Insect Molecular Biology

Insect Molecular Biology (2019) **28**(1), 23–34 doi: 10.1111/imb.12523

An odorant receptor mediates the attractiveness of *cis*-jasmone to *Campoletis chlorideae,* the endoparasitoid of *Helicoverpa armigera*

Y.-L. Sun*, J.-F. Dong*‡, C. Ning*, P.-P. Ding*, L.-Q. Huang*, J.-G. Sun*§ and C.-Z. Wang*†

**State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; †College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China; ‡Forestry College, Henan University of Science and Technology, Luoyang, Henan Province, China; and §Biology and Food Engineering College, Anyang Institute of Technology, Anyang, Henan Province, China*

Abstract

Parasitic wasps rely on olfaction to locate their hosts in complex chemical environments. Odorant receptors (ORs) function together with well-conserved odorant coreceptors (ORcos) to determine the sensitivity and specificity of olfactory reception. *Campoletis chlorideae* (Hymenoptera: Ichneumonidae) is a solitary larval endoparasitoid of the cotton bollworm, *Helicoverpa armigera*, and some other noctuid species. To understand the molecular basis of *C. chlorideae*'s olfactory reception, we sequenced the transcriptome of adult male and female heads (including antennae) and identified 211 *OR* transcripts, with 95 being putatively full length. The tissue expression profiles, as assessed by reverse-transcription PCR, showed that seven *OR*s were expressed only or more highly in female antennae. Their functions were analysed using the *Xenopus laevis* oocyte expression system and two-electrode voltage-clamp recordings. CchlOR62 was tuned to *cis*-jasmone, which was attractive to female *C. chlorideae* adults and *H. armigera* larvae in the subsequent behavioural assays. Further bioassays using caged plants showed that the parasitism rate of *H. armigera* larvae by *C. chlorideae* on *cis*-jasmone-treated tobacco plants was higher than on the control plants. Thus, *cis*-jasmone appears to be an important infochemical involved in the interactions of plants, *H. armigera* and *C. chlorideae*, and CchlOR62 mediates the attractiveness of *cis*-jasmone to *C. chlorideae*.

Keywords: *Campoletis chlorideae*, odorant receptor, *Xenopus* expression system, two-electrode voltage-clamp recording, behavioural assay.

Introduction

Parasitoids serve as important control agents of herbivore pests in agricultural ecosystems. The success and effectiveness of parasitic wasps in suppressing pest populations depend on their abilities to locate hosts in complex chemical environments (Dicke, 1999; Turlings *et al.*, 2001). Herbivore-induced plant volatiles (HIPVs) are important signals that aid in guiding parasitic wasps to their host herbivores (Turlings *et al.*, 1990; Dicke, 2009; McCormick *et al.*, 2012; Aartsma *et al.*, 2017; Turlings and Erb, 2018). In the past three decades, components of HIPVs emitted by herbivore-infested plants and their roles as host location cues in the foraging behaviour of parasitic wasps have been extensively studied in the laboratory and small-scale field experiments (De Moraes *et al.*, 1998; D'Alessandro *et al.*, 2009). In some systems, blends of complex HIPVs were responsible for the attraction of the parasitoids (Birkett *et al.*, 2003; Morawo and Fadamiro, 2016). In other systems, the presence of a single component is sufficient to attract parasitoids (Wei *et al.*, 2007; Yu *et al.*, 2010).

The olfactory system mediates the attractive behaviour of parasitoids to HIPVs. Odorants in the surrounding environment are detected by olfactory sensilla mainly distributed on the antennae. Electrophysiological studies using electroantennograms (EAGs) and single sensillum recordings revealed that the olfactory sensory neurones

First published online 5 October 2018.

Correspondence: Chen-Zhu Wang, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. Tel.: + 86 10 64807115; fax: + 86 10 64807099; e-mail: czwang@ioz.ac.cn

in the antennal sensilla of parasitoids respond to certain HIPV components (Dweck *et al*., 2013; Tamiru *et al*., 2015). Odorant receptors (ORs) function with well-conserved odorant coreceptors (ORcos) in the dendrites of olfactory sensory neurones to determine the sensitivity and specificity of olfactory reception (Larsson *et al*., 2004; Benton *et al*., 2006; Hallem and Carlson, 2006). *Orco* double-stranded RNA-treated *Microplitis mediator* displayed a lower attraction-based flying rate to nonanal and farnesene when compared with controls in a Y-tube olfactometer bioassay, suggesting that the OR-based signalling pathways play important roles in parasitoids' attraction to HIPVs (Li *et al*., 2012). Genome analyses and transcriptome sequencing have been used to characterize the OR repertoires in a number of parasitoid species (Robertson *et al*., 2010; Zhou *et al*., 2015; Wang *et al*., 2017a, 2017b), increasing our understanding of the roles of ORs in the perception of chemosensory stimuli. However, at present, just one OR in one species that tunes to HIPVs has been deorphanized. RNA interference experiments coupled with EAG and behavioural studies on *Anastatus japonicus*, the parasitic wasp of the litchi pest *Tessaratoma papillosa*, showed that AjapOR35 was tuned to the oviposition attractants β-caryophyllene and (*E*)-α-farnesene (Wang *et al*., 2017b). The functions of the majority of ORs are still unknown in most parasitoid species.

Campoletis chlorideae (Hymenoptera: Ichneunmonidae) is a solitary larval endoparasitoid of many noctuid species, including *Helicoverpa armigera*, *Helicoverpa assulta*, *Mythimna separata*, *Spodoptera exigua*, *Spodoptera litura*, *Agrotis ypsilon* and *Anomis flava* in China (Li *et al*., 1997; You *et al*., 2002; Han *et al*., 2015). It is widespread in the Yellow River and Yangtze River Valleys as a main natural enemy of *H. armigera* (You *et al*., 2002). Early studies in our laboratory mainly focused on the analysis of the HIPVs released from the herbivore-infested host plants, as well as the behavioural and electrophysiological responses of *C. chlorideae* to these compounds (Yan *et al*., 2005; Yan and Wang, 2006a, 2006b; Yan *et al*., 2006). Maize plants infested by *H. armigera* and *My. separata* were more attractive to *C. chlorideae* than undamaged plants (Yan and Wang, 2006a). The molecular mechanism responsible for *C. chlorideae*'s sensing of HIPVs needs to be investigated.

To understand the molecular basis of the olfactory-based attraction of this endoparasitoid species to HIPVs, we initially sequenced the transcriptome of the *C. chlorideae* head using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA), and then we analysed the expression profiles of putative full-length *OR*s using reverse-transcription PCR. Next, we used the *Xenopus laevis* oocyte expression system and two-electrode voltage-clamp recordings to study the functions of female antennae-biased ORs when challenged with a range of HIPVs. CchlOR62 responded robustly to *cis*-jasmone. Therefore, we tested the EAG responses of the antennae of mated female parasitoids to *cis*-jasmone. We also studied the behavioural responses of mated female parasitoids and *H. armigera* larvae to this chemical. Finally, we conducted cage experiments to compare the parasitism rates of *H. armigera* larvae by *C. chlorideae* on *cis*-jasmone-treated and untreated tobacco plants. The study indicated that *cis*-jasmone could be used in the biological control of herbivore pests.

Results

The candidate ORs in C. chlorideae

Based on the sequence similarity to insect ORs, we identified 211 candidate *OR* genes from Illumina HiSeq 2000 transcriptome data of female and male heads (including antennae and mouthparts). All of these genes were submitted to the National Center for Biotechnology Information (NCBI) database, under the accession numbers MG859290–MG859500. We selected 151 *C. chlorideae* ORs, together with the 151 *Mi. mediator* ORs (Wang *et al*., 2017a), 173 *Apis mellifera* ORs (Robertson and Wanner, 2006; Zhou *et al*., 2015) and 301 *Nasonia vitripennis* ORs (Robertson *et al*., 2010; Zhou *et al*., 2012) to analyse their phylogenetic relationships in a neighbour-joining tree. The ORcos of the four species are highly conserved and clustered in one branch, but the other ORs are extremely divergent amongst species, and most ORs from the same species formed monophyletic groups. Only 13% of the CchlORs are firstly grouped with *Mi. mediator*, *Ap. mellifera* or *N. vitripennis* ORs (Fig. S1).

