Identification of Putative Carboxylesterase and Glutathione S-transferase Genes from the Antennae of the *Chilo suppressalis* (Lepidoptera: Pyralidae)

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**ABSTRACT.** In insects, rapid degradation of odorants in antennae is extremely important for the sensitivity of olfactory receptor neurons. Odorant degradation in insect antennae is mediated by multiple enzymes, especially the carboxylesterases (CXEs) and glutathione S-transferases (GSTs). The Asiatic rice borer, *Chilo suppressalis*, is an economically important lepidopteran pest which causes great economic damage to cultivated rice crops in many Asian countries. In this study, we identified 19 putative CXE and 16 GST genes by analyzing previously constructed antennal transcriptomes of *C. suppressalis*. BLASTX best hit results showed that these genes are most homologous to their respective orthologs in other lepidopteran species. Phylogenetic analyses revealed that these CXE and GST genes were clustered into various clades. Reverse-transcription quantitative polymerase chain reaction assays showed that three CXE genes (*CsupCXE8, CsupCXE13*, and *CsupCXE18*) are antennae-enriched. These genes are candidates for involvement in odorant degradation. Unexpectedly, none of the GST genes were found to be antennae-specific. Our results pave the way for future researches of the odorant degradation mechanism of *C. suppressalis* at the molecular level.

**Key Words:** *Chilo suppressalis*, odorant-degrading enzyme, phylogenetic analysis, expression pattern

Carboxylesterases (CXEs) and glutathione S-transferases (GSTs) are two major families of metabolic enzymes widely distributed in animals, plants, insects, and microbes (Hayes et al. 2005, Oakeshott et al. 2005). In insects, CXEs and GSTs have a broad range of functions. They play essential roles not only in the metabolism of a variety of physiologically important endogenous compounds, but also in the detoxification of a diversity of harmful exogenous compounds, such as plant allelochemicals and insecticides, leading to rapid adaptation of insects to host plants and insecticide resistance (Enayati et al. 2005, Li et al. 2007).

Apart from the functions of metabolism and detoxification of endobiotics and xenobiotics, insect CXEs and GSTs also play critical roles in the olfaction that maintains the sensitivity of the olfactory receptor neurons (ORNs) by rapidly degrading stray odorants and protect the vulnerable ORNs against harmful volatile xenobiotics (Leal 2013). These were also called odorant-degrading enzymes (ODEs) (Vogt 2003). To date, an ever-growing number of genes encoding CXEs and GSTs have been identified in the antennae of various insect species, including *Antheraea polyphemus* (Vogt and Riddiford 1981, Vogt et al. 1985, Ishida and Leal 2005), *Manduca sexta* (Rogers et al. 1999), *Mamestra brassicae* (Maibeche-Coisne et al. 2004), *Sesamia nonagrioides* (Merlin et al. 2007), *Popillia japonica* (Ishida and Leal 2008), *Epiphasys postvittana* (Jordan et al. 2008), *Spodoptera littoralis* (Merlin et al. 2007, Durand et al. 2010a), *Agrius planipennis* (Mamidala et al. 2013), *Spodoptera litura* (He et al. 2014a, Spodoptera exigua (He et al. 2014a,b,c; He et al. 2015), Bombyx mori (Tan et al. 2014), and *Drosophila melanogaster* (Chertemps et al. 2012, Younus et al. 2014), and their functions in olfaction were investigated. For example, the first characterized *A. polyphemus* pheromone-degrading enzyme (ApollPDE) is a CXE. This enzyme could effectively degrade the acetate component of the sex pheromone in a series of in vitro studies (Vogt and Riddiford 1981, Vogt et al. 1985, Ishida and Leal 2005). Other studies also revealed that the purified native or recombinant antennal CXEs from *P. japonica* and *D. melanogaster* could degrade sex pheromone constituents (Ishida and Leal 2008, Younus et al. 2014).

Furthermore, antennal CXEs from *Sp. littoralis*, *S. exigua* and *Sp. litura* seem involved not only in the degradation of sex pheromones but also plant volatiles (Durand et al. 2010b, 2011; He et al. 2014a,c). GSTs have also been implicated in odorant degradation. For instance, in *M. sexta*, a GST (GST-msolf1) is localized in the pheromone-sensitive sensilla and inactivates volatile plant aldehydes (Rogers et al. 1999). In *B. mori*, a GST (BmGSTD4) is specifically distributed in the sensillum lymph of male antennae, indicating the enzyme might be involved in pheromone degradation (Tan et al. 2014).

