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# AN OLFACTORY-SPECIFIC GLUTATHIONE-*S*-TRANSFERASE IN THE SPHINX MOTH *MANDUCA SEXTA*

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## Summary

**Insect antennae have a primary function of detecting odors including sex pheromones and plant volatiles. The assumption that genes uniquely expressed in these antennae have an olfactory role has led to the identification of several genes that are integral components of odorant transduction. In the present study, differential display polymerase chain reaction (ddPCR) was used to isolate 25 antennal-specific mRNAs from the male sphinx moth *Manduca sexta*. Northern blot analyses revealed that one clone, designated G7-9, was antennal-specific and was highly enriched in male antennae relative to female antennae. *In situ* hybridization indicated that G7-9 expression was restricted to a spatial domain of the olfactory epithelium occupied exclusively by sex-pheromone-sensitive olfactory sensilla. Amino acid homology and phylogenetic analyses identified G7-9 as a glutathione-*S*-transferase (GST); we have named the full-length clone GST-msolf1. GSTs are known to function primarily in the detoxification of noxious compounds. Spectrophotometric and chromatographic analyses of total GST activity indicate that the endogenous GSTs of male and female antennae can modify *trans*-2-hexenal, a plant-derived green leaf aldehyde known to stimulate the olfactory system of *M. sexta*. The restricted localization of GST-msolf1 to sex-pheromone-sensitive sensilla, the fact that the sex pheromone of *M. sexta* consists of a complex mixture of aldehyde components, and the observation that antennal GSTs can modify an aldehyde odorant suggest that GST-msolf1 may have a role in signal termination. In the light of the more commonly observed role of GSTs in xenobiotic metabolism, we propose that GST-msolf1 may play a dual role of protecting the olfactory system from harmful xenobiotics and inactivating aldehyde odorants, especially components of the *M. sexta* sex pheromone.**

Key words: sphinx moth, *Manduca sexta*, olfaction, glutathione-*S*-transferase, antennal-specific mRNA, odorant, odorant degrading enzyme, pheromone, xenobiotic.

## Introduction

The olfactory systems of terrestrial animals are designed to trap and sample volatile hydrophobic molecules. Some of these molecules are odorants, such as volatile plant compounds and pheromones emitted from other organisms. However, many are cytotoxic xenobiotics, such as plant-derived monoterpenoids and isothiocyanates (Gershenzon and Croteau, 1991; Louda and Mole, 1991) and synthetic pesticides and herbicides (Grant and Matsumara, 1989; Hayes and Pulford, 1995). The sensory neurons of the olfactory epithelium are among the few cells of the nervous system in direct contact with the external environment. Olfactory neurons are therefore directly exposed to both odorants and xenobiotics. Given the importance of the olfactory system in controlling critical behavior patterns such as reproduction and feeding, it might be expected that olfactory systems would have evolved both mechanisms for detoxifying xenobiotics to minimize cytotoxicity and mechanisms for inactivating odors to minimize signal saturation. Furthermore, it is possible that some of these mechanisms serve both ends, performing a dual role by inactivating both xenobiotics and odorants.

In insects, olfactory neurons are present within sensilla located on the antennae. These sensilla are cuticular hair-like structures with a fluid-filled lumen that contains the ciliated dendrites of one to several olfactory neurons; the neuronal cell bodies and accompanying support cells of these neurons are situated at the base of the sensillum lumen (see Fig. 7A). Numerous studies have revealed that the luminal fluid surrounding the sensory dendrites contains high concentrations of odorant-binding proteins (OBPs) as well as potent odorant-degrading enzymes (ODEs) (Vogt and Riddiford, 1981; Rybczynski et al., 1989, 1990; Vogt et al., 1991a,b; Maida et al., 1993; Steinbrecht, 1996); these OBPs and ODEs are expressed in the support cells and secreted into the sensilla lumen (Steinbrecht et al., 1992). Moth antennae typically possess two general classes of olfactory sensillum, those that are sensitive to sex pheromone and those that are sensitive to general environmental odorants such as volatile plant compounds (Boeckh et al., 1965; Keil, 1989; Lee and Strausfeld, 1990). In the sphinx moth *Manduca sexta*, individual antennae of both males and females have

approximately 100 000 olfactory sensilla. However, the representation and distribution of these sensilla are sexually dimorphic; male antennae have an estimated 43 000 sex-pheromone-sensitive and 55 000 general-odorant-sensitive sensilla (Sanes and Hildebrand, 1976; Keil, 1989; Lee and Strausfeld, 1990), while female antennae have primarily general-odorant-sensitive sensilla (Oland et al., 1988).

Behaviorally relevant odorant molecules and hazardous xenobiotics alike enter olfactory sensilla through pores in the cuticular hair wall. Odorant molecules are thought to interact with OBPs, which solubilize and facilitate the transport of the odorants to and from receptor proteins situated in the membranes of the ciliated dendrites (Vogt, 1995; Pelosi and Maida, 1995; Kaissling, 1998; R. G. Vogt, F. E. Callaghan, M. E. Rogers and J. C. Dickens, in preparation). Odor molecules are ultimately degraded and thus permanently inactivated by ODEs. Two types of antennal-specific ODEs, esterases and aldehyde oxidases, have been characterized in several moth species (Vogt et al., 1985; Rybczynski et al., 1989, 1990).

