Bulletin of Entomological Research

cambridge.org/ber

Identification and expression patterns of candidate carboxylesterases in *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), an important pest of fruit trees

Jia Li[1] and Long Zhang[2]

[1]Plant Protection College, Shenyang Agricultural University, Shenyang, China and [2]College of Grassland Science and Technology, China Agricultural University, Beijing, China

Research Paper

Cite this article: Li J, Zhang L (2022). Identification and expression patterns of candidate carboxylesterases in *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), an important pest of fruit trees. *Bulletin of Entomological Research* **112**, 567–573. https://doi.org/10.1017/S0007485322000244

Received: 10 October 2021
Revised: 5 April 2022
Accepted: 28 April 2022
First published online: 7 June 2022

Keywords:
Carboxylesterase; expression pattern; *Carposina sasakii*; transcriptomic data

Author for correspondence:
Jia Li, Email: teacher135@sina.cn

Abstract

Carposina sasakii Matsumura (Lepidoptera: Carposinidae) is an important pest of fruit trees in a large area of Asia. The adults mainly depend on olfaction to communicate with the environment, but the olfactory mechanism has not been well known. Odorant degrading enzymes (ODEs) are important olfactory proteins, which inactivate and degrade odorants to free odorant receptors for maintaining olfactory sensitivity. Carboxylesterases (CXEs) are considered to be a major group of moth ODEs. In this study, four candidate CXEs (*Csas*CXE1 ~ *Csas*CXE4) were identified by using head transcriptomic data from *C. sasakii* adult females and males. Sequence alignment showed conserved amino acid residues and their variations in *C. sasakii* CXEs. Phylogenetic analysis indicated the CXEs with the variations cluster well, and each *C. sasakii* CXE clusters in a clade with some of the other lepidopteran CXEs, with a high enough bootstrap value. Gene expression analysis revealed that *Csas*CXE2 and *Csas*CXE3 have similar tissue and sex expression patterns in *C. sasakii* adults. The two CXEs have relatively high expression levels in the heads and are expressed more abundantly in the female heads than male heads. *Csas*CXE1 and *Csas*CXE4 are expressed at higher levels in the male heads than female heads, but not dominantly expressed in the heads among the different tissues. Whether these CXEs function as ODEs remains to be further researched. This study laid the foundation for exploring functions of *C. sasakii* CXEs.

Introduction

The peach fruit borer *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae) is an important pest that seriously damages fruit trees such as apples, pears and jujubes in a large area of Asia (Zhang *et al.*, 2017*a*), with a damage rate of fruits up to 80% or more (Li *et al.*, 2013). The adults mainly depend on olfaction to communicate with the environment. *C. sasakii* adult females release (Z)-7-nonadecen-11-one and (Z)-7-eicosen-11-one as sex pheromone to attract the adult males for mating (Han *et al.*, 2000; Zhang *et al.*, 2017*a*). Nine carboxylic esters from apple fruits attract *C. sasakii* adult females or elicit their olfactory physiological responses, which may help the adult females choose oviposition sites (Wang *et al.*, 2011; Sun and Wang, 2015). However, the olfactory mechanism has not been well known, which limits the development of new technology for controlling the pest.

Olfaction in insects is mediated by specific odorant receptors (ORs) on dendrite membrane of olfactory neurons, which are involved in recognizing environmental odorants (Leal, 2013; Fleischer *et al.*, 2018). However, ORs usually need the assistance of other olfactory proteins such as odorant-binding proteins (OBPs) and odorant degrading enzymes (ODEs). OBPs bind the odorants and transport them to ORs (Pelosi *et al.*, 2006, 2018). After ORs are activated by odorants, ODEs inactivate and degrade the odorants to free ORs for maintaining olfactory sensitivity (Pelosi *et al.*, 2006; Leal, 2013). ODEs include many enzyme families such as carboxylesterases (CXEs), aldehyde oxidases (AOXs), alcohol dehydrogenase (ADs), glutathione S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs) and cytochrome P450s (CYPs) (Leal, 2013; Younus *et al.*, 2014; Huang *et al.*, 2016). The first insect ODE was identified from the moth *Antheraea polyphemus* (Lepidoptera: Saturniidae), which is a CXE used to degrade the female sex-pheromone component (E,Z)-6,11-hexadecadienyl acetate (Vogt and Riddiford, 1981; Ishida and Leal, 2005). Carboxylic esters are common components of moth sex-pheromones and host plant odorants, so CXEs are considered to be a major group of moth ODEs (Leal, 2013; Groot *et al.*, 2016). CXEs usually contain conserved amino acid residues such as the oxyanion hole (Gly-Gly, Ala), the pentapeptide Gly-X-Ser-X-Gly, a Glu and a His, of which the Ser, Glu and His form a catalytic triad (Oakeshott *et al.*, 2005). In fact, CXEs are distributed widely in different insect tissues and play many important roles (Oakeshott *et al.*, 2005). For example, besides functioning as ODEs, they are also involved

CAMBRIDGE UNIVERSITY PRESS

in resistance to ester insecticides (Hemingway *et al*., 2004), fat metabolism and mediation of juvenile hormone titer (Jones and Bancroft, 1986).

