, and carried out RT-PCR on the tissues of antennae, heads without antennae, legs and remaining bodies. The high expression level of *ApisOr23* was found in the antennae, while considerably lower expression level was detected in the legs. No expression was found in the tissues from the heads and bodies (Fig. 4).

3.4. Functional characterization of ApisOr23/Orco

The *ApisOr23*/*Orco* co-expressing *Xenopus* oocytes were used for functional characterization by two-electrode voltage clamps. A total of 57 plant volatiles were tested (listed in Appendix C). ApisOr23 mainly tuned to five of the chemicals, including aromatic ketone (4′-ethylacetophenone) and aliphatic compounds (*cis*-2-hexen-1-ol, 1-heptanol, hexyl acetate and *trans*-2-hexen-1-al) (Fig. 5-A and B; Table 1). However, there were no measurable responses to the other tested chemicals. The highest response of ApisOr23/Orco was induced by *trans*-2-hexen-1-al (207.81±17.63 nA), while 4′-ethylacetophenone and hexyl acetate activated the low responses with current values of 44.18±5.03 and 45.12±6.71 nA, respectively. Oocytes co-expressing ApisOr23/Orco had moderate responses to *cis*-2-hexen-1-ol and 1-heptanol (87.40±8.61 and 28.20±7.82 nA, respectively) (Fig. 5-C).

4. Discussion

Ors play an important role in the process of host plant volatile detection among various insect species. The functions of Ors from the model species *Drosophila melanogaster*, as well as many other species from Lepidoptera, Hemiptera and