Little is known concerning the fate of xenobiotics entering the olfactory sensilla. Tissue damage by potentially harmful xenobiotics might be minimized through specific biochemical intervention. One possibility for restricting entry of toxic or behaviorally irrelevant molecules into the sensillum may involve the OBPs. For example, there have been suggestions that ligand-specific binding properties of the OBPs might limit the diversity of volatiles that can readily gain access to the sensillum interior (Vogt et al., 1989, 1991a; Prestwich and Du, 1995; Steinbrecht, 1996). However, few detailed ligand binding studies have been performed, and there is no evidence that OBPs restrict entry of volatile substances *in vivo* or that chronic exposure of sufficiently high concentrations of volatile substances would not saturate or bypass the OBP transport system.

Alternatively, xenobiotic detoxification *via* enzymatic processes might limit the concentration of these agents to non-damaging levels. The biochemical pathways involved in xenobiotic metabolism have been well characterized (for reviews, see Dahl, 1988; Mannervik and Danielson, 1988). Xenobiotic metabolism is accomplished through the action of the biotransformation enzymes. Three classes of such enzymes, cytochrome P-450 oxygenases, UDP-glucuronosyltransferases (UGTs) and glutathione-*S*-transferases (GSTs), have been identified in olfactory epithelia of mammals (Tu et al., 1984; Baron et al., 1986; Nef et al., 1989; Lazard et al., 1990; Ding et al., 1991; Longo et al., 1991; Zupko et al., 1991; Ben-Arie et al., 1993). The cytochrome P-450 oxygenases belong to the so-called phase I transformation enzymes and generally catalyze hydroxylation reactions; the products of these reactions can then become substrates for the phase II transformation enzymes, which include the UGTs and GSTs. On the basis of the range of odorant types that can be modified by these enzymes and their presence in olfactory tissue, it was proposed that the biotransformation enzymes catalyze the transformation of odorants as a means of terminating the olfactory signal (Nef et al., 1989; Burchell, 1991; Lazard et al., 1991; Ding et al., 1991; Ben-Arie et al., 1993).

Using differential display polymerase chain reaction (ddPCR), we have identified an olfactory-specific GST from the antenna of the sphinx moth *Manduca sexta* and have named the protein GST-msolf1. Taking advantage of the functional organization of the male antennal epithelium into discrete sex-pheromone- and general-odorant-sensitive domains, we have examined GST-msolf1 expression by northern blot analysis in male and female antennae and by *in situ* hybridization, and have analyzed the antennal GSTs for their ability to modify aldehyde odorants. These analyses suggest that antennal GSTs may play a dual role of detoxifying xenobiotic compounds attacking the olfactory tissue and contributing to signal termination of the aldehyde sex-pheromone odorants.

## Materials and methods

### *Animals and tissues*

*Manduca sexta* were obtained as fertilized eggs (a gift from Dr L. M. Riddiford, University of Washington, Seattle, USA) and reared at 27 °C on a 16 h:18 h (L:D) light cycle. Male and female antennae were taken from pharate (pre-emerged) adult animals. Midguts were obtained from day 4 fifth-instar larvae and were dissected free of peritrophic membranes. All other tissues were obtained from newly emerged males. Dissected tissue was immediately frozen on dry ice and stored at −80 °C until use.

### *Isolation of potential olfactory-specific mRNAs by ddPCR*

Differential display polymerase chain reaction (ddPCR) was performed using the RNAimage Kit (Genhunter) following recommended protocols and comparing mRNAs from three tissues: adult male antenna, adult female antenna and adult male leg. RNA was isolated using the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi, 1987); dissected tissues were initially ground with guanidinium solution under liquid nitrogen using a mortar and pestle. Residual genomic DNA was degraded using DNAase I prior to cDNA synthesis. Complementary DNA synthesis reactions (20 µl) contained 0.2 µg of total RNA, 250 µmol $l^{-1}$ dNTPs, 100 units of MMLV RNAase $H^-$ reverse transcriptase, 5× RT buffer and 4 pmol of the appropriate anchored oligo(dT) primer, all supplied with the kit. Three cDNA reactions were performed for each mRNA pool using the three different anchored oligo(dT) primers.

Each cDNA product was then subjected to eight PCR amplifications using different arbitrary sense primers *versus* the appropriate anchored oligo(dT) primer. The PCR (20 µl) was performed in flame-sealed glass capillary tubes on an Idaho Technology Thermocycler. PCR used *Taq* DNA polymerase (1 unit, Promega), 10× buffer (500 mmol $l^{-1}$ Tris-HCl, pH 8.3, 2.5 mg $ml^{-1}$ bovine serum albumin, 10 % Ficoll, 10 mmol $l^{-1}$ tartrazine), 2 mmol $l^{-1}$ $MgCl_2$, 2 µmol $l^{-1}$ dNTP, 460 MBq of [$^{35}$S]dATP, 4 pmol of each primer and 2 µl of cDNA from the above reaction. Reaction conditions were: 2 min denaturation at 94 °C, 40 cycles at 94 °C (15 s), 40 °C (15 s) and 74 °C (30 s), and a final elongation at 74 °C (2 min).

PCR products were analyzed using 6 % polyacrylamide/urea sequencing gels; gels were dried on Whatman paper, and products were visualized by autoradiography on X-ray film (Kodak, X-Omat). The positions of bands of interest were marked on acetate sheets; marked sheets were aligned with registration marks on dried gels, and the underlying gel was excised using razor blades. Gels were re-exposed to X-ray film to confirm the quality of the excisions. DNA was eluted from the gel fragments in 100 µl of water at 100 °C and following recommended protocols (Genhunter).