In total, 95 of the *CchlORs* were putative full-length cDNAs encoding more than 380 amino acids. Details for the 95 *OR*s, including gene names, lengths and BLASTx algorithm-based best hits, are listed in Table S1. Their transcript levels were further verified with reverse transcription-PCR (RT-PCR) in female and male heads (Fig. S2). The results of the study showed that *CchlOR14*, *CchlOR52*, *CchlOR53*, *CchlOR60*, *CchlOR62*, *CchlOR63* and *CchlOR85* were predominately present in the female head [the ratio of Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) between female head and male heads (F/M) $\geq$ 9.99]. *CchlOR20*, *CchlOR26*, *CchlOR30*, *CchlOR47*, *CchlOR56* and *CchlOR70* were highly enriched in the male head (F/M $\leq$ 0.05). By contrast, the expression levels of the other 82 *OR*s, including *CchlORco*, were not significantly different between the two sexes (0.05 < F/M < 9.99; Table S1; Fig. S2).

Tissue distribution of female head-biased OR genes in C. chlorideae

The host location of *C. chlorideae* through HIPV cues is a female-specific behaviour, so we hypothesize the ORs involved in this behaviour would be upregulated in

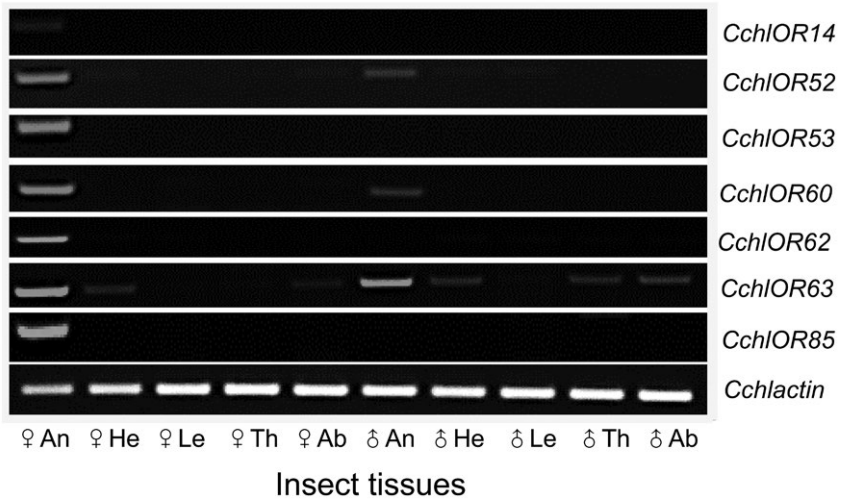

Figure 1. Expression patterns of female head-biased odorant receptor (OR) genes in different *Campoletis chlorideae* (*Cchl*) tissues. ♀An, female antennae; ♀He, female heads; ♀Le, female legs; ♀Th, female thoraxes; ♀Ab, female abdomens; ♂An, male antennae; ♂He, male heads; ♂Le, male legs; ♂Th, male thoraxes; ♂Ab, male abdomens.

females' antennae compared to males. The expression profiles of the seven female head-biased *OR*s were further investigated in different *C. chlorideae* tissues using RT-PCR. By comparing the expression levels in female antennae, male antennae and other body tissues, we found that all seven *OR*s were mainly expressed in the female antennae. In addition, *CchlOR52*, *CchlOR60* and *CchlOR63* were slightly expressed in male antennae. There were also weak PCR bands corresponding to *CchlOR63* in the male thorax, male and female heads, and male and female abdomens (Fig. 1).

Functional analysis of female antennae-biased ORs

To investigate the functions of the seven female antennae-biased *OR*s, we expressed them with *CchlORco* in *X. laevis* oocytes, following a strategy successfully used in functional studies of *OR*s from other insect species (Nakagawa *et al.*, 2005; Wang *et al.*, 2010; Yang *et al.*, 2017). We chose 19 compounds as stimulants for two-electrode voltage-clamp recordings. These chemicals included green leaf volatiles, terpenoids, and both aliphatic and aromatic compounds, which were detected as being emitted from *H. armigera*- and *My. separata*-infested maize (Yan and Wang, 2006a), *H. armigera*- and *H. assulta*-infested tobacco (Yan *et al.*, 2005) and *Spodoptera exigua*-infested cotton (Loughrin *et al.*, 1994) (Table 1). Two compounds, jasmonic acid and methyl jasmonate, as plant defence hormones, were also included. Information regarding the standard compounds is listed in Table 1. The expressed ORs were stimulated with 10^{-4} M concentrations of compounds. The *CchlOR62/CchlORco*-expressed oocytes responded with a high sensitivity (~700 nA) to *cis*-jasmone (Fig. 2A, B). *CchlOR52/CchlORco*-, *CchlOR53/CchlORco*-, *CchlOR60/CchlORco*-, *CchlOR63/CchlORco*- and *CchlOR85/CchlORco*-expressing oocytes did not exhibit measurable electrophysiological responses to any of the 19 chemicals tested (Fig. S3). *CchlOR14*, the plasmid of which cannot be linearized by enzymes, was not tested in the subsequent recording assays. We focused on *CchlOR62* and conducted dose-response experiments for *cis*-jasmone. The oocytes expressing *CchlOR62/CchlORco* elicited dose-dependent responses from 10^{-4} M to 10^{-3} M concentrations (Fig. 2C). The dose-response test was stopped at 10^{-3} M because the control cells (not injected with OR cRNA) responded to 10^{-2} M *cis*-jasmone.

C. chlorideae *females' EAG responses to cis-jasmone*

As CchlOR62 tuning to *cis*-jasmone is highly expressed in female antennae, we investigated EAG dose-response relationships of mated female *C. chlorideae* to *cis*-jasmone. At doses ≥ 100 ng in the odour cartridge, *cis*-jasmone elicited significant EAG responses compared with the control. The EAG voltage was saturated at the 1 μg dose (Fig. 3).

Attractiveness of cis*-jasmone to* C. chlorideae *and* H. armigera *larvae*

Based on the high expression of CchlOR62 and EAG responses to *cis*-jasmone in the female adults, we speculate that *C. chlorideae* females must have behavioural responses to *cis*-jasmone. When the attractiveness of *cis*-jasmone was investigated in a Y-tube olfactometer with mated male and female wasps, we found that *cis*-jasmone (100 μg) was significantly attractive to

Table 1. Test compounds in behavioural assay of *Campoletis chlorideae* and *Helicoverpa armigera* larvae and functional analysis of odorant receptors of *C. chlorideae*.

Odour	CAS number*	Maize (Yan and Wang, 2006a)	Tobacco (Yan *et al.*, 2005)	Cotton (Loughrin *et al.*, 1994)
GLV*				
(*Z*)-3-hexen-1-ol	928-96-1	✓	✓	✓
(*Z*)-3-hexenyl acetate	3681-71-8	✓	✓	✓
(*E*)-2-hexen-1-ol	928-95-0	✓	✓	-
(*E*)-2-hexenal	6728-26-3	✓	✓	✓
(*E*)-2-hexenyl acetate	2497-18-9	✓	✓	-
Terpenoid				
Myrcene	123-35-3	✓	-	✓
β-pinene	99-83-2	✓	✓	✓
D-limonene	5989-54-8	✓	-	✓
Farnesene	502614	✓	-	✓
Ocimene	13877-91-3	-	-	✓
(*E*)-caryophyllene	87-44-5	-	-	✓
Linalool	78-70-6	✓	-	✓
Aliphatic				
Nonanal	124-19-6	-	✓	-
Methyl jasmonate	39924-52-2	-	-	-
Cis-jasmone	488-10-8	-	-	✓
Jasmonic acid	221682-41-3	-	-	-
Aromatic				
Methyl salicylate	119-36-8	-	✓	-
Indole	120-72-9	✓	-	✓
Phenylethyl acetate	103-45-7	✓	-	-

Note: ✓means the odorants identified in the plant reported in cited paper.