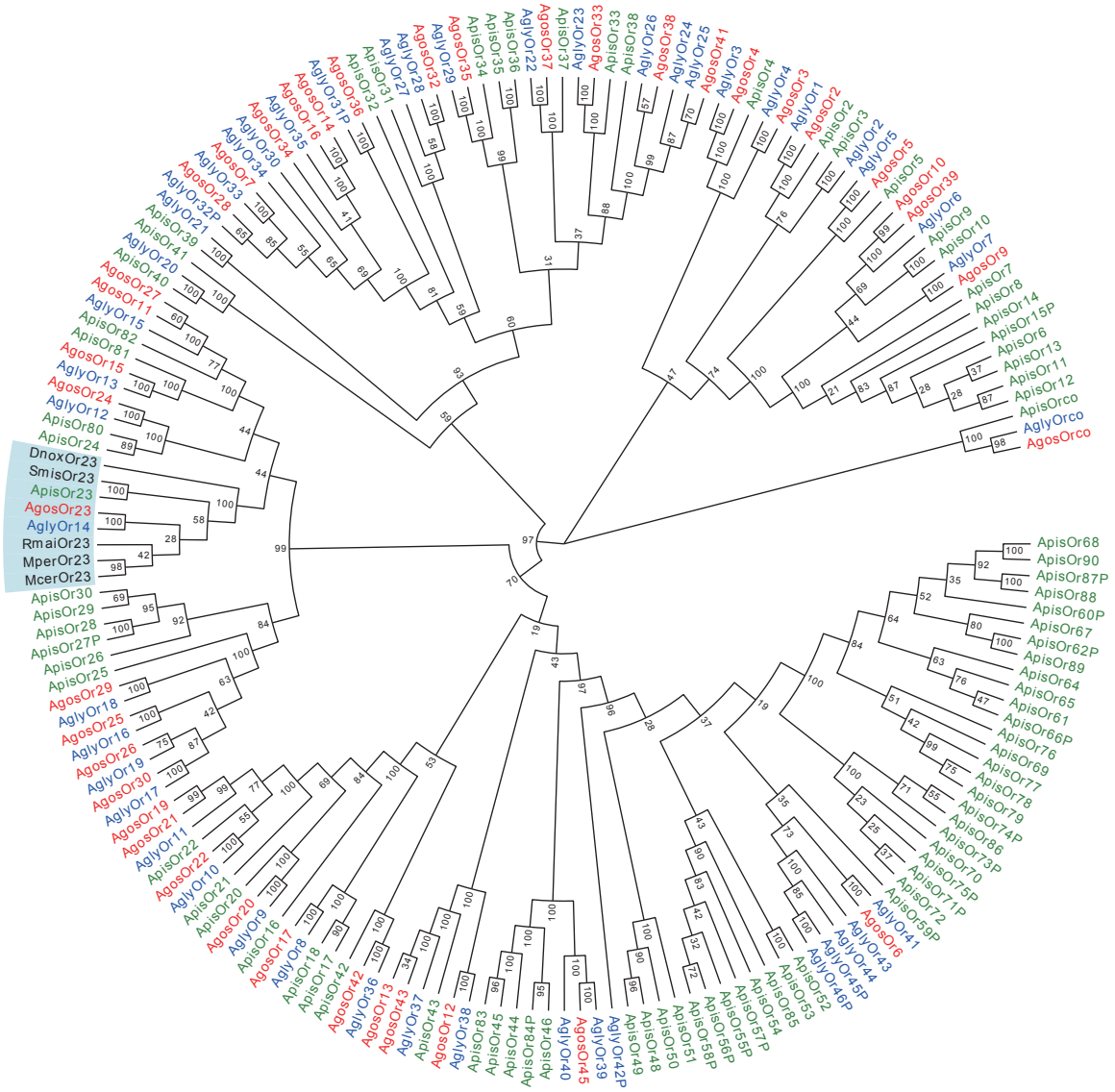


Fig. 1 Phylogenetic analysis of odorant receptors (Ors) in *Acyrthosiphon pisum*, *Aphis gossypii* and *Aphis glycines* in addition to five Or23s from *Rhopalosiphum maidis*, *Sitobion miscanthi*, *Diuraphis noxia*, *Myzus cerasi* and *Myzus persicae*. The predicted amino acid sequences of the Ors were aligned using the Mafft V7.0 Program. The phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1 000 bootstrap replicates by MEGA 5.0. This neighbor-joining tree was rooted with the Orco proteins, and indicated one highly conserved Or clade within many insect species. Abbreviations of *A. pisum*, *A. gossypii* and *A. glycines* are shown respectively as Apis in green, Agos in red, Agly in blue, and the five species listed above as Rmai, Smis, Dnox, Mcer, and Mper in black. The ApisOR23 clade is masked with light blue shadow.

Diptera, have been studied in recent years (Wicher *et al.* 2008; Dweck *et al.* 2015; Cui *et al.* 2018; Khashaveh *et al.* 2020; Liu *et al.* 2020; Wang *et al.* 2020). Nevertheless, only few studies were conducted on the function of Ors in aphids. The pea aphid has a complex plant-specialized population, displaying a highly adaptive evolution (Duvaux *et al.* 2015; Eyres *et al.* 2016). It can feed on multiple legumes, while many other aphids are reported to be specialists (Ragsdale *et al.* 2004). With the support of increasingly available genomics and transcriptomics data, more than 70 *Or* genes have been identified from the *A. pisum* genome (Robertson *et al.* 2019), and most *Or* genes have experienced recent and rapid expansion, which might indicate that such gene expansion is essential for host plant acceptance (Caillaud and Via 2000; Smadja *et al.* 2009; Smadja *et al.* 2012).

The odorant receptor gene family evolves under a birth-and-death process, which means *Ors* genes undergo many evolutionary events, including duplications, deletions, pseudogenizations and positive selection (McBride *et al.* 2007; Smadja *et al.* 2009). Comparisons of the *Or* gene family members from diverse insect species have revealed striking differences in gene family size (Robertson