Because heads are usually responsible for olfaction in insects, with the sensilla mainly on antennae and secondarily on palps, we analyzed head transcriptomes of *C. sasakii* adult females and males to identify olfactory proteins such as OBPs (Li *et al*., 2019). In this study, we identified candidate *C. sasakii* CXEs by using the head transcriptomic data, and then analyzed their phylogenetic characteristics and expression patterns. Whether these CXEs function as ODEs remains to be further researched. This study laid the foundation for exploring the functions of *C. sasakii* CXEs.

Materials and methods

Insects and tissue collection

C. sasakii adults (1–3 days after emergence) were supplied by the Institute of Pomology, Chinese Academy of Agricultural Sciences. Their different tissues were respectively collected and stored at −80 °C until use, including intact heads (with antennae and palps), wings, thoraxes (without wings) and abdomens.

Gene identification and rapid amplification of cDNA ends

Candidate CXEs were identified by using unigene annotations from the head transcriptomic data of *C. sasakii* adult females and males (Li *et al*., 2019). OrfPredictor 1.0 software was used to predict the open reading frames (ORFs) with default parameters. Because the ORFs of three *C. sasakii* CXEs were incomplete at the 3′ terminus, several conserved amino acid residues could not be identified. We used rapid amplification of cDNA 3′ ends (3′ RACE) to solve the problem. At first, total RNA was extracted from the heads of *C. sasakii* adults by using Trizol RNA Extraction Kit (Sangon, Shanghai, China). The integrity of the total RNA was judged by 1.5% agarose gel electrophoresis. Purity and concentration of the total RNA were checked using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Shanghai, China). Complementary DNA (cDNA) was synthesized from the total RNA by using RevertAid Premium Reverse Transcriptase (Thermo Fisher Scientific, Shanghai, China) and 3′ adaptor primer (table S1). The first round of PCR was performed in a system (25 µl) including template cDNA (1 µl), gene-specific primer 1 (10 µM, 0.5 µl), outer universal primer (10 µM, 0.5 µl), dNTP (2.5 mM, 4 µl), Taq enzyme (5 U µl^{-1}, 0.2 µl), 2× GC Buffer I (12.5 µl) and ddH_2O (6.3 µl). The PCR product was used as template DNA in the second round of PCR. The second round of PCR was performed in a system (50 µl) including template DNA (2 µl), gene-specific primer 2 (10 µM, 1 µl), inner universal primer (10 µM, 1 µl), dNTP (2.5 mM, 8 µl), Taq enzyme (5 U µl^{-1}, 0.5 µl), 2 × GC Buffer I (25 µl) and ddH_2O (12.5 µl). The specific and universal primers are shown in table S1. The two rounds of PCR shared the same program: 95 °C for 3 min, 94 °C for 30 s, 58 °C for 30 s, 72 °C for 60 s (loop to 94 °C for 30 s, 33 times), and then 72 °C for 7 min. The final PCR product was checked by 1% agarose gel electrophoresis and then purified using Column DNA Gel Extraction Kit (Sangon, Shanghai, China) for sequencing.

Sequence alignment and phylogenetic analysis

The amino acid sequences of CXEs from *C. sasakii* were aligned by using the software ClustalX 2.1 with default parameters. MEGA 5.0 software was used to construct a phylogenetic tree for amino acid sequences of the CXEs from *C. sasakii* and three other lepidopteran species: *Mythimna separata*, *Sesamia inferens* and *Spodoptera litura* (table S2). The neighbor-joining method with Poisson correction of distances was chosen for constructing the tree, using a bootstrap procedure based on 1000 replicates to assess node support.

Gene expression analysis using transcriptomic data

Salmon 0.8.2 and DEGseq 1.26.0 softwares were used to analyze expression levels of *C. sasakii* CXEs in head transcriptomes of the adult females and males. Salmon was used for obtaining read counts and values of transcripts per million (TPM). The read counts were normalized with trimmed mean of *M*-values. DEGseq was used to explore expression differentiations of the CXEs between *C. sasakii* adult females and males. A differential expression will be determined if TPM ≥5 at least in one sex, |FoldChange| >2 and $Q < 0.05$ at the same time.