Selected gel-eluted PCR products (1 µl) were reamplified using identical conditions to those described above except that the dNTP concentration was 20 µmol $l^{-1}$ and [$^{35}$S]dATP was omitted. Reamplified products were ligated into pCR-Script vector (pCR-Script Cloning Kit, Stratagene). The resulting clones were used to generate probes for northern blot analysis, for whole-mount *in situ* hybridization and as a template for sequencing reactions.

### *Probes for* in situ *hybridization and northern blot analyses*

Cloned ddPCR products were initially screened by whole-mount *in situ* hybridization using digoxigenin-DNA probes synthesized from plasmid DNA using PCR. Cloned inserts were amplified from purified (Qiagen) plasmid DNA using the appropriate ddPCR primers (RNAimage kit) and in the presence of 2 mmol $l^{-1}$ dATP, dGTP, dCTP, 0.6 mmol $l^{-1}$ dTTP and 0.3 mmol $l^{-1}$ digoxigenin-dUTP (Boehringer-Mannheim). PCR (10 µl) was performed in an Idaho Technology Thermocycler: denaturation at 94 °C (1 min); 35 cycles at 94 °C (1 s), 40 °C (5 s), 74 °C (15 s), and a final elongation at 74 °C (1 min).

Digoxigenin-RNA probes were synthesized from clones of interest following the initial screening. Antisense and sense RNAs were synthesized from insert DNA (Genus System, Boehringer Mannheim; Vogt et al., 1997). Templates for RNA syntheses were generated by PCR from purified plasmids (Qiagen) using either M13 forward or reverse primer *versus* an appropriate insert-specific primer (RNAimage kit). The resulting PCR product was gel-purified (Geneclean II kit, BIO 101), and RNA was synthesized from 1 µg of product in the presence of 2 mmol $l^{-1}$ ATP, CTP, GTP, 0.6 mmol $l^{-1}$ UTP and 0.3 mmol $l^{-1}$ digoxigenin-UTP using T7 or T3 RNA polymerase (Genus system, Boehringer-Mannheim).

G7-9 (GST-msolf1) probe was transcribed *in vitro* from the full-length ddPCR clone (base pairs 484–822; see Fig. 2). The general-odorant-binding protein GOBP-2 and pheromone-binding protein (PBP) probes were transcribed from the *M. sexta* GOBP2-7 (Vogt et al., 1991a; bases −1 to 483) and PBP clones (Györgyi et al., 1988; bases 588–910), respectively. The control DNA probe used in the northern blot analysis (see Fig. 1) derived from an *M. sexta* 18S rRNA clone (Rogers et al., GenBank accession U88190) in pBluescript II (Stratagene) and was generated using PCR according to Rogers et al. (1997).

### In situ *hybridization*

*In situ* hybridizations were performed on newly emerged adult male tissue, following protocols modified from Vogt et al. (1997). Antennae were partially dissected prior to whole-mount analysis. Pharate antennae were pinned to Sylgard (Dupont) dishes under phosphate-buffered saline (PBS) with their leading edge up (scales down) and bisected longitudinally using a no. 11 scalpel blade (somewhat analogous to opening a clam), exposing the epithelial cells underlying the antennal cuticle. Tissue was immediately treated with 4 % paraformaldehyde (PFA) in PBS overnight on ice. Fixed tissue was washed several times in PBS containing 0.1 % Tween 20 (PBST), dehydrated to 70 % methanol (in PBST) and stored at −20 °C.

For *in situ* hybridization, tissue was rehydrated to PBST, digested with proteinase K (10 µg $ml^{-1}$ in PBST, 2 h at 37 °C), post-fixed in 4 % PFA, 0.5 % glutaraldehyde in PBST (20 min, 4 °C) and reacted with acetic anhydride (0.25 % in 100 mmol $l^{-1}$ triethanolamine, 20 min, 20 °C). When using DNA probes, tissue was prehybridized overnight at 50 °C (0.6 mol $l^{-1}$ NaCl; 10 mmol $l^{-1}$ Tris, pH 7.5, 2 mmol $l^{-1}$ EDTA, 1× Denhardt's solution, 50 µg $ml^{-1}$ herring sperm DNA, 50 µg $ml^{-1}$ tRNA and 0.05 % Tween-20) and hybridized under the same conditions with 100 ng $ml^{-1}$ digoxigenin-labeled probes in prehybridization solution containing formamide (50 %). When using RNA probes, tissue was treated as above, except that hybridization was performed at 60 °C in 65 % formamide. Following hybridization, the tissue was washed in hybridization solution (minus Denhardt's solution, DNA, tRNA and probe) progressively diluted with PBST at the hybridization temperature. Antennae were incubated in blocking solution (5 % non-fat dried milk in PBST, 2 h, 20 °C) and then with alkaline-phosphatase-coupled anti-digoxigenin antibody (Boehringer Mannheim; diluted 1:5000 in blocking solution, overnight, 4 °C). Following washing in PBST, alkaline phosphatase was visualized using Nitroblue Tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) at 20 °C. Staining was stopped in 10 mmol $l^{-1}$ Tris-HCl, 1 mmol $l^{-1}$ EDTA (pH 7.5, 4 °C). Tissue was photographed in whole mount under dark-field illumination.