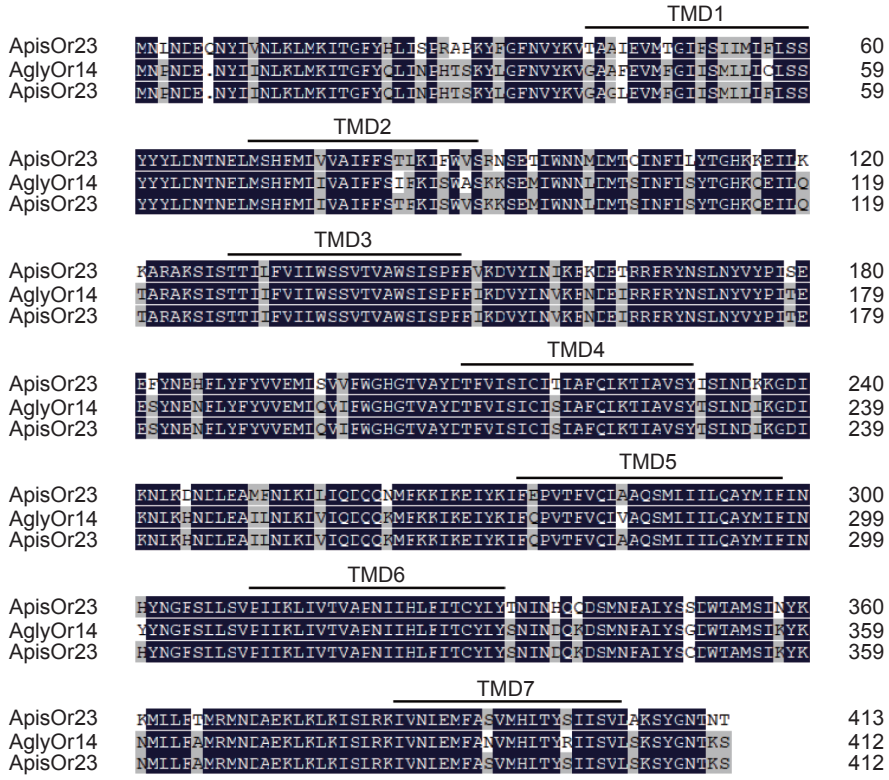


Fig. 2 Sequence alignment of ApisOr23, AglyOr14, and AgosOr23. The amino acid identity of the three sequences is 94.28%. Conserved amino acids are covered in black boxes while the unique amino acid sites are represented by grey and white boxes. Seven transmembrane domains (TMD) are predicted and marked with black lines.

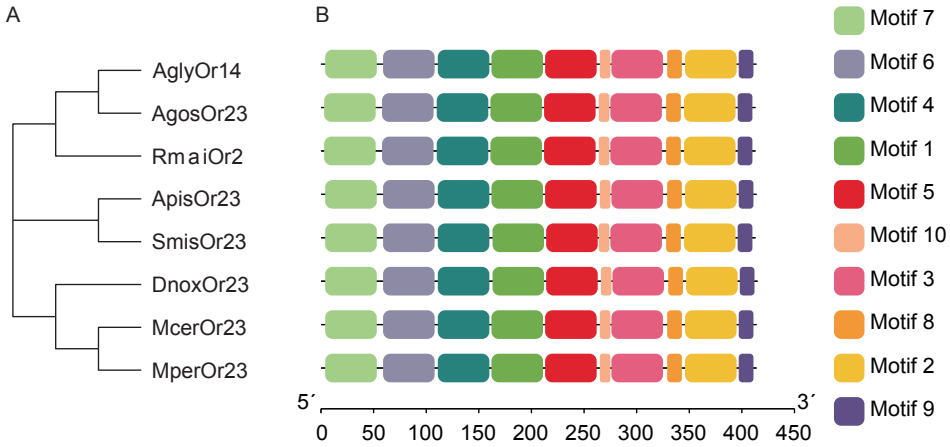


Fig. 3 Phylogenetic analysis and conserved motifs of the Or23 clade of eight aphid species. A, phylogenetic tree of the eight species in the Or23 clade. Agly, *Aphis glycines*; Agos, *Aphis gossypii*; Rmai, *Rhopalosiphum maidis*; Apis, *Acyrthosiphon pisum*; Smis, *Sitobion miscanthi*; Dnox, *Diuraphis noxia*; Mcer, *Myzus cerasi*; Mper, *Myzus persicae*. B, schematic distribution of conserved motifs in the Or23 clade. Motif analysis was carried out using MEME Software. The colored boxes represent conserved motifs that were located in the corresponding location of each Or.