To date, benefiting from expressed sequence tag (EST) and transcriptome sequencing approaches, more and more odorant-processing genes from insect antennae have become recognized (Grosse-Wilde et al. 2011, Gu et al. 2011, Leal et al. 2013, Cao et al. 2014, Gong et al. 2015). However, these studies mainly focused on binding proteins (including odorant-binding proteins and chemosensory proteins) and chemoreceptors (including olfactory receptors and ionotropic receptors) and identification of genes implicated in odorant degradation has not yet been performed. So far, the CXE and GST repertoires have only been identified in the antennae of five insect species: *E. postvittana* (Jordan et al. 2008), *Sp. littoralis* (Legeai et al. 2011), *S. nonagrioides* (Glaser et al. 2013), Ag. planipennis (Mamidala et al. 2013), and *D. melanogaster* (Younus et al. 2014). Consequently, additional insect species need to be investigated.

The Asiatic rice borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), is a severe lepidopteran rice pest in many Asian countries, causing great economic losses to rice crops (Su et al. 2014). The establishment of two antennal transcriptomes of *C. suppressalis* by Cao et al. (2014) have provided a great opportunity for systematically identifying antennal CXE and GST genes and assessing their roles in moth species. In this article, we report: (1) identification and sequence analyses of 19 CXE and 16 GST genes from the *C. suppressalis* antennal transcriptome; (2) phylogenetic analyses of these genes; (3) investigation of tissue-specific expression patterns of these genes. We found three CXE genes were specifically expressed in the antennae of *C. suppressalis*. These genes are excellent candidates for involvement in odorant degradation.
Materials and Methods

Insect Rearing and Tissue Collection. The C. suppressalis colony used in this study originated from a field population collected in an experimental plot of Anhui Agricultural University, Hefei, Anhui, China in June, 2014. Larvae were reared on rice variety Taichung Native 1 (TN1, susceptible to almost all herbivores of rice) in an insectary at 26 ± 1°C, 75% relative humidity under a 16:8 (L:D) h photoperiod. Adults were provided with 10% (weight/volume) honey solution on sterile cotton swabs. Male antennae, female antennae, abdomens, and legs were dissected from 3-d-old unmated adults, immediately frozen in liquid nitrogen, and stored at −80°C before use.

Homology Searches. Previously, two antennal transcriptome datasets of C. suppressalis were released (NCBI accession numbers: SRX497236 and SRX497239, Cao et al. 2014). Here, we use the basic local alignment search tool (BLAST, Altschul et al. 1997) to identify putative CXE and GST gene sequences from the two datasets. The annotated CXE and GST protein sequences from representative insect species, such as D. melanogaster, B. mori and Sp. littoralis, were retrieved from GenBank and used as queries for TBLASTN searches. The cutoff E-value of TBLASTN algorithm was set as 1 × 10−5.

Bioinformatic Analyses. The open reading frames (ORFs) of genes were predicted using ORF finder (http://www.ncbi.nlm.nih.gov/orf/gorf/gorf.html). Theoretical isoelectric points and molecular weights of deduced proteins were calculated using an ExPaSy tool (http://web.expasy.org/compute_pi/). Signal peptide and transmembrane domain were predicted using SignalP (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), respectively. Homology searches were performed using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi). Catalytic residues were predicted by searching the NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/structure/cds/cds.shtml). The protein sequences of CXEs and GSTs from C. suppressalis and other insect species were aligned using Clustal Omega (http://www.ebi.ac.uk/tools/msa/clustalo/). Phylogenetic trees were constructed by MEGA 5.05 software using the neighbor-joining method with 1,000 bootstrap replications (Tamura et al. 2011). The GenBank accession numbers of sequences used are listed in Supp Table 1 (online only).

RNA Isolation and cDNA Synthesis. Total RNA was isolated from different tissues (male antennae, female antennae, abdomens, and legs) of C. suppressalis adults using RNAiso Plus (TaKaRa, Dalian, China) following the manufacturer’s protocol. Each RNA sample was treated with the RNase-free DNase I (TaKaRa, Dalian, China) to eliminate potential contamination of genome DNA. RNA integrity was confirmed by gel electrophoresis and RNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Each RNA sample was reverse-transcribed to first-strand cDNA using the PrimeScript First Strand cDNA Synthesis Kit (Takara, Dalian, China).