Gene expression analysis using quantitative PCR data

Quantitative PCR was used to analyze expression levels of *C. sasakii* CXEs in different tissues (heads, wings, thoraxes and abdomens) of the adults. Total RNA was extracted from these tissues using UNIQ-10 Column Trizol RNA Extraction Kit (Sangon, Shanghai, China), and then checked by electrophoresis. Concentration and purity of the total RNA were tested with a SMA4000 micro-spectrophotometer (Merinton, Beijing, China). Subsequently, Maxima Reverse Transcriptase (Thermo Fisher Scientific, Shanghai, China) was used to synthesize cDNA from the total RNA. The cDNA was diluted ten times, and then used as a PCR template. Quantitative PCR was run on an ABI StepOne Plus device (Foster, CA, USA) with a system (20 µl) including template (2 µl), a pair of specific primers (table S3; 10 µM, 0.4 µl per primer), 2 × SG Master Mix (BBI, Shanghai, China; 10 µl) and ddH_2O (7.2 µl). Actin identified from the head transcriptomic data (Li *et al*., 2019) was used as an endogenous reference. The reaction program was 95 °C for 3 min, and then 45 cycles of 95 °C for 5 s and 60 °C for 30 s. Dissociation curves were obtained based on device guidelines. Every test was performed in biological triplicate. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to calculate relative expression levels of *C. sasakii* CXEs among different tissues. The data were compared by one-way ANOVA and LSD test at $P = 0.05$, using the software SPSS 17.0.

Results

Gene identification and sequence analysis

We identified four *C. sasakii* CXEs (*Csas*CXE1 ~ *Csas*CXE4) from head transcriptomes of the adult females and males (table 1). After 3′ RACE, the new sequence lengths are shown in table 1. Sequence alignment showed conserved amino acid residues that include the disulfide bridge (Cys, Cys), oxyanion hole (Gly-Gly, Ala), pentapeptide Gly-X-Ser-X-Gly, and catalytic triad (Ser, Glu, His) (fig. 1). However, variations of conserved amino acid residues occur in *Csas*CXE3 and *Csas*CXE4. The pentapeptide Gly-X-Ser-X-Gly is changed to a Gly-X-X-Ser-Gly motif in *Csas*CXE3, while the first glycine of the oxyanion hole is replaced by an alanine in *Csas*CXE4 (fig. 1).

https://doi.org/10.1017/S0007485322000244 Published online by Cambridge University Press

Table 1. Candidate CXEs identified from head transcriptomes of *C. sasakii* adult females and males

Name	Gene ID	Length (bp)	ORF (aa)	Best blast match			After 3′ RACE	
				Accession number and name	*E*-value	Identity (%)	Length (bp)	ORF (aa)
*Csas*CXE1	DN65297_c0_g1	531	177	gb|AEL33699.1|carboxylesterase CXE26 [*Spodoptera littoralis*]	3×10^{-106}	82.49	1464	481
*Csas*CXE2	DN68817_c0_g2	863	287	gb|AIY69044.1|carboxylesterase, partial [*Chilo suppressalis*]	1×10^{-128}	65.84	1959	552
*Csas*CXE3	DN67748_c1_g2	1062	353	gb|AEL33703.2|carboxylesterase CXE30 [*Spodoptera littoralis*]	2×10^{-150}	65.72	1960	617
*Csas*CXE4	DN69454_c0_g1	1770	564	gb|QLI62115.1|carboxylesterase 3 [*Streltzoviella insularis*]	0	71.99	1770	564