### *Northern blot analysis*

For each tissue sample, 10 µg of total RNA was electrophoresed on a 1 % agarose gel containing formaldehyde and electrophoretically transferred (Trans-Blot Cell; BioRad) in 1× TAE (Maniatis et al., 1982) onto nylon membrane (Amersham, HyBond-N). Membranes were prehybridized for 2.5 h at 68 °C [5× SSC (Maniatis et al., 1982); 0.1 % *N*-lauroylsarcosine, 2× Denhardt's solution; 0.02 % SDS, 100 µg $ml^{-1}$ herring sperm DNA] and hybridized with 25 ng $ml^{-1}$ digoxigenin-labeled antisense RNA probe under the same conditions in prehybridization solution containing formamide (50 %). To show that equivalent amounts of RNA were present in all lanes, *M. sexta* rRNA DNA probe was hybridized to the portion of the blot containing target rRNAs and processed separately. Hybridization was at 50 °C but otherwise under identical conditions. Membranes were washed at the hybridization temperature in 0.1× SSC, 0.1 % SDS, and

probes were visualized by luminous detection (CDP-star; Boehringer Mannheim) on X-ray film (Kodak, X-OMAT).

*Sequencing and identification of ddPCR clones*

Selected clones were sequenced using either vector primers or primers designed to an internal sequence. Sequencing was performed either at the University of Florida DNA Sequencing Core Laboratory (Gainsville, FL, USA) or at the Medical University of South Carolina Biotechnology Resource Laboratory (Charleston, SC, USA) using ABI prism dye terminator cycle sequencing protocols (Applied Biosystems). Sequences were initially characterized using the NCBI BLAST network server (Pearson and Lipman, 1988; Altschul et al., 1990). All sequencing concerning GST-msolf1 was performed fully in both directions.

*Cloning the full-length G7-9 (GST-msolf1) coding region by 5′ RACE PCR*

Full-length G7-9 coding region was obtained by 5′ RACE PCR using the 5′/3′ RACE Kit (Boehringer Mannheim). Antennal cDNA was A-tailed at its 5′ end and amplified by PCR using an anchored oligo(dT) sense primer (5′/3′ RACE kit) *versus* a G7-9 antisense primer (TTCGTAAGATTTTC-GTGTTCACTAAAGTC, base pairs 613–641; see Fig. 2). A 50 µl PCR reaction containing 50 ng of cDNA was performed using the expand long template PCR system (Boehringer Mannheim). A sample of this initial reaction was reamplified using an internal G7-9 antisense primer (CACTCTTTTC-CAGGAAGATGTTAAGCC, base pairs 506–530; Fig. 2) *versus* a supplied sense anchor primer (5′/3′ RACE Kit). PCR reactions were performed on a Cetus thermocycler under an oil overlay: 2.5 min denaturation at 94 °C, 30 cycles at 94 °C (25 s), 57 °C (40 s), 68 °C (4 min for the initial 10 cycles with an additional 20 s extension added to each remaining cycle), and a final elongation at 68 °C (7 min). The resulting PCR product was ligated into pCR-Script [pCR-Script (SK+) Cloning Kit; Stratagene] and sequenced as described above.

*Phylogenetic sequence analysis of GST-msolf1*

Amino acid sequences of GST-msolf1 and previously identified insect and vertebrate GSTs were aligned using the Clustal W multiple alignment program (Thompson et al., 1994). The same alignment was used to produce the pairwise comparison values presented in Table 1, the phylogeny presented in Fig. 3 and the alignment shown in Fig. 4. GST sequences were obtained from NCBI using the ENTREZ search engine. Sequence identities with the database source and accession number are as follows: *Anopheles gambiae* GST 1-1, EMBL Z71481; *Anopheles gambiae* GST 1-5, EMBL Z81291; *Anopheles gambiae* GST 1-6, EMBL Z81292; *Anopheles gambiae* GST 2-1, SWISS-PROT P46428; *Blatella germanica* Bla g 5, GENBANK U92412; *Bombyx mori* GST, EMBL AJ006502; *Caenorhabditis elegans* GST P, SWISS-PROT P10299; *Drosophila melanogaster* D1, PIR G46681; D21, PIR D46681; D23, PIR E46681; D25, PIR B46681; D26, PIR H46681; D27, PIR F46681); *Drosophila melanogaster* GST 2, PIR A48982; *Homo sapiens* GST A2-2, SWISS-PROT P09210; *Homo sapiens* GST M1-1, SWISS-PROT P09488; *Homo sapiens* GST P1-1, SWISS-PROT P09211; *Homo sapiens* GST 2, SWISS-PROT P30712); *Lucilia cuprina* GST 1-1, SWISS-PROT P42860; *Manduca sexta* GST 1, SWISS-PROT P46430; GST 2 SWISS-PROT P46429; *Musca domestica* GST 1, EMBL X61302; *Musca domestica* GST 2, SWISS-PROT P46431; GST 4, SWISS-PROT P46433; *Musca domestica* Sigma, SWISS-PROT P46437; *Rattus norvegicus* GST ya2, PIR A26653; *Rattus norvegicus* GST Yb1, PIR A29794; *Rattus norvegicus* GST 7-7, SWISS-PROT P04906; *Rattus norvegicus* GST 5-5, SWISS-PROT Q01579.