Reverse-Transcription Quantitative Polymerase Chain Reaction. Primer pairs for quantitative polymerase chain reaction (qPCR) were designed using BatchPrimer3 (http://probes.ws.usda.gov/batch-primer3/) and are listed in Supp Table 2 (online only). The housekeepers actin A1 and ribosomal protein 49 (rp49) were used as reference genes (Teng et al. 2012). qPCR assays were performed using iTag Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). 20 μl reaction volumes contained 10 μl iTag Universal SYBR Green Supermix, 0.4 μl (0.2 μM) of each primer, 1 μl (10 ng) cDNA template, and 8.2 μl nuclease-free water. qPCRs were run on a Bio-Rad CFX96 Real-Time System (Bio-Rad, Hercules, CA) with the following parameters: one cycle of 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 25 s. To confirm that only single gene was detected by fluorescence dye, a heat-dissociation protocol was added at the end of thermal cycle, and the amplified products were analyzed by gel electrophoresis. In addition, each product was sequenced to verify the amplification of correct target. A no-template control and a no-reverse transcriptase control were both included on each reaction plate to detect possible contamination. Three independent biological replicates were performed, approximately 150 male antennae, 150 female antennae, 30 abdomens (15 from male and 15 from female, pooled together), and 240 legs (120 from male and 120 from female, pooled together) were used in each replicate. These replicates were tested on multiple reaction plates and on each plate two reference genes (actin A1 and rp49) were all included.

Quantification of gene expression was conducted according to a modified version of the ΔCq method (Pfaffl 2001; Hagström et al. 2014). The steps are briefly described as follows: the efficiency (E) of a primer pair in a sample was determined from the slope of the log-linear portion of the calibration curve: $E = 10^{\frac{-1}{\text{slope}}}$ Then the formula was used to calculate the relative expression ratio for a particular gene in a sample versus a control in comparison to reference genes. In the formula, $E_{\text{target}}$ is the efficiency of target gene transcript; $E_{\text{ref}}$ is the efficiency of reference gene transcript; ΔCq_target is the Cq deviation of control–sample of the target gene transcript; and ΔCq_ref is the Cq deviation of control–sample of the reference gene transcript. Because two reference genes (actin A1 and rp49) were used in qPCR, here $(E_{\text{ref}})^{\Delta Cq_{\text{ref}}}$ is the average value for actin A1 and rp49 in each sample.

Data Statistics. Statistical analyses of data were carried out using Data Processing System (DPS) software v9.5 (Tang and Zhang 2013). The relative expression levels from three independent biological replicates were log-transformed and analyzed by one-way analysis of variance (ANOVA) followed by the Tukey’s test. The level of significance was set at $P < 0.05$.

Results and Discussion

Identification of CXE Genes from C. suppressalis Antennae. So far, genome-wide analyses of CXE genes have been performed for model insect species, such as D. melanogaster, Anopheles gambiae, B. mori, Apis mellifera, Nasonia vitripennis, and Tribolium castaneum. From these 35, 51, 76, 24, 41, and 49 CXE genes were identified, respectively (Yu et al. 2009, Oakeshott et al. 2010). However, researches on the large-scale identification of the CXE gene families in insect antennae are limited. In this study, a total of 19 sequences with high identities and low E-values to CXE genes from known insects were identified from the antennal transcriptome of C. suppressalis (Table 1, Supp Fig. 1 [online only]). These sequences were named as CsupCXE1 to CsupCXE19 (Table 1). Of these, 18 sequences had full-length ORFs while one sequence (CsupCXE8) was an incomplete cDNA with truncations in both 5’- and 3’-regions. The GenBank accession numbers and lengths of all the 19 CsupCXE genes are presented in Table 1. The lengths of the 18 deduced full-length CsupCXE proteins ranged from 524 to 709 amino acid residues. These had predicted theoretical isoelectric points ranging from pH 4.66 to 8.28, and predicted molecular weights ranged from 59.61 to 78.36 kDa (Table 1). The numbers of antennal CXE genes in C. suppressalis are more than those obtained from E. postvittana (five genes, Jordan et al. 2008), but less than those from Sp. littoralis (20 genes, Durand et al. 2010a), D. melanogaster (26 genes, Younus et al. 2014), and Ag. planipennis (56 genes, Maimida et al. 2013). There is a possibility that other CsupCXE genes may have been undetected by this transcriptomic approach but still be expressed in the antennae.