Although such a feature is remarkably common, even in closely related species, we still noticed that many receptors remain quite conserved among different aphid species. These conserved ORs not only showed sequence similarity, but also possessed highly consistent odorant response profiles (Cao *et al.* 2016). Moreover, the highly conserved Ors may play an important role in the key life processes of insects. For example, the major component

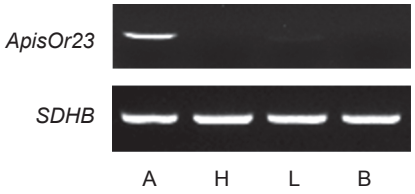


Fig. 4 Tissue expression pattern of *ApisOr23* using semi-quantitative RT-PCR. The succinate dehydrogenase B (*SDHB*) gene (GenBank: NM_001162436) was selected as reference. A, antennae; H, heads (antennae removed); L, legs; B, bodies.

of aphid alarm pheromone, (*E*)-β-farnesene, was detected by two highly conserved odorant receptors from *A. pisum* and *A. gossypii* (Zhang *et al.* 2017b). Here, we identified another odorant receptor of *A. pisum*, named *ApisOr23*. The amino acid sequences are conserved among three different aphid species, which utilize relatively distinctive host species. In order to further confirm that the Or23 clade is conserved among different aphids, we annotated ApisOr23 homologous genes from five other aphids, and performed the conserved motif analysis. The Or23 clade was shown to be considerably conserved among the different species, suggesting that the *Or23* genes of various aphid species might play an essential role in host plant location or other behaviors, such as oviposition site-selection. Future works on the functions of other *Or23* clade members would provide more evidences for this hypothesis.

It has reported that *cis*-2-hexen-1-ol, hexyl acetate and *trans*-2-hexen-1-al are the most common green leaf volatiles (GLVs) from plants. Specially the latter one is the main volatile released from legumes (Pareja *et al.* 2009). The attractiveness of GLVs (including *trans*-2-hexen-1-al) has been reported in the black bean aphid *Aphis fabae* (Webster *et al.* 2008). In addition, *trans*-2-hexen-1-al showed a significant attractiveness to the tea aphid *Toxoptera aurantii* (Han *et al.* 2012; Bian *et al.* 2014), suggesting that *trans*-2-hexen-1-al is involved in the attraction of various aphid species. Therefore, we hypothesized that *trans*-2-hexen-1-al may also attract the pea aphid. In this study, we found that *trans*-2-hexen-1-al was the best ligand for ApisOr23, and the Or23 clade is significantly conserved among the eight aphid species, indicating that Or23 in aphids is involved in signal discrimination of *trans*-2-hexen-1-al. However, we cannot exclude the possibility that there may be other odorant receptors which also respond to *trans*-2-hexen-1-al, leading to some kind of combinatorial coding that could affect the aphid's behavior. For example, numerous Ors from *D. melanogaster* showed responses to *trans*-2-hexen-1-al, including Or7a, Or35a, Or42a and Or67b, and others, among which DmelOr7a was the main receptor tuned to *trans*-2-hexen-1-al, and it is involved in aggregation behavior and oviposition site-selection (Kreher *et al.* 2005, 2008; Lin *et al.* 2015). Therefore, future work should systematically study the peripheral coding map of aphids to odorant detection and host selection behavior in order to elucidate the molecular mechanisms of the detection of host plant volatiles.