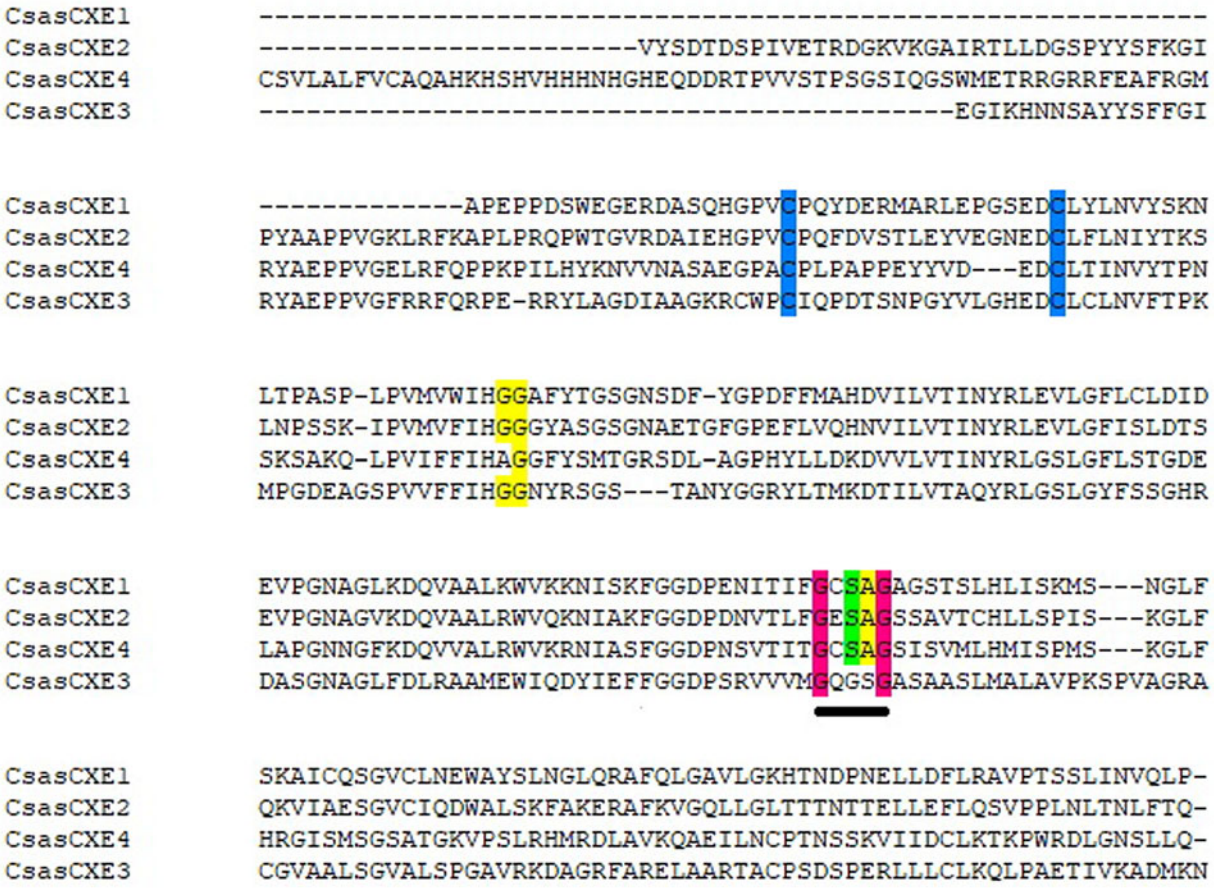

Figure 1 Sequence alignment of candidate *C. sasakii* CXEs, showing conserved amino acid residues: the disulfide bridge (Cys, Cys; blue), oxyanion hole (Gly-Gly, Ala; yellow), pentapeptide Gly-X-Ser-X-Gly (underlined), and catalytic triad (Ser, Glu, His; green).

Phylogenetic analysis

Phylogenetic analysis indicated that the CXEs with the Gly-X-X-Ser-Gly pentapeptide motif cluster in a major clade, including *Csas*CXE3, a *M. separata* CXE (*Msep*CXE6) and a *S. litura* CXE (*Slit*CXE30) (fig. 2). *Csas*CXE4, a *M. separata* CXE (*Msep*CXE3), two *S. inferens* CXEs (*Sinf*CXE5 and *Sinf*CXE16) and two *S. litura* CXEs (*Slit*CXE5 and *Slit*CXE16)

https://doi.org/10.1017/S0007485322000244 Published online by Cambridge University Press

```
CsasCXE1        ------FIQNDIMDITDAILFAPVIEKSSLNVEKFLTEPPPELVLRGDIAKVPIIVGYTS
CsasCXE2        ------TATPDESLRGLPRSFAPIVEKRFKNVQSFLDQEPIDLLVSGKINKVPLLLGFNS
CsasCXE4        ------FYEFG---YDPVGIWMPIVELDFG-QERFLPIEPIDAIREGKMHAVPWIVSQTT
CsasCXE3        SEGLVDSAKFLEQISGKSGAGARVEGADDKRGLPPMVVESPTDALDKKANRIPLLTGVTS

CsasCXE1        REGIEIGRKLQNILPLLQ-MKGG-VVPRELKYKFTS--EELDVADLQ-IRKHYFGE----
CsasCXE2        AEGLLMLFDTVNKLNFTN-KNPNYFIPHDIAEKLNYNNETLTAMGER-VIKYYSGR----
CsasCXE4        REFFWLAYTALRNETLLNRMKTEWDTIAPISFMLPREKTPENAEKVRRLTEHYLGP----
CsasCXE3        AETSRAVFGKFSTFLVDRLKNVKNFIKEELVGGLQAIIKDVQGLVPIKLPKNHTILPLAE

CsasCXE1        ---EPIS-----ENKLEDVIDLESDLLFSYNIYRFCRYHTHHTGKPAFVYKFTADTERN-
CsasCXE2        ---NMFT-----SNDSEAIVNMTTDAYFLYNTHRFAYLYSQLA--KVYMYEFSYDTDLN-
CsasCXE4        ---KGSSSLANNEESATALGNFYVDSIEGLPVHRMANLMCRHSPQPVYYYEFAYIGNHS-
CsasCXE3        YYQSIFDNSLKASDGLKQIVEATGDALFNFPAYQTVKSWSAGG--HAYLYSFEHVGNLSK

CsasCXE1        ----------FTKRQYQMEEIEGVCHADDLPYLFN--VTSPP---VPLTEESLPIIRQFV
CsasCXE2        ----------FVKRALNITDIKGACHADELFYIFYSPLNQGP---YEEQDELKVAAYRVS
CsasCXE4        ----------HYEDPVTNKPVA-AAHHDDLIYLFTLSYRFPT---IPLDSPDNVLVERMT
CsasCXE3        GSHFLRGVSLAEKSDESIEESKGPAHGDELAYIFEPLDDESNPLNEETSETDARVRENFV

CsasCXE1        RLWTNFATFGEPTTQEDK-----EKWASFTNKDRNCFIIG-KTSRCATTETKSLDLWDKI
CsasCXE2        KIWADFAKTGNPTPDRSLG----VKWPRYTAKCKEYLNINEQVSIGKYAEQERTNLWNKL
CsasCXE4        SIWYNFARYGDPNPREGQPELASLSWPVMKPSDRKYLQIGKEFTVKEKLFEEKYTIWEEL
CsasCXE3        NIIAKFAHDLNPLETKKPK---FANFMPFSEKNNQFIKIGEEVAVEKDFRYCQMGLWGNM
```