N-terminal signal peptides were predicted for 11 of the 18 CsupCXE protein sequences, except for CsupCXE8 due to its incomplete N-terminal region (Table 1). The presence of a signal peptide indicated that the corresponding enzyme could be secreted. BLASTX best hits in GenBank are all sequences from Lepidopteran species with sequence identities from 52 to 73% and E-values from 0.0 to 3 × 10−169 (Table 1). Multiple sequence alignments showed that all the
19 CsupCXEs proteins, including the truncated CsupCXE8, displayed a conserved sequence motif, such as the conserved pentapeptide Gly-X-Ser-X-Gly and oxanion hole residues (Gly, Gly and Ala), although these residues in some sequences were variable (Table 2). These residues are essential for the enzymatic activity of CXE proteins (Oakeshott et al. 2005). In addition to the serine residues, all the CsupCXEs possess the conserved glutamate and histidine residues of the catalytic triad (Table 2) and therefore might be active enzymes.

**Phylogenetic Analysis of Insect CXEs.** Based on the sequence similarity and substrate specificity, insect CXEs can be divided into three major clades: intracellular catalytic, secreted catalytic, and neurodevelopmental. They can also be subdivided into eight classes: α-estersases, β-estersases, juvenile hormone esterases (JHEs), acetylcholinesterases (AChEs), gliotactins, neurotactins, neuroligins, and glutactins (Oakeshott et al. 2005). Here, we performed a phylogenetic analysis for the 19 antennal CXEs from *C. suppressalis* together with CXEs from *D. melanogaster*, *A. mellifera*, *Sp. littoralis*, and some other related species (Fig. 1). The topology of the neighbor-joining tree was very similar to those obtained in previous studies (Yu et al. 2009, Durand et al. 2010a), and clearly some of the CXEs are the putative orthologs, such as CsupCXE11, SlitCXE11 and SnonCXE11, and CsupCXE13, ApolPDE, SlitCXE13, SnonCXE13 and SexiCXE13 (Fig. 1).

In this tree, insect CXEs can be divided into eight different clades (Fig. 1). Of these, eight CsupCXEs (CsupCXE1, 3, 8, 9, 12, 14, 17, and 18) with signal peptides were clustered into a clade together with moth α-estersases and antennal esterases: the former are well known for their detoxification functions to degrade insecticides (Jackson et al. 2013, Li et al. 2013). CsupCXE1 fell into a branch together with the *A. polyphemus* ODE (ApolODE, Ishida and Leal 2002) and *M. brassicae* odorant-degrading esterase (MbraEAST, Maëbêche-Coise et al. 2004). The two CXEs are specifically expressed in antennae and possibly involved in odorant degradation. CsupCXE14 fell into a branch together with the SexiCXE4 and SexiCXE14 of *Sp. exigua* (Fig. 1). The two enzymes have function in the degradation of plant volatiles and sex pheromones (He et al. 2014a,c). The SlitCXE7 of *Sp. littoralis* also fell into this branch (Fig. 1). The gene encodes an enzyme which can efficiently hydrolyze pheromone compounds as well as plant volatiles (Durand et al. 2011).

CsupCXE16 and CsupCXE19 were clustered into the ‘intestinal esterases’ clade (Fig. 1). This clade includes the *A. polyphemus* intestinal esterase (ApolIE, Ishida and Leal 2002). ApoIE is expressed throughout the body of adult males and females, and may be involved in the clearance of pheromones from the integument (Vogt and Riddiford 1986). CsupCXE13 was clustered into the ‘β-estersases and pheromone-degrading esterases’ clade (Fig. 1). This clade includes a well-characterized pheromone-degrading enzyme of *A. polyphemus* (ApolPDE). The enzyme is male antennae-specific and involved in the rapid inactivation of sex pheromones (Ishida and Leal 2005).

Seven CsupCXEs (CsupCXE4, 5, 6, 7, 10, 11, and 15) lack signal peptides were clustered into the ‘α-estersases’ clade (Fig. 1). In this clade, CsupCXE10 fell into a branch together with two well-characterized ODEs: SlitCXE10 and SexiCXE10 (Durand et al. 2010b; He et al. 2015), suggested these CXEs are orthologues and may have similar functions in odorant degradation. CsupCXE2 fell into a clade together with lepidopteran JHEs (Fig. 1). JHEs have critical roles in regulating larval to adult transition in insects and other arthropods (Oakeshott et al. 2005). The phylogenetic diversity of these *C. suppressalis* CXEs suggested that they might play different roles in the metabolism of odorants, endobiotics or xenobiotics.