- represents the compounds not reported in cited paper.

*CAS number: chemical abstracts service number; GLV: green leaf volatile

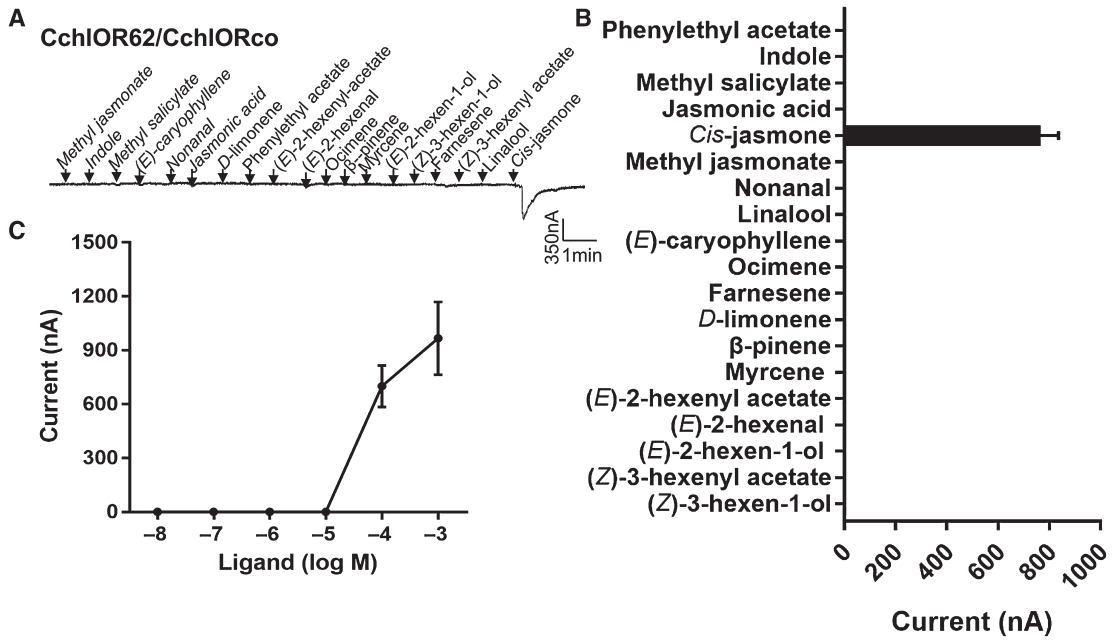

Figure 2. Functional analysis of different female antennae-biased *Campoletis chlorideae* odorant receptors (CchlORs) in *Xenopus laevis* oocytes. (A) Inward current responses of *X. laevis* oocytes in response to odorants (10^{-4} M solutions). Applied odorants are indicated by arrowheads. (B) Odorant-response spectrum of CchlOR62/odorant coreceptor (ORco). Responses were measured as induced inward currents, expressed in nA. Error bars represent the standard errors of the means ($n = 5$). (C) Dose-response of *CchlOR62*/*ORco*-expressing oocytes stimulated with *cis*-jasmone (10^{-8} M–10^{-3} M) ($n = 3$–5).

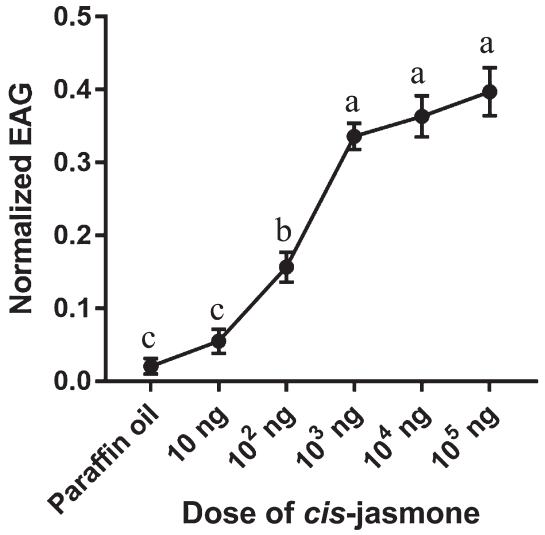

Figure 3. Electroantennogram (EAG) dose-response curves of female *Campoletis chlorideae* to *cis*-jasmone. Error bars indicate standard errors of the means for $n = 10$ mated female parasitoids. Different letters indicate significant differences (Tukey's multiple comparison test after a one-way analysis of variance; $P < 0.05$).

females ($\chi^2 = 9.22$, $P = 0.002$), but not to males (Fig. 4). Next, we tested behavioural dose-response relationships of *C. chlorideae* females to *cis*-jasmone. Doses of 10 μg ($\chi^2 = 6.69$, $P = 0.009$) and 1 μg ($\chi^2 = 3.99$, $P = 0.045$) also attracted the mated female parasitoids (Fig. 4).

The early-instar larvae of *H. armigera* are the dominant hosts of *C. chlorideae* in the field (You *et al*., 2002; Han *et al*., 2013). Di *et al.* (2017) showed that *cis*-jasmone is also attractive to the first-instar larvae of *H. armigera* and HarmOR41 is the tuning receptor. We further proved the chemotaxis of the third-instar larvae of *H. armigera* in response to different concentrations of *cis*-jasmone. *cis*-jasmone attracted the larvae at 100 ng ($P = 0.004$) and 1 μg doses ($P = 0.019$) (Fig. 5).

Behavioural studies of C. chlorideae *responses to cis-jasmone in cages*

We further investigated the effects of *cis*-jasmone on the searching behaviour and parasitic power of *C. chlorideae* in a cage setting (Fig. 6). The mated female *C. chlorideae* were more likely to be attracted by *cis*-jasmone-treated plants ($\chi^2 = 18.36$, $P < 0.0001$; Fig. 7A; Video S1). The parasitism rate of *H. armigera* in the *cis*-jasmone-treated plants was 28 ± 5.42%, which was higher than in the untreated group (12 ± 2.10%, $P = 0.023$; Fig. 7B).

Discussion

Chemoreceptors are the most critical elements mediating HIPVs in the peripheral olfactory signal transduction pathway. In this study, we identified 95 *OR*s with putative full length from the transcriptomes of male and female *C. chlorideae* heads. One of the female antennae-biased ORs, CchlOR62, was functionally characterized as responding to *cis*-jasmone. This compound was attractive to both mated female *C. chlorideae* and third-instar *H. armigera* larvae but in different dose ranges. Cage studies showed that *cis*-jasmone increased the parasitism rate of *H. armigera* by *C. chlorideae*.