A distance-based phylogeny (neighbor-joining; Saitou and Nei, 1987) was constructed from aligned amino acid sequences using the algorithm implemented in MEGA (Version 1.0; Kumar et al., 1993). Positions that were not recognizably homologous among the in-group taxa were excluded from the analysis. Pairwise gamma distances were calculated according to the formula of Nei et al. (1976) with the *a* parameter set to a value of 2.05 (Uzzel and Corbin, 1971). The pairwise deletion option for gaps was used throughout the distance analyses. Bootstrapping (Felsenstein, 1985) was used to evaluate the degree of support for particular groupings.

*Enzyme assays*

All enzyme assays were performed on the soluble fractions of adult antennal homogenates. Frozen adult antennae were ground under liquid nitrogen (in 1 mmol $l^{-1}$ phosphate buffer, pH 6.5, 1 mmol $l^{-1}$ phenylmethylsulfonylfluoride) with a mortar and pestle and homogenized on ice using a ground-glass homogenizer. Homogenates were centrifuged at low speed to remove cuticle (12 000 ***g***, 10 min, 4 °C) and then subjected to high-speed ultracentrifugation (100 000 ***g***, 60 min, 4 °C). The soluble fraction (supernatant) was divided into samples (10 antennal equivalents per tube), lyophilized to dryness and stored at −80 °C. Prior to use, lyophilized antennal homogenates were resuspended in sterile water (0.01 original volume) to yield a final buffer concentration of 100 mmol $l^{-1}$.

Total GST conjugation activity in male and female antennae was measured spectrophotometrically using glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates (Habig et al., 1974). Antennal homogenates (0.1 antenna equivalent) were incubated in a 1 ml reaction mixture containing 1 mmol $l^{-1}$ glutathione and 1 mmol $l^{-1}$ CDNB (5 min, 20 °C, in 100 mmol $l^{-1}$ phosphate buffer; pH 6.5). The reaction was stopped by the addition of acetic acid to a final concentration of 0.2 mol $l^{-1}$, and the resulting GSH–CDNB reaction products were detected at 340 nm using a double-beam spectrophotometer (model U-2000, Hitachi). All reactions were corrected for non-enzymatic conjugation by measuring the activity of the same stock solution without protein.

GST conjugation activity towards the aldehyde odorant *trans*-2-hexenal was determined using the thin-layer chromatography assay described by Ben-Arie et al. (1993). A 50 µl reaction mixture containing 10 mmol $l^{-1}$ *trans*-2-hexenal (0.01 vol of 1 mol $l^{-1}$ absolute ethanol stock), 10 mmol $l^{-1}$

glutathione and two antennal equivalents of olfactory homogenates was incubated in 100 mmol $l^{-1}$ phosphate buffer (pH 6.5) for 60 min (20 °C). To visualize the reaction products, a 3 µl sample was loaded onto 0.25 mm thin-layer chromatography silica plates (Macherey and Nagel Polygram SIL G/$UV_{254}$, 4 cm×8 cm, coated plastic; Beckman 661120-4), developed using butanol:acetic acid:water (12:3:5 by volume) and stained with ninhydrin (0.25 % w/v in acetone; 42 °C). Control reactions using CDNB substrate were performed in a similar manner using 1 mmol $l^{-1}$ CDNB and GSH.

### *Image processing*

All X-ray film and photographic images were digitized and processed using Adobe Photoshop and printed using a dye-sublimation printer.

### *Nomenclature*

Previous phylogenetic studies suggest that there are multiple classes of GSTs, including mammalian alpha, mu, pi, theta and invertebrate sigma classes. Available sequences of insect GSTs have previously been shown to form two groups, the first associating with a division that includes the mammalian alpha, mu, pi and invertebrate sigma GSTs, and the second with a division that includes the mammalian theta class (Buetler and Eaton, 1992; for a review, see Hayes and Pulford, 1995). A recent study by Snyder and Maddison (1997) suggests that the theta-related GSTs are not well supported as a single lineage. For clarity, but consistent with the view of Snyder and Maddison (1997), we refer to the alpha/mu/pi-related insect GSTs as insect Type-1 GSTs and to the second group of insect GSTs as insect Type-2 GSTs.

## Results

### *Characterization of antennal-derived ddPCR products*

Twenty-five ddPCR products were cloned and characterized by whole-mount *in situ* hybridization to determine the spatial location of their mRNAs in male antennae. *In situ* hybridization revealed several ddPCR probes hybridizing to mRNA expressed throughout the antennal epithelium (data not shown). Two clones, designated G7-9 and G7-5, hybridized to mRNA expressed in non-overlapping regions of the olfactory sensory epithelium and were characterized further. Upon sequencing, the G7-5 clone was revealed to be GOBP1, which has previously been identified and characterized (Vogt et al., 1991a,b). Sequence analysis of the G7-9 clone (bases 484–822 in Fig. 2) indicated that it was homologous to the glutathione-*S*-transferase (GST) family of proteins; the full-length clone was subsequently named GST-msolf1.