Figure 1 Continued.

```
CsasCXE1        YDMN----I-----------------------------------------------------
CsasCXE2        YCEAGLPCIHHHEHHHHHHGH-----------------------------------------
CsasCXE4        YPIQY---------------------------------------------------------
CsasCXE3        VEKVTGVLCKNILGNLLKVVDLPLPITLPVITNSKDNNKIPILESISSGISEAKDQKTER

CsasCXE1        ----------
CsasCXE2        ----------
CsasCXE4        ----------
CsasCXE3        ITRRPLIKIF
```

Figure 1 Continued.

form a clade in which the first glycine of the oxyanion hole is replaced by another amino acid (fig. 2). *Csas*CXE1 and *Csas*CXE2 are close to each other, but both of them are far away from *Csas*CXE3 and *Csas*CXE4 (fig. 2). Each of the four *C. sasakii* CXEs clusters in a clade with some of the other lepidopteran CXEs, with the bootstrap values 100, 91, 100 and 79 respectively (fig. 2).

Gene expression analysis using transcriptomic data

Gene expression analysis using head transcriptomic data revealed that all the four *C. sasakii* CXEs have differential expressions between the adult females and males, of which *Csas*CXE2 and *Csas*CXE3 are expressed more abundantly in the females than males, but *Csas*CXE1 and *Csas*CXE4 are expressed at higher levels

https://doi.org/10.1017/S0007485322000244 Published online by Cambridge University Press

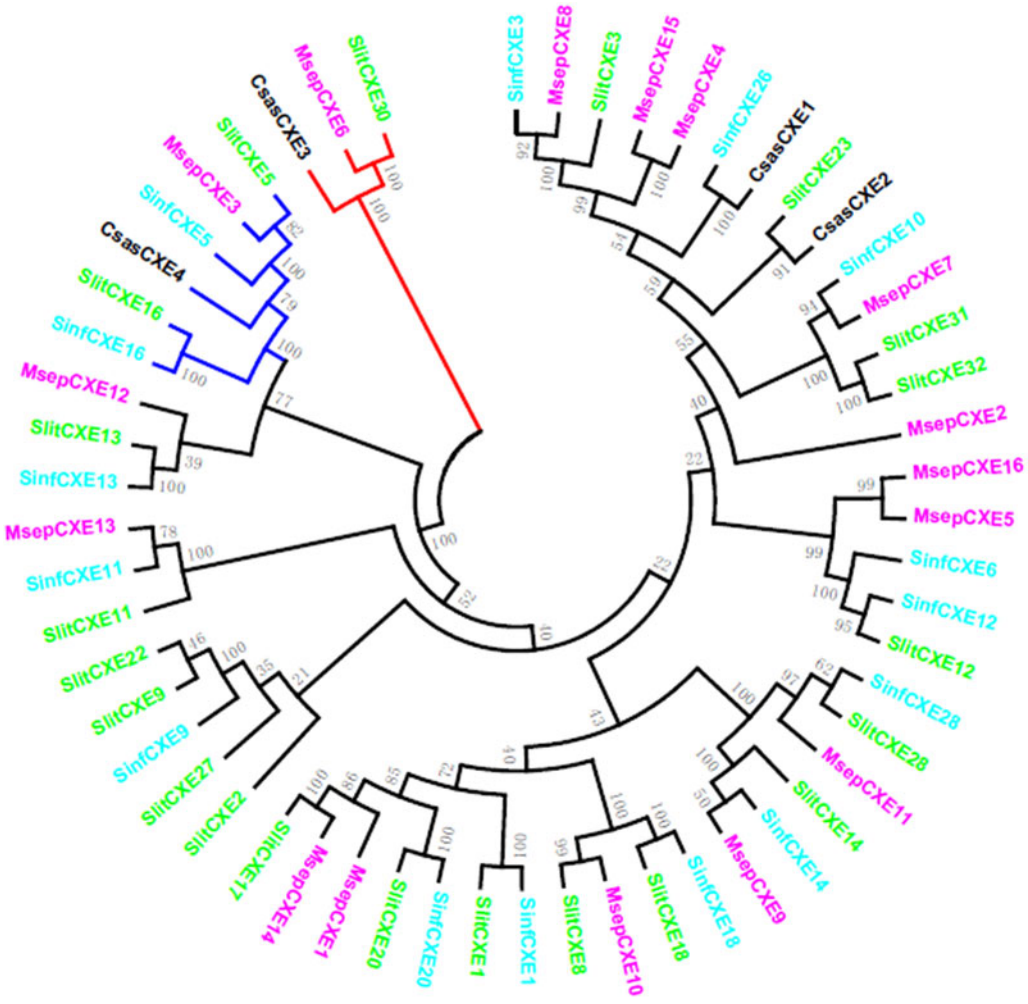

Figure 2 Phylogenetic tree constructed for amino acid sequences of the CXEs from *C. sasakii* (black font) and three other lepidopteran species: *M. separata* (Msep, purple font), *S. inferens* (Sinf, cyan font) and *S. litura* (Slit, green font). Red lines show the clades with the Gly-X-X-Ser-Gly pentapeptide motif, while blue lines show the clades in which the first glycine of the oxyanion hole is replaced by another amino acid. Bootstrap values (%) are indicated at the nodes.