Families of ORs from genome or transcriptome sequences have been obtained in several species of Hymenoptera. A study of the *Microplitis demolitor* genome revealed 203 *OR* genes in this braconid species (Zhou *et al*., 2015). The number of *OR* family genes in another braconid species, *Mi. mediator*, was 169 (Wang *et al*., 2017a), whereas the jewel wasp *N. vitripennis* has an even more expanded *OR* family of 301 genes (Robertson *et al*., 2010). The analysis of the chemoreceptor superfamily in the *Ap. mellifera* genome revealed an *OR* family of 174 genes (Robertson and Wanner, 2006). Another two representative species of Hymenoptera, *Camponotus floridanus* and *Harpegnathos saltator*, have 477 and 307

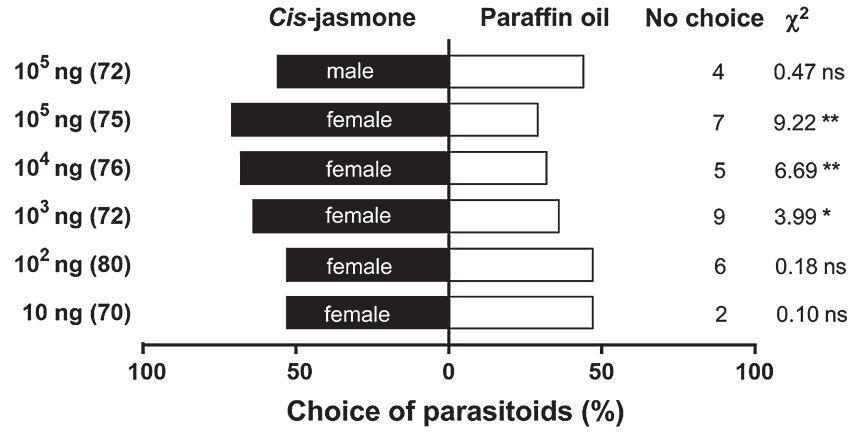

Figure 4. Behavioural responses of female *Campoletis chlorideae* in a Y-tube olfactometer to *cis*-jasmone. Experiments were conducted on mated female parasitoids. Numbers in parentheses represent sample sizes. (Chi-squared test; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.)

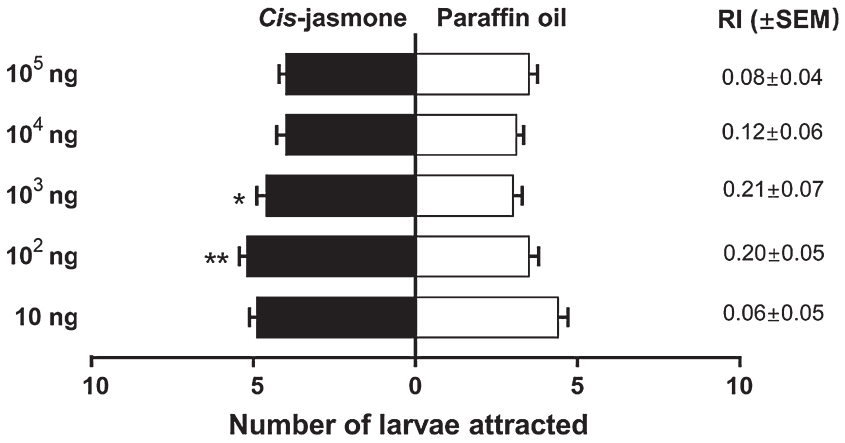

Figure 5. Chemotaxis behavioural assay of *Helicoverpa armigera* larval responses to different doses of *cis*-jasmone. Error bars indicate standard errors of the means (SEMs) for $n = 10$ trials (paired Student's *t*-test; **, $P < 0.01$; *, $P < 0.05$). A response index (RI) was also calculated using (O – C)/(O + C), where O represents the number of larvae in the odorant zone and C represents the number of larvae in the control zone.

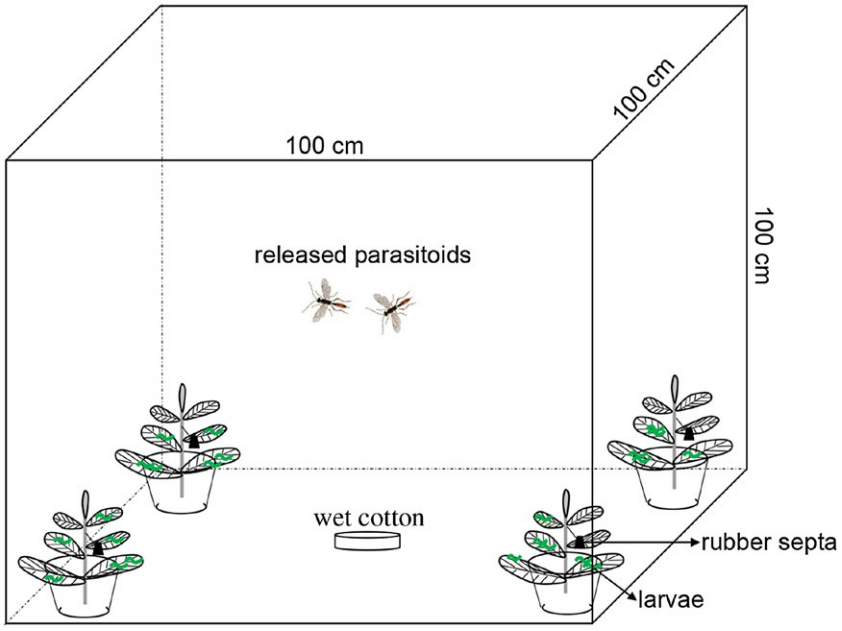

Figure 6. Schematic visualization of the set-up of the cage experiment. Four vegetative tobacco plants were caged with metal wires that were covered with a fine nylon net. Each plant was infested by 15 third-instar *Helicoverpa armigera* larvae. Two dispensers along the diagonals of each cage contained 3 mg *cis*-jasmone for the treatment group, whereas the remaining two dispensers contained 10 µl hexane for the control group. [Colour figure can be viewed at wileyonlinelibrary.com]

*OR*s, respectively (Zhou *et al.*, 2012). In this work, the transcriptome of the *C. chlorideae* head was sequenced, and one coreceptor, *ORco*, and 210 specific *OR*s were identified, including 95 *OR*s having putative full-length sequences (Table S1). In comparison with the numbers of *OR* genes in the species of other insect orders, eg 62 in *Drosophila melanogaster* (Clyne *et al.*, 1999; Vosshall *et al.*, 1999; Robertson *et al.*, 2003) and 68 *in Bombyx mori* (Anderson *et al.*, 2009), there is a remarkably larger repertoire of *OR*s in hymenopteran species (including parasitic wasps). Although a link between the number of ORs and the complexity of olfactory systems seems not immediately obvious in some cases, eg the red flour beetle, *Tribolium castaneum* (Engsontia *et al.*, 2008), the expansion of the OR family in hymenopteran species indicates that the olfactory system is complicated in this insect group. The increased number of *OR* genes mediates the perception of a wide range of social pheromones and complex HIPVs.

HIPVs are important cues that help parasitoids find their hosts (Turlings *et al.*, 2001; Aartsma *et al.*, 2017; Turlings and Erb, 2018). They consist of complex compounds from

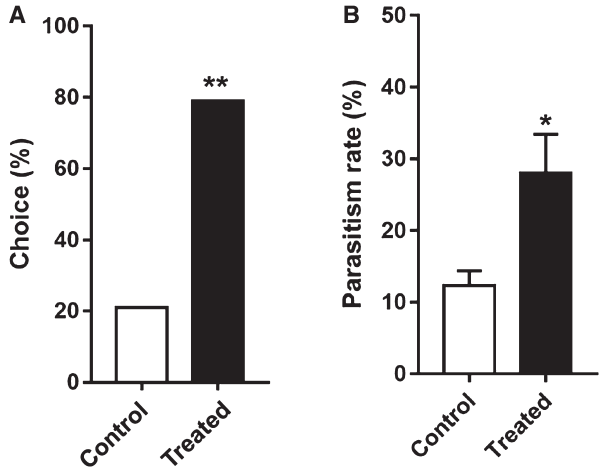

Figure 7. Cage bioassays of the attractiveness of *cis*-jasmone and the parasitism rate of *Helicoverpa armigera* larvae by *Campoletis chlorideae* on tobacco plants treated with *cis*-jasmone vs. untreated plants. (Chi-squared test for the attractiveness analysis; paired Student's *t*-test for parasitism rate experiments. Error bars indicate standard errors of the means; **, $P < 0.01$; *, $P < 0.05$; $n = 8$.)