### Table 2. Catalytic motifs of the *C. suppressalis* CXEs

| Gene name | GxSxG version | E | H | Oxyanion hole version |
|-----------|---------------|---|---|-----------------------|
| CsupCXE1  | GESAG         | + | + | GGA                   |
| CsupCXE2  | GESAG         | + | + | GGA                   |
| CsupCXE3  | GESAG         | + | + | GGY                   |
| CsupCXE4  | GESAG         | + | + | GGA                   |
| CsupCXE5  | GESAG         | + | + | GGA                   |
| CsupCXE6  | GESAG         | + | + | GGG                   |
| CsupCXE7  | GESAG         | + | + | GGA                   |
| CsupCXE8  | GESAG         | + | + | GGG                   |
| CsupCXE9  | GESAG         | + | + | GGA                   |
| CsupCXE10 | GESAG         | + | + | GGG                   |
| CsupCXE11 | GESAG         | + | + | GGA                   |
| CsupCXE12 | GESAG         | + | + | GGG                   |
| CsupCXE13 | GESAG         | + | + | GGA                   |
| CsupCXE14 | GESAG         | + | + | GGG                   |
| CsupCXE15 | GESAG         | + | + | GGA                   |
| CsupCXE16 | GESAG         | + | + | GGG                   |
| CsupCXE17 | GESAG         | + | + | GGA                   |
| CsupCXE18 | GESAG         | + | + | GGG                   |
| CsupCXE19 | GESAG         | + | + | GGA                   |
23, 8, 19, and 35 GST genes were isolated, respectively (Yu et al. 2008, Oakeshott et al. 2010). In this study, we identified a total of 16 cDNA fragments encoding putative GSTs from the *C. suppressalis* antennal transcriptome (Table 3, Supp Fig. 1 [online only]). Of these, 14 sequences had complete ORFs while two sequences (*CsupGSTd4* and *CsupGSTe2*) were incomplete cDNAs with truncated 3′-regions (Table 3). These *C. suppressalis* antennal GST genes were named *CsupGSTd1* to *CsupGSTu1* according to the classification system and

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Fig. 1. Phylogenetic analysis of CXEs from various insect species including *Antheraea polyphemus*, *Bombyx mori*, *Chilo suppressalis*, *Epiphyas postvittana*, *Mamestra brassicae*, *Spodoptera exigua*, *Spodoptera littoralis*, and *Sesamia nonagrioides*. Bootstrap values above 50% are shown for each node. The *C. suppressalis* CXEs are highlighted in red.
order of discovery (Table 3). These sequences have been deposited into GenBank, the accession numbers listed in Table 3. The length of the 14 deduced full-length proteins ranged from 204 to 284 amino acid residues and had predicted theoretical isoelectric points ranging from pH 4.93 to 6.10, with predicted molecular weights ranging from 23.23 and 32.43 kDa (Table 3). The number of the C. suppressalis antennal GSTs are more than those identified from the antennae of E. postvittanae (11 genes, Jordan et al. 2008), S. lituraalis (14 genes, Legeai et al. 2011), and A. planipennis (13 genes, Mamidala et al. 2013), but fewer than those isolated from D. melanogaster (31 genes, Younus et al. 2014). We cannot rule out that transcriptome sequencing may not be powerful enough to discover all the GST genes, especially those transcripts with extremely low abundance in the antennae.

BLASTx best hits results of all the 16 GSTs were shown in Table 3. All of the GSTs had high identities (60–98% identities) and low E-values (4 × 10^{-168} to 3 × 10^{-93}) to their respective orthologs from Lepidopteran species. Of these, CspGSTd1 showed 73% identity to the previously identified antennal GST (AstraGST) of A. transtella (Leal et al. 2009) (Table 3, Supp Fig. 2 [online only]). CspGSTd1 also showed 70 and 69% identities to the olfactory-specific GST (GST-msolf) from M. sexta (Rogers et al. 1999) and a delta class GST (BmorgGSTd1) from B. mori, respectively (Supp Fig. 2 [online only]). CspGSTs1, is identical to a sigma class GST gene (CsGStSigma) previously identified in C. suppressalis by Huang et al. (2011a) (Table 3).