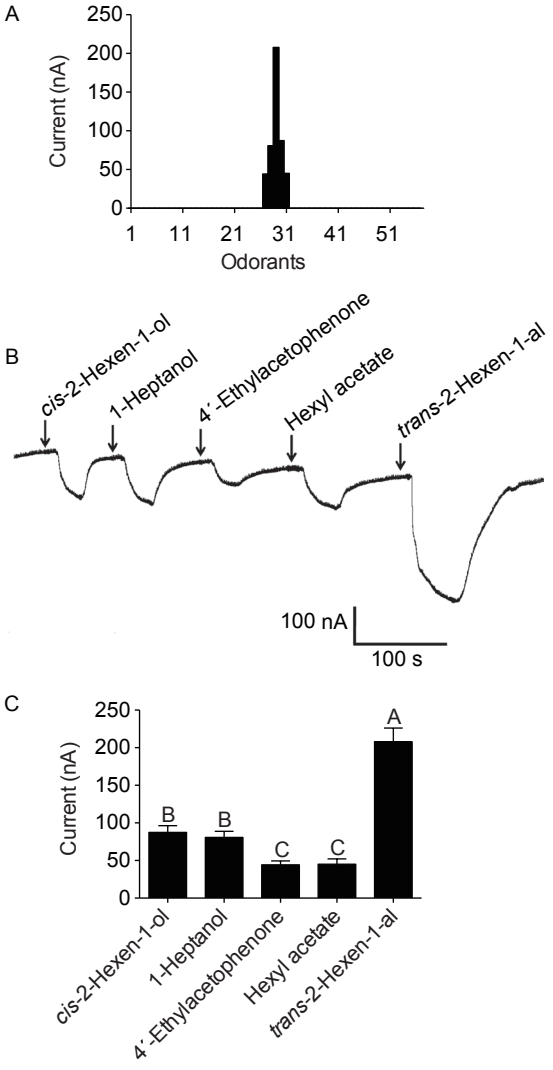


Fig. 5 Functional characterization of ApisOr23/Orco response to odorants in the *Xenopus* oocyte system. A, tuning curves of ApisOr23 to 57 individual plant volatiles. The *x*-axis shows the number of the tested odorants (Appendix C). The *y*-axis shows the strength of current values (nA) of the ApisOr23/Orco upon exposure to the odorants, with the strongest response in the center. B, inward current responses of ApisOr23/Orco stimulated with five odorants as 10^{-4} mol L^{-1} solutions. C, response profile of ApisOr23/Orco. Error bars indicate mean±SEM (n=13). Different uppercase letters indicate significant differences of the current values stimulated by different odorants (P<0.05).

Interestingly, *trans*-2-hexen-1-al has also proven to be one of the main herbivore-induced plant volatiles (HIPVs) induced by a chewing herbivore, the beet armyworm caterpillar, *Spodoptera exigua* (Schwartzberg *et al.* 2011). So, it may act as an indirect defensive signal of plants by

repelling pests or attracting natural enemies (Allmann and Baldwin 2010). When caterpillars and aphids co-occur on the same plant, caterpillar-induced *trans*-2-hexen-1-al may act as a negative signal reducing the aggregation of aphids because they prefer undamaged plants rather than caterpillar-infested plants (Ray *et al.* 2020). Therefore, we hypothesize that *trans*-2-hexen-1-al acting as common GLVs (at low concentration) may attract *A. pisum*. However, *trans*-2-hexen-1-al acting as HIPVs are induced in quantity (at high concentration) displaying repellent effect on the aphids when caterpillars and aphids co-occur on the same niche. This phenomenon has been proved recently that the attractions and aversions of *D. melanogaster* to alcohol are mediated by three separate neural pathways, as DmelOr42b and DmelOr59b are necessary for attraction to alcohol at low concentration, while aversion behavior to high concentration level of alcohol is detected by DmelOr42a (Keesey *et al.* 2020). Therefore, we speculate that other ORs in aphids, besides ApisOR23, may involve in mediating the attraction or aversion to *trans*-2-hexen-1-al in aphids.

5. Conclusion

In this study, we annotated the *Or23* genes from five aphid genomes, and the subsequent phylogenetic and conserved motif analysis showed that the Or23 clade was highly conserved among the different aphids. By using a heterologous expression system in *Xenopus* oocytes, the response of ApisOr23/Orco was activated by five plant volatiles, of which *trans*-2-hexen-1-al released from legume plants presented the highest response level. This result indicated that *trans*-2-hexen-1-al might act as an important chemical cue for host selection of aphid.

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