Table 2. Comparison of gene expressions between *C. sasakii* adult females and males, an analysis using head transcriptomic data

Gene	TPM-female	TPM-male	|FoldChange|	*Q* value	Differential expression
*Csas*CXE1	0.97	5.21	5.35	5.18×10^{-8}	Yes
*Csas*CXE2	9.59	3.87	2.48	9.53×10^{-7}	Yes
*Csas*CXE3	5.49	2.01	2.73	1.48×10^{-2}	Yes
*Csas*CXE4	8.56	21.15	2.47	3.53×10^{-14}	Yes

in the males than females (TPM ≥5 at least in one sex, |FoldChange| >2 and $Q < 0.05$ at the same time; table 2).

Gene expression analysis using quantitative PCR data

Gene expression analysis using quantitative PCR data revealed that the expression levels of *Csas*CXE2 and *Csas*CXE3 in the heads are higher than those in the thoraxes and abdomens, but are not significantly different from those in the wings (fig. 3). The expression level of *Csas*CXE1 is lower in the heads than in the wings and thoraxes, but higher than that in the abdomens (fig. 3). *Csas*CXE4 is equally expressed in the heads, wings and abdomens, but has the highest level of expression in the thoraxes (fig. 3).

Discussion

C. sasakii adults mainly depend on olfaction to communicate with the environment, sensing sex pheromone (Han *et al.*, 2000; Zhang *et al.*, 2017*a*) and host plant odorants (Wang *et al.*, 2011; Sun and Wang, 2015). However, the olfactory mechanism has not been well known. ODEs are important olfactory proteins, which inactivate and degrade odorants to free ORs for maintaining olfactory sensitivity (Pelosi *et al.*, 2006; Leal, 2013). CXEs are considered to be a major group of moth ODEs (Leal, 2013; Groot *et al.*,

https://doi.org/10.1017/S0007485322000244 Published online by Cambridge University Press