different chemical classes, but little is known about the ORs that are tuned to these cues. In the present work, to better understand the functions of the identified *CchlOR* genes, we first conducted a study of the *CchlOR*s' expression pattern. According to the FPKM values (Table S1) and RT-PCR analysis (Fig. S2), most of the *OR*s in *C. chlorideae* have similar expression levels in the heads of both genders. Six *OR*s were mainly expressed in the male head (F/M $\leq$ 0.05), and seven *OR*s showed female head-biased expression profiles (F/M $\geq$ 9.99). Based on the biological characteristics of *C. chlorideae*, we hypothesize the male head-biased ORs could be the candidate receptors for detecting food source semiochemicals or mate-associated compounds (Xu and Turlings, 2018). Because female parasitoids use HIPV cues for host location and foraging (Dicke, 2009; Aartsma *et al*., 2017), the high expression levels of *OR*s in female *C. chlorideae* antennae could be important in recognition of HIPVs. Consequently, we focused on these *OR*s and investigated their functions. We found that CchlOR62 was exclusively tuned to *cis*-jasmone (Fig. 2). EAG results subsequently demonstrated that antennae of mated *C. chlorideae* females responded to different doses of *cis*-jasmone (Fig. 3). Five other female-biased ORs did not exhibit measurable responses to any of the tested chemicals (Fig. S3), probably because the concentrations of these compounds did not reach the receptors' response thresholds, or their ligands were not included in the tested chemicals.

cis-jasmone, as a member of the jasmonate class of compounds, is released from various plant flowers to attract pollinators (Knudsen and Tollsten, 1993). It is also an important HIPV released from cotton upon feeding by *S. exigua* or *Helicoverpa zea* larvae (Loughrin *et al*., 1994, 1995 ; Röse and Tumlinson 2004). The application of oral secretions from *Manduca sexta* larvae to mechanically wounded leaves triggers *cis*-jasmone emissions from various species of *Nicotiana* (Lou and Baldwin, 2003). Additionally, *cis*-jasmone acts as an attractant to the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in laboratory tests (Birkett *et al*., 2000). In this study, we demonstrated that *cis*-jasmone is also attractive to mated female *C. chlorideae*, but not to males, at a dose range of 1 to 100 μg in the filter paper (Fig. 4). We also tested the unmated females in the experiments, and their behavioural response to *cis*-jasmone was similar to that of the mated ones (data not shown). It is reported that the amount of *cis*-jasmone collected from one cotton plant subjected to beet armyworm larvae-feeding for 2 days in a laboratory setting was 458 ng (Loughrin *et al*., 1994). *cis*-jasmone appears to be used as a valuable cue by *C. chlorideae* females to locate crowded colonies of host caterpillars feeding on plants.

cis-jasmone was also found to attract *H. armigera* larvae, but at lower doses (100 ng and 1 μg; Fig. 5). This chemical may be used as a signal molecule by the hosts of *C. chlorideae* to find appropriate feeding sites on plants. The OR that tunes to *cis*-jasmone on the antennae of *H. armigera* larvae has been identified as HarmOR41 (Di *et al*., 2017). Because the two receptors that tune to the same chemical share a quite low identity (20.69%), convergent evolution appears to occur for these two unrelated ORs from *C. chlorideae* and *H. armigera*. This suggests that *cis*-jasmone has a strong selection for both the parasitoid and its host in tritrophic interactions.

In the cage experiment, synthetic *cis*-jasmone not only proved to be attractive to mated female *C. chlorideae* but it also increased the parasitism rate of *H. armigera* larvae (Figs 6, 7; Video S1). Although the quantity of *cis*-jasmone in each rubber septa we used is much higher than the amount released by the plant, the chemical we loaded was controlled-released. Thus, it appears that *cis*-jasmone can be used to enhance the effectiveness of parasitoids in manipulating the population of host pest species. One potential strategy is genetically modifying host plants to produce more *cis*-jasmone (Pickett and Khan, 2016). Advances in uncovering the biosynthetic mechanisms of *cis*-jasmone in plants now renders this targeting possible. It is reported that *cis*-jasmone can be biosynthesized through the isomerization of *cis*-oxophytodienoic acid (*cis*-OPDA) to *iso*-OPDA, which yields *cis*-jasmone by spontaneous decarboxylation (Dabrowska and Boland, 2007). Therefore, overexpressing the isomerase in appropriate plant systems could provide an interesting biotechnological route to increase the attractiveness of plants to the parasitoids of caterpillars. Additionally, the exposure of intact plants to *cis*-jasmone can induce their indirect defence

metabolisms (Pickett *et al.*, 2007; Bruce *et al.*, 2008; Moraes *et al.*, 2009; Dewhirst *et al.*, 2012). For example, wheat (*Triticum aestivum*) plants sprayed with formulated *cis*-jasmone were less susceptible after 24 h to attack by *Sitobion avenae* than control plants (Bruce *et al.*, 2003). Soybean, *Glycine max*, after being treated with *cis*-jasmone, emits a blend of volatiles that attracts the stink bug egg parasitoid *Telenomus podisi* (Moraes et al, 2009). Thus, it is also possible that gene modifications in plants resulting in the emission of large amounts of *cis*-jasmone could not only elevate the parasitism rate of *H. armigera* larvae on modified plants but could also act as an external signal, alerting recipient plants and enabling them to prepare their own defences prior to insect attack.

In summary, we identified 95 full-length *OR*s from male and female heads of the parasitoid *C. chlorideae*, an important natural enemy of the agricultural pest *H. armigera*. Amongst these ORs, we identified CchlOR62, which is specifically tuned to *cis*-jasmone, an herbivore-induced plant odorant. This compound is attractive to mated *C. chlorideae* females and *H. armigera* larvae in different dose ranges. The cage bioassays showed that *cis*-jasmone can increase the parasitism rate of *H. armigera* by *C. chlorideae*. This approach reveals the molecular basis of how *C. chlorideae* detects an infochemical released from damaged plants and also provides support for the application of *cis*-jasmone in the biological control of agricultural pests with parasitoids.

Experimental procedures

Animal rearing

H. armigera larvae were reared on an artificial diet at 27 ± 1 °C, with 75 ± 5% relative humidity (RH) and a 16-h light (L)/8-h dark (D) cycle. Adults were given 10% by volume honey solution. A colony of the parasitoid *C. chlorideae* was started with cocoons collected from Luoyang, Henan Province, China. The colony was maintained on *H. armigera* larvae. Parasitoids were kept under conditions of 24 ± 1 °C, with 75 ± 5% RH and a 16-h L/8-h D day cycle. A honey solution (20% by volume) was provided every day as a food source.

X. laevis frogs were kindly provided by Prof. Qinghua Tao's laboratory in the School of Life Sciences, Tsinghua University, Beijing, China, and reared with pork liver as food in our laboratory at 20 ± 1 °C. All procedures in this study were approved by the Institute of Zoology Animal Care and Use Committee (protocol number IOZ17090-A).

Chemicals

(*Z*)-3-hexen-ol (98%), (*Z*)-3-hexenyl acetate (97%) and myrcene (90%), were obtained from the Roth KG Company (Roth, Karlsruhe, Germany); (*E*)-2-hexen-1-ol (95%), (*E*)-2-hexenal (97%), *D*-limonene (98%), (*E*)-caryophyllene (99%), linalool (97%) and phenylethyl acetate (99%) from the Fluka Chemie Company (Fluka, Buchs, Switzerland); (*E*)-2-hexenyl acetate (98%), β-pinene (99%), ocimene (90%), nonanal (95%), methyl jasmonate (95%), *cis*-jasmone (85%), methyl-salicylate (99%) and farnesene (98%) from Sigma-Aldrich (Sigma-Aldrich, St Louis, MO, USA); jasmonic acid (85%) from TCI (Shanghai, China); and indole (99.5%) from Aladdin (Shanghai, China).