Northern blot analysis using G7-9 antisense RNA probe was performed using mRNAs isolated from male antennae, midgut, head, leg and wing. The G7-9 probe hybridized to an abundant 1.4 kilobase (kb) and a less abundant 3.6 kb message in male antennae; no hybridization was detected to mRNAs isolated from midgut, head, leg or wing (Fig. 1A). These results support the initial observation from the ddPCR suggesting that G7-9 is olfactory-specific. Northern blot analysis comparing male and female antennae showed that G7-9 is expressed in both sexes; however, expression is much stronger in male than in female (Fig. 1B). This pattern is similar to the expression pattern of the male-enriched PBP (1.4 kb; Fig. 1B), which associates with the sex-pheromone-specific sensilla of male antennae and a small uncharacterized subset of sensilla in female antennae (Györgyi et al., 1988; R. G. Vogt and M. E. Rogers, unpublished results). These expression patterns contrast with that of GOBP2, the concentration of which appears to be slightly elevated in female antennae relative to male antennae (0.8 kb; Fig. 1B); GOBP2 associates with the general-odorant-sensitive sensilla common to both male and female antennae (Vogt et al., 1991a; R. G. Vogt and M. E. Rogers, unpublished results).

### *Sequence analysis of GST-msolf1 (G7-9) cDNA*

The full-length coding region of GST-msolf1 was obtained by 5′ RACE PCR using antisense primers derived from the G7-9 ddPCR clone. Four resulting clones were analyzed, yielding

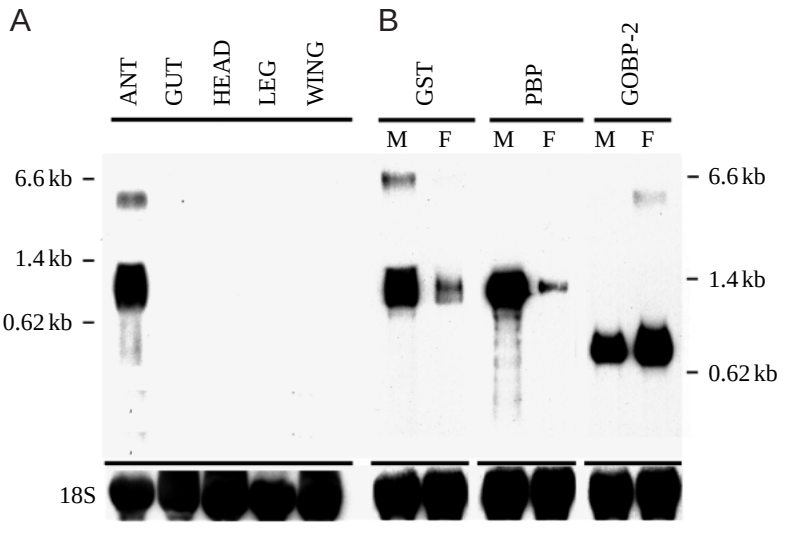


Fig. 1. Northern blot analysis of GST-msolf1 expression in adult *Manduca sexta*. (A) Hybridization of GST-msolf1 antisense RNA probe with mRNA isolated from various male adult tissues: antenna (ANT), midgut (GUT), head, leg and wing. (B) Comparative hybridizations of GST-msolf1, pheromone-binding protein (PBP) and GOBP2 (general-odorant-binding protein 2) (antisense RNA probes with antennal mRNAs of adult males (M) and females (F). Each lane contained an equivalent amount of total RNA, but exposure times varied for each probe to compensate for the relative amounts of target mRNA: GST-msolf1 (20 min); PBP (5 min); GOBP2 (5 min). Hybridizations using *M. sexta* 18S rRNA control probe are shown to indicate the relative amounts of total RNA in each lane. RNA markers for both panels are, from top to bottom, 6.6 kb, 1.4 kb and 0.62 kb.

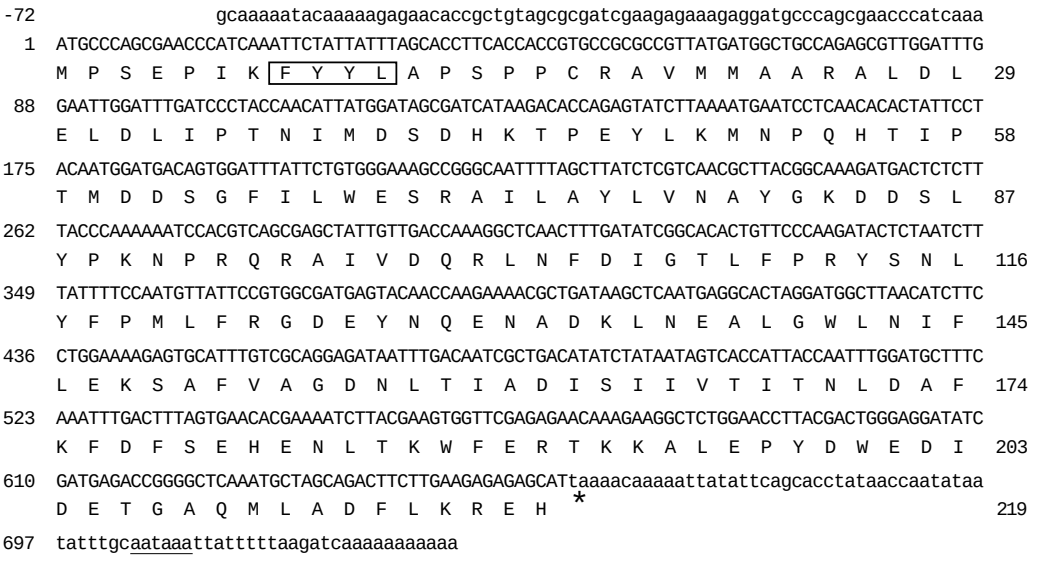


Fig. 2. Nucleotide sequence and deduced amino acid sequence of GST-msolf1. The predicted nucleotide-coding region is shown in capital letters. The assignment of the initiation codon was based on the presence of an in-frame ATG located seven codons 5′ of a conserved amino acid domain (boxed FYYL) common to the cytosolic insect glutathione-*S*-transferases (GSTs). The first in-frame stop codon (asterisk, nucleotide 659) suggested the 3′ terminus. The consensus sequence for polyadenylation is indicated (underlined).