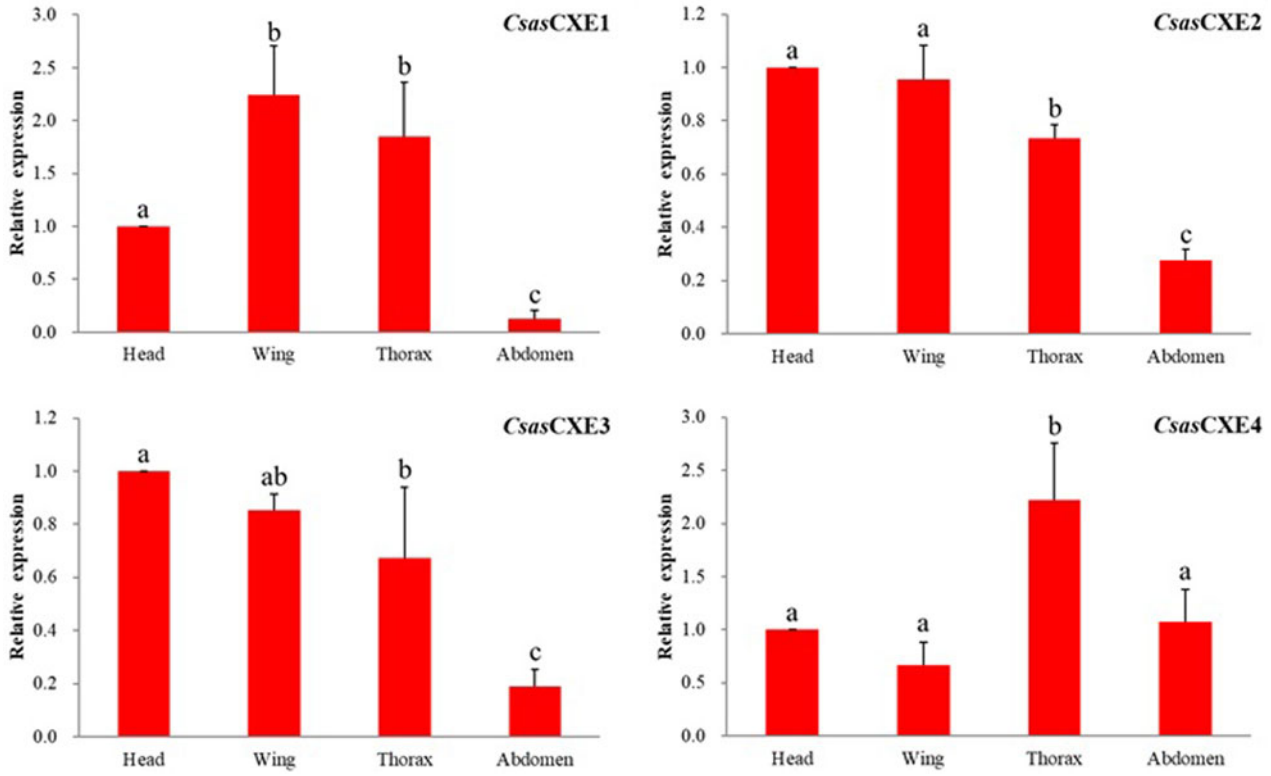

Figure 3 Relative expression levels of *C. sasakii* CXEs among different tissues (head, wing, thorax and abdomen) of the adults. Expressions of each CXE in different tissues are normalized, relative to that in head. Data are shown as mean of triplicates with standard deviation (the bar). Different letters at the bars indicate significant differences in the expression levels between different tissues ($P < 0.05$, LSD test).

2016). In this study, four candidate CXEs (*Csas*CXE1 ~ *Csas*CXE4) were identified by using head transcriptomic data from *C. sasakii* adult females and males. Transcriptome analysis has also been used to identify CXEs in other moths such as *Hyphantria cunea* (Ye *et al.*, 2021), *Cnaphalocrocis medinalis* (Zhang *et al.*, 2017*b*), *S. litura* (Zhang *et al.*, 2016), *Chilo suppressalis* (Liu *et al.*, 2015) and *S. inferens* (Zhang *et al.*, 2014).

Sequence alignment indicated variations of conserved amino acid residues in two *C. sasakii* CXEs (*Csas*CXE3 and *Csas*CXE4). The pentapeptide Gly-X-Ser-X-Gly is changed to a Gly-X-X-Ser-Gly motif in *Csas*CXE3, while the first glycine of the oxyanion hole is replaced by an alanine in *Csas*CXE4. Such variations occur not only in *C. sasakii*, but also in other species. The CXEs with such variations cluster well in the phylogenetic tree. Remarkably, we wonder whether such variations affect CXEs' functions. The oxyanion hole is an important functional site of CXEs, which may be affected by the change of the Gly-X-Ser-X-Gly pentapeptide motif. This is because the second X in the motif is usually an alanine involved in the formation of the oxyanion hole, but the alanine often does not exist in the Gly-X-X-Ser-Gly motif. In addition, the replacement of the first glycine of the oxyanion hole may also affect its formation.

Phylogenetic analysis indicated that each of the four *C. sasakii* CXEs clusters in a clade with some of the other lepidopteran CXEs, with the bootstrap values 100, 91, 100 and 79 respectively. Although the bootstrap values are high enough to suggest possible functional similarities, the functions of these CXEs have not been determined. Two *S. inferens* CXEs (*Sinf*CXE26 and *Sinf*CXE5) clustered with *Csas*CXE1 and *Csas*CXE4 respectively were considered as candidate ODEs (Zhang *et al.*, 2014), but their functions remain to be further researched.

Gene expression analysis using head transcriptomic data revealed that all the four *C. sasakii* CXEs have differential expressions between the adult females and males. The results suggest that these CXEs are likely to be involved in sex-related activities. *Csas*CXE2 and *Csas*CXE3 have similar tissue and sex expression patterns. The two CXEs have relatively high expression levels in the heads and are expressed more abundantly in the female heads than male heads. If the two CXEs function as ODEs, they may be able to inactivate and degrade carboxylic esters from host fruits in *C. sasakii* adult females. Although *Csas*CXE1 and *Csas*CXE4 are not dominantly expressed in the heads, the possibility that they serve as ODEs cannot be excluded. The two CXEs are expressed at higher levels in the male heads than female heads, so we wonder whether they are involved in inactivation and degradation of the sex pheromone in *C. sasakii* adult males. *C. sasakii* sex-pheromone components are not esters, so they should not be directly degraded by CXEs. However, maybe CXEs cooperate with other enzymes to participate in the processes. It was reported that different enzyme families may work together in inactivation and degradation of the same type of odorants (Steiner *et al.*, 2019). Our study laid the foundation for exploring functions of *C. sasakii* CXEs.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485322000244

Acknowledgements. This work was supported by the Research Start-up Foundation for Introduced Talents of Shenyang Agricultural University (Grant number: 20153016) and the Research Start-up Foundation for Doctors of Liaoning Province (Grant number: 20170520379).

Conflict of interest. The authors declare none.

References

Fleischer J, Pregitzer P, Breer H and Krieger J (2018) Access to the odor world: olfactory receptors and their role for signal transduction in insects. *Cellular and Molecular Life Sciences* **75**, 485–508.

Groot AT, Dekker T and Heckel DG (2016) The genetic basis of pheromone evolution in moths. *Annual Review of Entomology* **61**, 99–117.