Cytosolic GST proteins have two domains in their secondary structure: an N-terminal thioredoxin-like domain, responsible for glutathione GSH binding (G-site) and a C-terminal domain, which contains a pocket of hydrophobic substrate binding site (H-site) (Ketterman et al. 2011). Multiple sequence alignment analyses showed that, for all the deduced CspGST proteins, a conserved G-site can be found in the N-terminal domain and a more variable H-site can be observed in the C-terminal domain (Supp Fig. 3 [online only]). Variations of the H-site enables GSTs to accommodate various electrophilic substrates (Lerkthirat and Ketterman 2008).

**Phylogenetic Analysis of Insect GSTs.** The insect cytosolic GSTs can be classified into six groups (delta, epsilon, omega, theta, zeta, and the ‘unclassified’ clade) based on sequence similarity, genomic organization, and biochemical properties (Ketterman et al. 2011). GSTs which could not be classified by the nomenclature system were assigned as an unclassified subgroup (Ketterman et al. 2011). To better understand the classification and phylogenetic relationships of the CspGSTs, a phylogenetic tree was constructed (Fig. 2). In this tree, the 16 CspGSTs were placed into seven clades, including six cytosolic classes: delta (four CspGSTs), epsilon (three), omega (four), sigma (two), theta (one) and zeta (one), and the ‘unclassified’ clade (one) (Fig. 2).

CspGSTd1, CspGSTe2, CspGSTd3, and CspGSTd4 fell into the delta class and CspGSTe1, CspGSTe2, and CspGSTe3 fell into the epsilon class (Fig. 2). Delta and epsilon are two most common classes in insects (Enayati et al. 2005). Members from these two classes are well-known for their detoxification functions and usually related to insecticide resistance (Enayati et al. 2005, Li et al. 2007). Delta class GSTs are also involved in the degradation of odorants. Two well-characterized olfactory-related GSTs in M. sexta and B. mori both grouped with the delta class (Rogers et al. 1999, Tan et al. 2014). In addition, a delta class GST (AtraGST) was found highly expressed in the antennae of A. transtella, indicating its possible involvement in inactivating odorants (Leal et al. 2009). In the tree, CspGSTd1 was clustered into a branch together with GST-msolf and AstraGST (Fig. 2), suggesting that they might be orthologs and play similar roles in the odorant inactivation. CspGSTs1 and CspGSTs2 were clustered into the sigma class (Fig. 2). Members belonging to the sigma class are considered to have housekeeping roles. For example, in D. melanogaster, B. mori and S. litura, sigma class GSTs could act as antioxidants, conjugating lipid peroxidation products (Singh et al. 2001, Yamamoto et al. 2006), or as signaling molecules recognizing invasive pathogens (Huang et al. 2011b). However, in L. migratoria, sigma class GSTs are also involved in the detoxification of insecticides (Qin et al. 2012, 2013).

GSTs belonging to the omega, theta and zeta classes and the unclassified subgroup are involved in the detoxification of xenobiotics. For instance, in Anopheles cracens, an omega class GST (AcGSTO1-1) bound but did not metabolize the organophosphate temephos (‘Abate’) (Wongtrakul et al. 2010). Also in A. cracens, a theta-class GST functioned not only as peroxidease but also as binding protein for organophosphates (Wongtrakul et al. 2010). A zeta-class GST (bmGSTz) in B. mori can metabolize the neurotoxin permethrin and may contribute to permethrin resistance (Yamamoto et al. 2009). Furthermore, in L. migratoria, an unclassified GST (LmGSTu1) is responsible for the detoxification of the organophosphate insecticide chlorpyrifos (Qin et al. 2013). However, olfactory-related functions of GSTs belonging to these classes have not yet been determined.