RNA extraction

For the transcriptome analysis, 100 male heads and 100 female heads were collected separately from *C. chlorideae* on the third day after eclosion. For RT-PCR, heads, antennae, heads (without antennae), legs, thoraxes and abdomens were collected separately from both sexes of 3-day-old adult *C. chlorideae*. All the tissues were collected in photophase. The total RNA extraction was performed following the manufacturer's instructions for Trizol reagent (Invitrogen, Carlsbad, CA, USA). The quantity of RNA samples was checked by 1.5% agarose gel electrophoresis and a Nano Drop 2000 spectrophotometer (Nano-Drop Products, Wilmington, DE, USA).

C. chlorideae *head transcriptome sequencing, OR identification and phylogenetic analysis*

cDNA library construction and Illumina sequencing of the samples were performed at the Beijing Institutes of Biological Sciences (Chinese Academy of Sciences), China. Briefly, messenger RNA (mRNA) was purified from 10 μg of total RNA (a mixture of RNAs from the heads). Using oligo deoxythymine (dT) magnetic beads, mRNA was enriched and then fragmented into short sequences in the presence of divalent cations at 94 °C for 5 min. Then, the first-strand cDNA was generated using random hexamer-primed reverse transcription, followed by the synthesis of second-strand cDNA using RNaseH (New England Biolabs, Ipswich, MA, USA) and DNA polymerase I (Promega, Madison, WI, USA). After the end-repair and ligation of adaptors, the products were amplified by PCR and purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) to create a cDNA library. Transcriptome *de novo* assembly was carried out with the short read assembly program Trinityrnaseu-r2013-02-25 (https://github.com/trinityrnaseq/trinityrnaseq/wiki). Then, the Trinity outputs were clustered by tgicl (https://sourceforge.net/projects/tgicl/files/tgicl%20v2.1) and finally capped using Cap3 to produce the unigenes. The consensus cluster sequences and singletons formed the unigene dataset. The annotation of unigenes was performed by NCBI's BLASTX algorithm-based search against a pooled nonredundant database with an E-value cut-off of 1e-5.

The OR phylogenetic tree was built based on amino acid sequences from the datasets of Hymenoptera species. Amino acid sequences were aligned using the program ClustalW. The neighbour-joining tree was constructed using the mega 7.0 program (Kumar *et al.*, 2016). The evolutionary distances were computed using the Jones-Taylor-Thornton (JTT) matrix-based method. All ambiguous positions were removed for each sequence pair.

Expression pattern analysis of ORs in C. chlorideae

Total RNA from different tissues was first treated with DNase I (TaKaRa, Otsu, Japan), then cDNA was produced using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). The *actin* gene of *C. chlorideae* was used as the control to monitor the quality of each cDNA sample. Amplification was performed using ExTaq DNA polymerase (TaKaRa). At least two biological replications for each OR were run, and the products were analysed using 1.5% agarose gels. The sense and antisense primers for RT-PCR were designed with Primer Premier 6.0 software and are listed in Table S2.

Functional characterization of C. chlorideae *ORs in Xenopus expression system*

Full-length *CchlORco* and female-biased *ORs* were amplified by PCR with Q5 High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA). Gene-specific primers are listed in in Table S2. PCR products of the *OR*s were first cloned into the pGEM-T easy vector (Promega) and then subcloned into pCS2+ vector. cRNAs were synthesized from linearized modified pCS2+ vectors with mMESSAGE mMACHINE SP6 (Ambion, Austin, TX, USA). Mature oocytes were treated with 2 mg/ml of collagenase type I (Sigma-Aldrich) saline solution [96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$ and 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.6] for 20–30 min at room temperature. Oocytes were later microinjected with 25 ng OR cRNA and 25 ng ORco cRNA. The injected oocytes were incubated for 3–7 days at 18 °C in Barth's solution (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 1.8 mM $CaCl_2$ and 5 mM HEPES, pH 7.5) supplemented with 5% dialysed horse serum, 50 mg/ml tetracycline, 100 mg/ml streptomycin and 550 mg/ml sodium pyruvate. Barth's solution containing 0.1% dimethyl sulphoxide was used as a negative control. Whole-cell currents were recorded with a two-electrode voltage-clamp. Intracellular glass electrodes were filled with 3 M KCl and presented resistances of 0.2–2.0 MΩ. Signals were amplified with an OC-725C amplifier (Warner Instruments, Hamden, CT, USA) at a holding potential of 80 mV, low-pass filtered at 50 Hz and digitized at 1 kHz. Data acquisition and analysis were carried out with Digidata 1322A and pCLAMP software (Axon Instruments Inc., Foster City, CA, USA). Every OR/ORco complex expressed in *X. laevis* oocytes was tested in at least 50 cells.

The EAG response to cis*-jasmone*

Three-day-old mated female parasitoids were used for EAG tests of *cis*-jasmone dose-responses in photophase. The antennae were cut off from the heads and used for signal recording. A few segments from the tips of antennae were clipped off and mounted on the antenna holder with two metal electrodes using conductive gel (Spectra 360, Parker Lab, NJ, USA), and then the electrode holder was inserted into the EAG probe. The EAG signal recorded with the electrode was first amplified with a DC/AC preamplifier (Syntech UN-06), and further processed with Autospike software (Syntech, Hilversum, The Netherlands). *cis*-jasmone was dissolved into paraffin oil (Fluka) to obtain 10 μg/μl solutions. The mother solution was diluted into 1, 0.1, 0.01 and 0.001 μg/μl solutions. The exposure proceeded from the lowest to the highest concentration to minimize the effects of olfactory adaptation possibly resulting from strong stimulation. In addition, 10 μl mineral oil and 100 μg (Z)-3-hexen-1-ol in 10 μl mineral oil were used as the control and standard stimulus, respectively.

An aliquot of 10 μl of test solution was pipetted onto a piece of filter paper (0.4 × 5 cm), which was inserted into a glass Pasteur pipette (0.5 cm in diameter, 15 cm in length). In a series of tests, the control and standard stimulus were applied subsequently after five successive stimulations. The results were normalized by dividing the peak EAG amplitude of the test by the average EAG amplitude of the two nearest standard stimulations after the subtraction of the amplitude recorded in response to the control. Each concentration gradient was tested on 10 individuals.

Attractiveness of cis*-jasmone to* C. chlorideae

A Y-tube olfactometer was used to test the attractiveness of *cis*-jasmone to mated *C. chlorideae* males and females (Yan and Wang, 2006b). *cis*-jasmone was dissolved into paraffin oil to obtain 10, 1, 0.1, 0.01 and 0.001 μg/μl solutions. Filter paper (1 × 2 cm) impregnated with 10 μl of sample or paraffin oil was placed in the flask. During the test, the room temperature was kept at 24 ± 1 °C, with 75 ± 5% RH. Light-emitting diode lamps (80 W) were suspended over the Y-tube to produce a light intensity of ~1000 lx. Parasitoids were individually released at the base of the central arm and observed for 5 min. If a parasitoid did not make a choice after this period, then it was recorded as no choice. Parasitoids that walked more than halfway to the lateral arm and stayed there at least 5 s were recorded as having made a choice. After five individuals were tested, the olfactometer was turned and the flasks were switched to avoid positional effects.

Attractiveness of cis*-jasmone to* H. armigera *larvae*

Chemotaxis assays were carried out as described in Di et al (2017). Filter paper (0.5 cm in diameter) impregnated with 10 μl sample was placed on one side of a Petri dish (10 cm in diameter, filled with 1% agarose.) and a filter paper impregnated with 10 μl paraffin oil was placed on the opposite side. The first day third-instar larvae were placed in the centre of the dish and the lid closed. After 10 min, larvae within a 2-cm radius of each filter paper were counted. This behavioural assay was repeated 10 times, with 10 larvae used for each replication. All of the larvae were starved for 15 min in advance.