Han KS, Jung JK, Choi KH, Lee SW and Boo KS (2000) Sex pheromone composition and male trapping of the peach fruit moth, *Carposina sasakii* (Matsumura) (Lepidoptera: Carposinidae) in Korea. *Journal of Asia-Pacific Entomology* **3**, 83–88.

Hemingway J, Hawkes NJ, McCarroll L and Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology* **34**, 653–665.

Huang X, Liu L, Su X and Feng J (2016) Identification of biotransformation enzymes in the antennae of codling moth *Cydia pomonella*. *Gene* **580**, 73–79.

Ishida Y and Leal WS (2005) Rapid inactivation of a moth pheromone. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14075–14079.

Jones BR and Bancroft HR (1986) Distribution and probable physiological role of esterases in reproductive, digestive and fat-body tissues of the adult cotton boll weevil, *Anthonomus grandis* Boh. *Biochemical Genetics* **24**, 499–508.

Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annual Review of Entomology* **58**, 373–391.

Li L, Zhang S, Zhang A, Zhou X, Men X, Zhuang Q and Yu Y (2013) Biological characteristics of *Beauveria bassiana* and its virulence to overwintering larvae of *Carposina niponensis* Walsingham. *Chinese Journal of Biological Control* **29**, 318–323.

Li J, Wang X and Zhang L (2019) Identification of putative odorant binding proteins in the peach fruit borer *Carposina sasakii* (Lepidoptera: Carposinidae) by transcriptome analysis and their expression profile. *Biochemical and Biophysical Research Communications* **508**, 1024–1030.

Liu S, Gong ZJ, Rao XJ, Li MY and Li SG (2015) Identification of putative carboxylesterase and glutathione S-transferase genes from the antennae of the *Chilo suppressalis* (Lepidoptera: Pyralidae). *Journal of Insect Science* **15**, 1–8.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods (San Diego, Calif.)* **25**, 402–408.

Oakeshott JG, Claudianos C, Campbell PM, Newcomb RD and Russell R (2005) Biochemical genetics and genomics of insect esterases. In Gilbert LI, Iatrou K and Gill SS (eds), *Comprehensive Molecular Insect Science*. London: Elsevier, pp. 309–361.

Pelosi P, Zhou JJ, Ban LP and Calvello M (2006) Soluble proteins in insect chemical communication. *Cellular and Molecular Life Sciences* **63**, 1658–1676.

Pelosi P, Iovinella I, Zhu J, Wang G and Dani FR (2018) Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. *Biological Reviews* **93**, 184–200.

Steiner C, Chertemps T and Maïbèche M (2019) Diversity of biotransformation enzymes in insect antennae: possible roles in odorant inactivation and xenobiotic processing. In Picimbon JF (ed.), *Olfactory Concepts of Insect Control – Alternative to Insecticides*. Cham, Switzerland: Springer, pp. 115–145.

Sun Y and Wang H (2015) Electroantennograms activities of *Carposina sasakii* Matsumura to volatiles of apples. *Liaoning Agricultural Sciences* 11–13.

Vogt RG and Riddiford LM (1981) Pheromone binding and inactivation by moth antennae. *Nature* **293**, 161–163.

Wang X, Wang H, Lv S, Yang F and Zhang Z (2011) Study on the host selection process of *Carposina niponensis* oviposition. *Hubei Agricultural Sciences* **50**, 2236–2239.

Ye J, Mang D, Kang K, Chen C, Zhang X, Tang Y, Purba ER, Song L, Zhang QH and Zhang L (2021) Putative carboxylesterase gene identification and their expression patterns in *Hyphantria cunea* (Drury). *PeerJ* **9**, e10919.

Younus F, Chertemps T, Pearce SL, Pandey G, Bozzolan F, Coppin CW, Russell RJ, Maïbèche-Coisne M and Oakeshott JG (2014) Identification of candidate odorant degrading gene/enzyme systems in the antennal transcriptome of *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **53**, 30–43.

Zhang YN, Xia YH, Zhu JY, Li SY and Dong SL (2014) Putative pathway of sex pheromone biosynthesis and degradation by expression patterns of genes identified from female pheromone gland and adult antenna of *Sesamia inferens* (Walker). *Journal of Chemical Ecology* **40**, 439–451.

Zhang YN, Li JB, He P, Sun L, Li ZQ, Fang LP, Ye ZF, Deng DG and Zhu XY (2016) Molecular identification and expression patterns of carboxylesterase genes based on transcriptome analysis of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Asia-Pacific Entomology* **19**, 989–994.

Zhang Z, Li X, Xue Y, Zhao Z, Li J and Ma R (2017*a*) Increased trapping efficiency for the peach fruit moth *Carposina sasakii* (Matsumura) with synthetic sex pheromone. *Agricultural and Forest Entomology* **19**, 424–432.

Zhang YX, Wang WL, Li MY, Li SG and Liu S (2017*b*) Identification of putative carboxylesterase and aldehyde oxidase genes from the antennae of the rice leaffolder, *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology* **20**, 907–913.

https://doi.org/10.1017/S0007485322000244 Published online by Cambridge University Press