identical sequences within the presumptive coding region. The full-length sequence of the GST-msolf1 coding region is presented in Fig. 2, along with its translated amino acid sequence. The location of the start methionine is suggested by an in-frame ATG located upstream of an FYYL amino acid sequence that is conserved among several other insect GSTs (see Fig. 4). The location of the 3′ terminus is suggested by an in-frame stop codon (TAA) at position 659. Alignment with related sequences indicated that the positions of these initiation and termination codons yield an open reading frame consistent in length with that of other cytosolic GSTs (see Fig. 4). This predicted GST-msolf1 coding region consists of 657 nucleotides, encoding a protein of 219 amino acids. Classification of GST-msolf1 as a cytosolic enzyme is supported by the lack of an obvious leader sequence motif within its initial 15–20 N-terminal amino acid residues and by its similarity in this region to other GSTs of known cytosolic location. A consensus polyadenylation signal (AATAAA) is present 14 nucleotides upstream of the C-anchored oligo(dT) primer that was used to amplify the G7-9 ddPCR product; this same signal motif was present in cDNAs encoding antennal-specific *M. sexta* GOBP1 and GOBP2 (Vogt et al., 1991a).

A database search (NCBI BLAST network server) using full-length GST-msolf1 strongly supported its relationship with members of the GST superfamily; probability values ranged from $10^{-69}$ to $10^{-5}$, where a value of less than 0.05 is considered statistically significant (Karlin and Altschul, 1990). Two groups of insect GSTs have previously been identified, distinguished by their association with (Type-1) or without (Type-2) the mammalian alpha, mu and pi GSTs (see Materials and methods). The percentage identity matrix presented in Table 1 indicates that the GST-msolf1 protein shares

Table 1. *Percentage amino acid identity between multiple insect glutathione-*S*-transferases*

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Type-2 | | | | | | | | | | | |
| **GST-msolf1** | 1 | | | | | | | | | | |
| *Bm*GST | 2 | 74.8 | | | | | | | | | |
| *Ms*GST 1 | 3 | 34.9 | 39.0 | | | | | | | | |
| *Ag*GST 1-1 | 4 | 35.6 | 35.1 | 30.0 | | | | | | | |
| *Dm*GST D1 | 5 | 41.3 | 44.4 | 37.1 | 37.7 | | | | | | |
| *Lc*GST 1-1 | 6 | 40.8 | 41.7 | 37.8 | 43.5 | 82.6 | | | | | |
| *Md*GST 1 | 7 | 34.2 | 42.5 | 35.6 | 44.9 | 83.7 | 92.3 | | | | |
| Type-1 | | | | | | | | | | | |
| *Ms*GST 2 | 8 | 12.6 | 15.7 | 16.9 | 11.3 | 16.5 | 16.6 | 16.9 | | | |
| *Md*GST Sigma | 9 | 11.0 | 12.0 | 17.9 | 11.0 | 13.3 | 11.3 | 14.3 | 38.8 | | |
| *Dm*GST 2 | 10 | 12.3 | 10.6 | 13.9 | 11.4 | 14.0 | 11.9 | 15.0 | 36.1 | 85.9 | |
| *Ag*GST 2-1 | 11 | 16.4 | 13.4 | 10.7 | 11.3 | 12.3 | 11.9 | 12.8 | 38.6 | 59.9 | 57.4 |

*Ag*, *Anopheles gambiae*; *Bm*, *Bombyx mori*; *Dm*, *Drosophila melanogaster*; *Lc*, *Lucilia cuprina*; *Md*, *Musca domestica*; *Ms*, *Manduca sexta*.

Percentage identity obtained from the same alignment used to generate Figs 3 and 4 is shown. Gaps (deletions) and insertions present in each pairwise comparison were omitted and not scored.

See Materials and methods for definitions of Type-1 and Type-2 sequences and for sequence identities.

34.2–74.8 % amino acid identity with the Type-2 insect GSTs, but only 11.0–16.4 % identity with the Type-1 insect GSTs. These identity values are well within the range of other comparisons both within and between the types (within type, 30.0–92.3 %; between type 10.6–17.9 %). GST-msolf1 shared the highest identity (74.8 %) with an olfactory GST isolated from the silk moth *Bombyx mori*.

Phylogenetic analysis of insect and vertebrate GSTs further supports the classification of GST-msolf1 as a member of the insect Type-2 GSTs. Fig. 3 shows a neighbor-joining tree of several GST family members. GST-msolf1 groups with the insect Type-2 and mammalian theta GSTs, while the insect Type-1 and mammalian mu, pi and alpha GSTs form a separate group (100 % bootstrap support). Within the insect Type-2 group, GST-msolf1 is strongly associated (100 % bootstrap support) with an antennal *Bombyx mori* GST (Krieger and Breer, 1998). These two olfactory GSTs are well supported to be distinct from the nearby dipteran GSTs (96 % bootstrap support) as well as another *M. sexta* GST isolated from larval midgut (*Manduca* 1, 77 % bootstrap support).