**Expression Patterns of CXE and GST Genes.** Tissue-specific expression patterns of the 19 CspCXE and 16 CspGST genes were evaluated by qPCR in various tissues, including male and female antennae, abdomens, and legs. The results showed that most CspCXE genes were highly expressed in the nonolfactory tissues (Fig. 3). Only three genes were mainly expressed in antennae: CspCXE8, CspCXE13, and CspCXE18. In addition, CspCXE8 and CspCXE13 were almost
equally expressed in male and female antennae, while the \textit{CsupCXE18} transcripts were more abundant in male antennae than female antennae (Fig. 3). The deduced proteins encoded by these genes all had signal peptides (Table 1), suggesting that these enzymes could be secreted into the sensillar lymph and possibly degrade odorants or pheromones. Indeed, a number of CXEs restricted to insect antennae showed capacity to degrade sex pheromone constituents and plant volatiles (Ishida and Leal 2005; Durand et al. 2010b, 2011; He et al. 2014b,c; He et al. 2015). Interestingly, the expression profile of \textit{CsupCXE13} (antennae-enriched) is intermediate between its orthologs: \textit{ApolPDE} of \textit{A. polyphemus} (male antennae-specific, Ishida and Leal 2005 ), \textit{SlitCXE13} of \textit{Sp. littoralis} (ubiquitous, Durand et al. 2010a), and \textit{SexiCXE13} (ubiquitous, He et al. 2014a). This phenomenon is possibly due to the within-species variation.

The key role of CXEs is hydrolyze esters of carboxylic acids. However, the pheromone blend produced by female \textit{C. suppressalis} was identified as a mixture of Z-11-hexadecenal (Z-11-16Ald), Z-13-octadecenal (Z-13-18Ald), and Z-9-hexadecenal (Z-9-16Ald) (Tatsuki et al. 1983); none of these compositions are esters. There is a possibility that \textit{CsupCXE8}, \textit{CsupCXE13}, and \textit{CsupCXE18} might be involved in the degradation of volatiles emitted by rice crops, i.e., methyl jasmonate or methyl salicylate (Bi et al. 2007). Apart from rice volatiles, acetate pheromones from sympatric insect species could also be substrates for these antennae specific CXEs. For example, the sex pheromone blend of the rice leaffolder, \textit{Cnaphalocrocis medinalis} (Lepidoptera: Pyralidae), consists of (Z)-11-hexadecaenyl acetate and (Z)-13-octadecaenyl acetate (Kawazu et al. 2009) and could possibly be degraded by antennal CXEs of \textit{C. suppressalis}.

Unexpectedly, we found that none of the \textit{CsupGST} genes was restricted to antennae (Fig. 4). Most of the \textit{CsupGST} genes were mainly expressed in the abdomen, or ubiquitously expressed in both antennae and nonolfactory tissues (Fig. 4). That none of the \textit{CsupGSTs} were antennae-specific is somewhat surprising given that olfactory-specific GSTs have been isolated from other moth species, such as \textit{M. sexta} and \textit{B. mori} (Rogers et al. 1999, Tan et al. 2014). We found \textit{CsupGSTd1} was ubiquitous in antennae, abdomen, and legs (Fig. 4). Considering
Fig. 3. Relative expression levels of CsupCXE genes in different tissues of C. suppressalis adults. Levels of gene expression were normalized relative to that in leg (onefold). Different lowercase letters indicate significant differences (one-way ANOVA with Tukey’s test, \( P < 0.05 \)).

Fig. 4. Relative expression levels of CsupGST genes in different adult tissues. Levels of gene expression were normalized relative to that in leg (onefold). Different lowercase letters indicate significant differences (one-way ANOVA with Tukey’s test, \( P < 0.05 \)).
that delta class GSTs are commonly associated with xenobiotic detoxification (Li et al. 2007), and that CsupGSTd1 is the orthologous locus of the olfactory-related GST-msof1 of M. sexta (Rogers et al. 1999), we hypothesized that CsupGSTd1 might play a dual role in degradation of odorants and detoxification of xenobiotics. However, whether CsupGSTd1, as well as other antennal GSTs, have a role in olfaction remains to be investigated.

In conclusion, this is the first study of genes involved in the degradation of odorants in the lepidopteran species C. suppressalis. We identified 19 putative CFEx and 16 GST genes in the antennal transcriptome of the moth, analyzed their phylogenetic relationships and investigated their expression profiles in different tissues. We found three CFEx genes are enriched in antennae, indicating their potential involvement in odorant degradation. This study suggests that our transcriptome analysis approach is efficient in identifying divergent genes and the results also make it possible for future research of these antennal degradation enzymes in inactivating diverse chemicals.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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CsupGSTd1, as well as other antennal GSTs, have a role in olfaction and detoxification of xenobiotics. However, whether CsupGSTd1 and other antennal GSTs play a dual role in degradation of odorants and detoxification of xenobiotics remains to be investigated.

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