Behavioural studies of C. chlorideae *responses to* cis*-jasmone in cages*

A cage experiment was conducted at Henan University of Science and Technology (34°36′ N, 112°25′ E, 163.9 m elevation). Four vegetative tobacco plants were caged with 1 × 1 × 1 m metal wires that were covered with a fine nylon net. The experiment was repeated eight times on different days. Each plant was infested by 15 third-instar *H. armigera* larvae that had been deprived of food supply for 15 min prior to the experiment. To dispense *cis*-jasmone, a rubber septum was tied to the tobacco stem at a distance of 10 cm above the pot edge. Two

dispensers along the diagonals of each cage contained 3 mg *cis*-jasmone (3 μl *cis*-jasmone + 7 μl hexane, mixed well before applied) for the treatment group, whereas the remaining two dispensers contained 10 μl hexane for the control group. The positions of each group were switched after each replication. The compound was renewed after each replication. Experiments were conducted in a climate-controlled room at 24 ± 1 °C, with 75 ± 5% RH and a 16-h L/8-h D cycle. The light intensity was 1000 lx in the photophase and 10 lx in the scotophase.

In the parasitism rate experiment, six 3-day-old mated female parasitoids and two 3-day-old mated male parasitoids were released at each replication. 24 h after the release of the parasitoids, *H. armigera* larvae were collected from the different infested plants. For the *C. chlorideae* behavioural assay, one 3-day-old mated female parasitoid was introduced into the cage in each test. We observed the behaviour of the parasitoid for 1 h. Parasitoids that stayed on the leaves of plants for more than 5 s were considered as having made a choice.

The experiments all used 70–80-day-old plants of the tobacco species NC89 with eight to nine leaves that were grown in fertile soil in flowerpots. All of the plants and parasitoids were used only once.

Statistical analysis

The data analyses were carried out using GraphPad Prism 6. The level of significance was set as $P < 0.05$.

Acknowledgements

We thank Rui Wang, Rui Tang and Lin Yang for their assistance in performing the EAG test, Ke Yang and Chang Di for their help in making the two-electrode voltage-clamp recordings. This work was supported by the National Key R & D Program of China (Grant number: 2017YFD0200400), the Strategic Priority Research Program of the Chinese Academy of Sciences (grant number: XDB11010300), the National Natural Science Foundation of China (grant number: 31471777) and the State Key Laboratory of Integrated Management of Pest Insects and Rodents (grant number: ChineseIPM1509).

References

Aartsma, Y., Bianchi, F.J.J.A., van der Werf, W., Poelman, E.H. and Dicke, M. (2017) Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytologist*, **216**, 1054–1063.

Anderson, A.R., Wanner, K.W., Trowell, S.C., Warr, C.G., Jaquin-Joly, E., Zagatti, P. *et al.* (2009) Molecular basis of female-specific odorant responses in *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, **39**, 189–197.

Benton, R., Sachse, S., Michnick, S.W. and Vosshall, L.B. (2006) Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. *PLoS Biology*, **4**, e20.

Birkett, M.A., Campbell, C.A.M., Chamberlain, K., Guerrieri, E., Hick, A.J., Martin, J.L. *et al.* (2000) New roles for *cis*-jasmone as an insect semiochemical and in plant defense. *Proceedings of the National Academy of Sciences*, **97**, 9329–9334.

Birkett, M.A., Chamberlain, K., Guerrieri, E., Pickett, J.A., Wadhams, L.J. and Yasuda, T. (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *Journal of Chemical Ecology*, **29**, 1589–1600.

Bruce, T.J.A., Martin, J.L., Pickett, J.A., Pye, B.J., Smart, L.E. and Wadhams, L.J. (2003) *cis*-Jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). *Pest Management Science*, **59**, 1031–1036.

Bruce, T.J.A., Matthes, M.C., Chamberlain, K., Woodcock, C.M., Mohib, A., Webster, B. *et al.* (2008) *cis*-Jasmone induces *Arabidopsis* genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. *Proceedings of the National Academy of Sciences*, **105**, 4553–4558.

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J. and Carlson, J.R. (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron*, **22**, 327–338.

Dabrowska, P. and Boland, W. (2007) Iso-OPDA: an early precursor of *cis*-jasmone in plants? *Chembiochem*, **8**, 2281–2285.

D'Alessandro, M., Brunner, V., von Mérey, G. and Turlings, T.C.J. (2009) Strong attraction of the parasitoid *Cotesia marginiventris* towards minor volatile compounds of maize. *Journal of Chemical Ecology*, **35**(9), 999–1008.

De Moraes, C.M., Lewis, W.J., Pare, P.W., Alborn, H.T. and Tumlinson, J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature*, **393**, 570–573.

Dewhirst, S.Y., Birkett, M.A., Loza-Reyes, E., Martin, J.L., Pye, B.J., Smart, L.E., *et al.* (2012) Activation of defence in sweet pepper, *Capsicum annum*, by *cis*-jasmone, and its impact on aphid and aphid parasitoid behavior. *Pest Management Science*, **68**, 1419–1429.

Di, C., Ning, C., Huang, L.Q. and Wang, C.Z. (2017) Design of larval chemical attractants based on odorant response spectra of odorant receptors in the cotton bollworm. *Insect Biochemistry and Molecular Biology*, **84**, 48–62.

Dicke, M. (1999) Evolution of induced indirect defence of plants. In: Tollrian, R. and Harvell, C.D. (Eds.) *The Ecology and Evolution of Inducible Defenses*. Princeton: Princeton University Press, pp. 62–88.

Dicke, M. (2009) Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment*, **32**(6), 654–665.

Dweck, H.K.M., Ebrahim, S.A.M., Kromann, S., Bown, D., Hillbur, Y., Sachse, S. *et al.* (2013) Olfactory preference for egg laying on citrus substrates in *Drosophila*. *Current Biology*, **23**(24), 2472–2480.

Engsontia, P., Sanderson, A.P., Cobb, M., Walden, K.K.O., Robertson, H.M. and Brown, S. (2008) The red flour beetle's large nose: an expanded odorant receptor gene family in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, **38**(4), 387–397.

Hallem, E.A. and Carlson, J.R. (2006) Coding of odors by a receptor repertoire. *Cell*, **125**(1), 143–160.

Han, L.B., Huang, L.Q. and Wang, C.Z. (2013) Host preference and suitability in the endoparasitoid *Campoletis chlorideae* is associated with its ability to suppress host immune responses. *Ecological Entomology*, **38**, 173–182.

Han, L.B., Yin, L.H., Huang, L.Q. and Wang, C.Z. (2015) Differential immunosuppression by *Campoletis chlorideae* eggs and ichnovirus in larvae of *Helicoverpa armigera* and *Spodoptera exigua*. *Journal of Invertebrate Pathology*, **130**, 88–96.

Knudsen, J.T. and Tollsten, L. (1993) Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. *Botanical Journal of the Linnean Society*, **113**(3), 263–284.

Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**(7), 1870–1874.

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H. and Vosshall, L.B. (2004) Or83b Encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron*, **43**(5), 703–714.

Li, K.M., Ren, L.Y., Zhang, Y.J., Wu, K.M. and Guo, Y.Y. (2012) Knockdown of *microplitis mediator* odorant receptor involved in the sensitive detection of two chemicals. *Journal of Chemical Ecology*, **38**(3), 287–294.

Li, Z.H., Wang, N.C., Zheng, F.Q., Yie, B.H., Liu, G.L. and Xu, W.A. (1997) A list of natural enemies of tobacco pests in Shandong. *Journal Shandong Agricultural University*, **28**, 391–400.

Lou, Y. and Baldwin, I.T. (2003) *Manduca sexta* recognition and resistance among allopolyploid *Nicotiana* host plants. *Proceedings of the National Academy of Sciences*, **100**(Supplement 2), 14581–14586.

Loughrin, J.H., Manukian, A., Heath, R.R., Turlings, T.C.J. and Tumlinson, J.H. (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proceedings of the National Academy of Sciences*, **91**, 11836–11840.

Loughrin, J.H., Manukian, A., Heath, R.R. and Tumlinson, J.H. (1995) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *Journal of Chemical Ecology*, **21**(8), 1217–1227.

McCormick, A.C., Unsicker, S.B. and Gershenzon, J. (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science*, **17**, 303–310.

Moraes, M.C.B., Laumann, R.A., Pareja, M., Sereno, F.T.P.S., Michereff, M.F.F., Birkett, M.A. *et al.* (2009) Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with *cis*-jasmone. *Entomologia Experimentalis et Applicata*, **131**, 178–188.

Morawo, T. and Fadamiro, H. (2016) Identification of key plant-associated volatiles emitted by *Heliothis virescens* larvae that attract the parasitoid, *Microplitis croceipes*: implications for parasitoid perception of odor blends. *Journal of Chemical Ecology*, **42**(11), 1112–1121.

Nakagawa, T., Sakurai, T., Nishioka, T. and Touhara, K. (2005) Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. *Science*, **307**, 1638–1642.

Pickett, J.A. and Khan, Z.R. (2016) Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytologist*, **212**(4), 856–870.

Pickett, J.A., Birkett, M.A., Bruce, T.J.A., Chamberlain, K., Gordon-Weeks, R., Matthes, M.C. *et al.* (2007) Developments in aspects of ecological phytochemistry: the role of *cis*-jasmone in inducible defence systems in plants. *Phytochemistry*, **68**, 2937–2945.

Robertson, H.M. and Wanner, K.W. (2006) The chemoreceptor superfamily in the honeybee *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Research*, **16**, 1395–1403.

Robertson, H.M., Warr, C.G. and Carlson, J.R. (2003) Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences*, **100**(Supplement 2), 14537–14542.

Robertson, H.M., Gadau, J. and Wanner, K.W. (2010) The insect chemoreceptor superfamily of the parasitoid jewel wasp *Nasonia vitripennis*. *Insect Molecular Biology*, **19**, 121–136.

Röse, U.S.R. and Tumlinson, J.H. (2004) Volatiles released from cotton plants in response to *Helicoverpa zea* feeding damage on cotton flower bud. *Planta*, **218**, 824–832.

Tamiru, A., Bruce, T.J.A., Woodcock, C.M., Birkett, M.A., Midega, C.A.O., Pickett, J.A. *et al.* (2015) Chemical cues modulating electrophysiological and behavioural responses in the parasitic wasp *Cotesia sesamiae*. *Canadian Journal of Zoology*, **93**, 281–287.

Turlings, T.C.J. and Erb, M. (2018) Tritrophic interactions mediated by herbivore induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annual Review of Entomology*, **63**(1), 433–452.

Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. *Science*, **250**, 1251–1253.

Turlings T.C.J., Gouinguené S., Degen T. and Fritzche Hoballah M.E. (2001) The chemical ecology of plant-caterpillar-parasitoid interactions. In: Tscharntke, T. and Hawkins, B.A. (Eds.) *Multitrophic Level Interactions*. Cambridge: Cambridge University Press, pp. 148–173.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A. and Axel, R. (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell*, **96**, 725–736.

Wang, G.R., Carey, A.F., Carlson, J.R. and Zwiebel, L.J. (2010) Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. *Proceedings of the National Academy of Sciences*, **107**(9), 4418–4423.

Wang, S.N., Shan, S., Zheng, Y., Peng, Y., Lu, Z.Y., Yang, Y.Q. *et al.* (2017a) Gene structure and expression characteristic of a novel odorant receptor gene cluster in the parasitoid wasp *Microplitis mediator* (Hymenoptera: Braconidae). *Insect Molecular Biology*, **26**, 420–431.

Wang, Y.L., Chen, Q., Guo, J.Q., Li, J., Wang, J.T., Wen, M. *et al.* (2017b) Molecular basis of peripheral olfactory sensing during oviposition in the behavior of the parasitic wasp *Anastatus japonicus*. *Insect Biochemistry and Molecular Biology*, **89**, 58–70.

Wei, J.N., Wang, L., Zhu, J., Zhang, S., Nandi, O.I. and Kang, L. (2007) Plants attract parasitic wasps to defend themselves against insect pests by releasing hexenol. *PLoS ONE*, **2**, e852.

Xu, H. and Turlings, T.C.J. (2018) Plant volatiles as mate-finding cues for insects. *Trends in Plant Science*, **23**, 100–111.

Yan, Z.G. and Wang, C.Z. (2006a) Similar attractiveness of maize volatiles induced by *Helicoverpa armigera* and *Pseudaletia separata* to the generalist parasitoid *Campoletis chlorideae*. *Entomologia Experimentalis et Applicata*, **118**, 87–96.

Yan, Z.G. and Wang, C.Z. (2006b) Identification of *Mythmna separata*-induced maize volatile synomones that attract the parasitoid *Campoletis chlorideae*. *Journal of Applied Entomology*, **130**, 213–219.

Yan, Z.G., Yan, Y.H. and Wang, C.Z. (2005) Attractiveness of tobacco volatiles induced by *Helicoverpa armigera* and *Helicoverpa assulta* to *Campoletis chlorideae*. *Chinese Science Bulletin*, **50**(13), 1334–1341.

Yan, Z.G., Yan, Y.H., Kang, L. and Wang, C.Z. (2006) EAG responses of *Campoletis chlorideae* (Uchida) to plant volatiles and host pheromone gland compounds. *Acta Entomologica Sinica*, **49**, 1-9.

Yang, K., Huang, L.Q., Ning, C. and Wang, C.Z. (2017) Two single-point mutations shift the ligand selectivity of a pheromone receptor between two closely related moth species. *eLife*, **6**, e29100.

You, L.S., Lei, R.H., Jiang, J.X., Bo, L.Y. and Xiao, Z.S. (2002) Bionomic of *Campoletis chlorideae* (Hymonoptera: Ichneumonidae) as a parasitoid of the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Entomologia Sinica*, **9**, 29–37.

Yu, H.L., Zhang, Y.J., Wyckhuys, K.A.G., Wu, K.M., Gao, X.W. and Wu, K.M. (2010) Electrophysiological and behavioral responses of *Microplitis mediator* (Hymenoptera: Braconidae) to caterpillar-induced volatiles from cotton. *Environmental Entomology*, **39**, 600–609.

Zhou, X., Slone, J.D., Rokas, A., Berger, S.L., Liebig, J., Ray, A. *et al*. (2012) Phylogenetic and transcriptomic analysis of chemosensory receptors in a pair of divergent ant species reveals sex-specific signatures of odor coding. *PLoS Genetics*, **8**(8), e1002930.

Zhou, X., Rokas, A., Berger, S.L., Liebig, J., Ray, A. and Zwiebel, L.J. (2015) Chemoreceptor evolution in Hymenoptera and its implications for the evolution of eusociality. *Genome Biology and Evolution*, **7**(8), 2407–2416.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Figure S1. Phylogenetic relationships of the odorant receptors from *C. chlorideae* and other Hymenoptera insects.

Figure S2. RT-PCR analysis of candidate OR genes in female and male *C. chlorideae* heads. FH: female head; MH: male head.

Figure S3. Functional analysis of female antennae-biased *CchlORs* (*OR85/ORco, OR63/ORco, OR60/ORco, OR53/ORco and OR52/ORco*) in *Xenopus laevis* oocytes.

Table S1. Unigenes of candidate odorant receptors in *C. chlorideae* head

Table S2. Primers for the RT-PCR of candidate *ORs* and the functional analysis of female antennae-biased *ORs.*

Video S1. Attraction of *C. chlorideae* to cis-jasmone in cage.