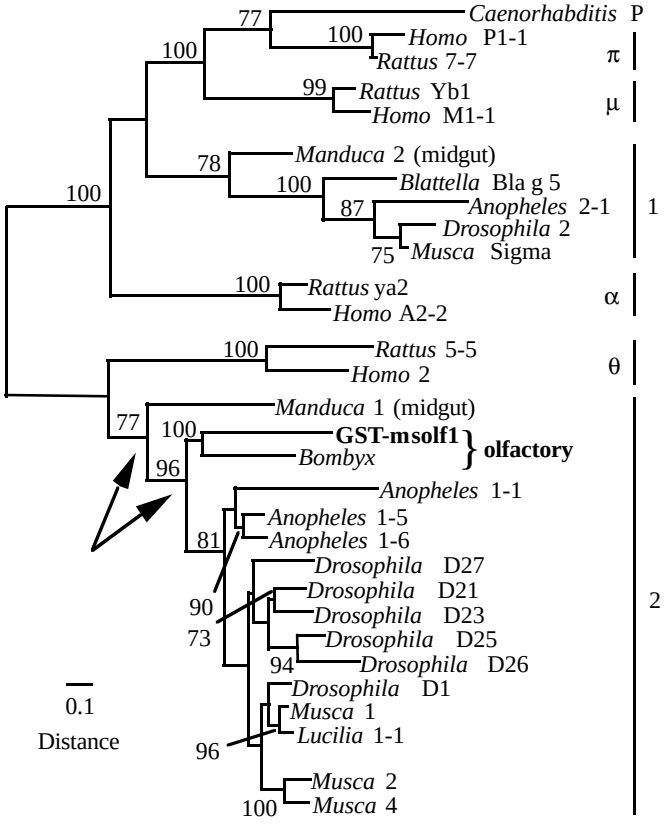


Fig. 3. Unrooted neighbor-joining phylogeny relating GST-msolf1 with several insect and vertebrate glutathione-*S*-transferases (GSTs). Branch lengths are proportional, and the scale of distance is indicated. Bootstrap support values (%) based on 1000 replicates are indicated; values below 70 % are not shown. The overall branching pattern is consistent with earlier analyses (Pembe and Taylor, 1992; Toung et al., 1993; Syvanen et al., 1994; Snyder and Maddison, 1997). Previously defined groups of mammalian (α, μ, π, θ) and insect Type-1 (1) and Type-2 (2) GSTs are indicated to the right of the sequence names. See Materials and methods for definitions of the insect types and for sequence identities. GST-msolf1 groups with the insect Type-2 and mammalian theta GSTs. Within the insect grouping, GST-msolf1 associates with an olfactory GST from the silk moth *Bombyx mori* (*Bombyx*); this olfactory group is distinct from the Type-2 dipteran GSTs and a *M. sexta* GST isolated from larval midgut (*Manduca* 1) (arrows), implying functional differences between GST-msolf1 and its non-olfactory homologs.

Amino acid alignments between GST-msolf1 and five other Type-2 GSTs are shown in Fig. 4. A conserved serine at GST-msolf1 position 14 and asparagine at GST-msolf1 position 52 were previously suggested to distinguish the insect Type-2 GSTs from the mammalian mu, pi, alpha and theta GSTs (Wilce et al., 1995). In the insect Type-1 GSTs, these sites contain different amino acids (data not shown) and may therefore be diagnostic in distinguishing these two insect types as well. Structural analysis of a Type-2 GST from the Australian sheep blowfly *Lucilia cuprina* suggested that a proline, glycine and aspartic acid at positions 53, 149 and 156, respectively, may be important for protein folding (Wilce et al., 1995); these same amino acids are conserved throughout the Type-2 GSTs and are present within GST-msolf1 at positions 58, 154 and 161 respectively.

### In situ *localization of GST-msolf1 within male antennae*

GST-msolf1 expression was localized within the antenna by hybridizing the G7-9 antisense RNA probe to partially dissected antennal tissue in whole mount (Fig. 5). The *M. sexta* male antenna is approximately 2.5 cm long, divided along its length into approximately 80 similar annular units. Because the antennal cuticle renders the tissue impervious to probes, antennae were cut open along their length to create symmetrically identical half-antennae, exposing the inner surface of the epithelial cells underlying the antennal cuticle. Fig. 5A shows such bisected tissue hybridized with G7-9 probe. Stained cells are revealed along the border of each annulus; no hybridization was observed in the central region of the annulus. Visualization under higher magnification suggested that this staining was in the cytosol of cells with large nuclei (data not shown); these cells resembled the trichogen support cells on the basis of their size and position within the epithelium (Sanes and Hildebrand, 1976; R. G. Vogt, unpublished results). Hybridization using control sense probe under identical conditions showed no staining (Fig. 5B).

Expression of the odorant-binding proteins PBP and GOBP2 identify regions in the olfactory epithelium containing either sex-pheromone-specific sensilla (PBP expression, Fig. 5C,Ei) or general-odorant-sensitive sensilla (GOBP2 expression, Fig. 5D,Eii). Initial screening of the ddPCR-derived clones showed that clone G7-5 hybridized to the region expressing GOBP2 (data not shown). G7-5 was subsequently identified as GOBP1, a homolog of GOBP2; GOBP1 and GOBP2 have previously been shown to have overlapping distributions that were distinct from the distribution of PBP (Vogt et al., 1991a). Expression of GST-msolf1 appears to be restricted to the same region and to the same cells as expression of PBP; it does not

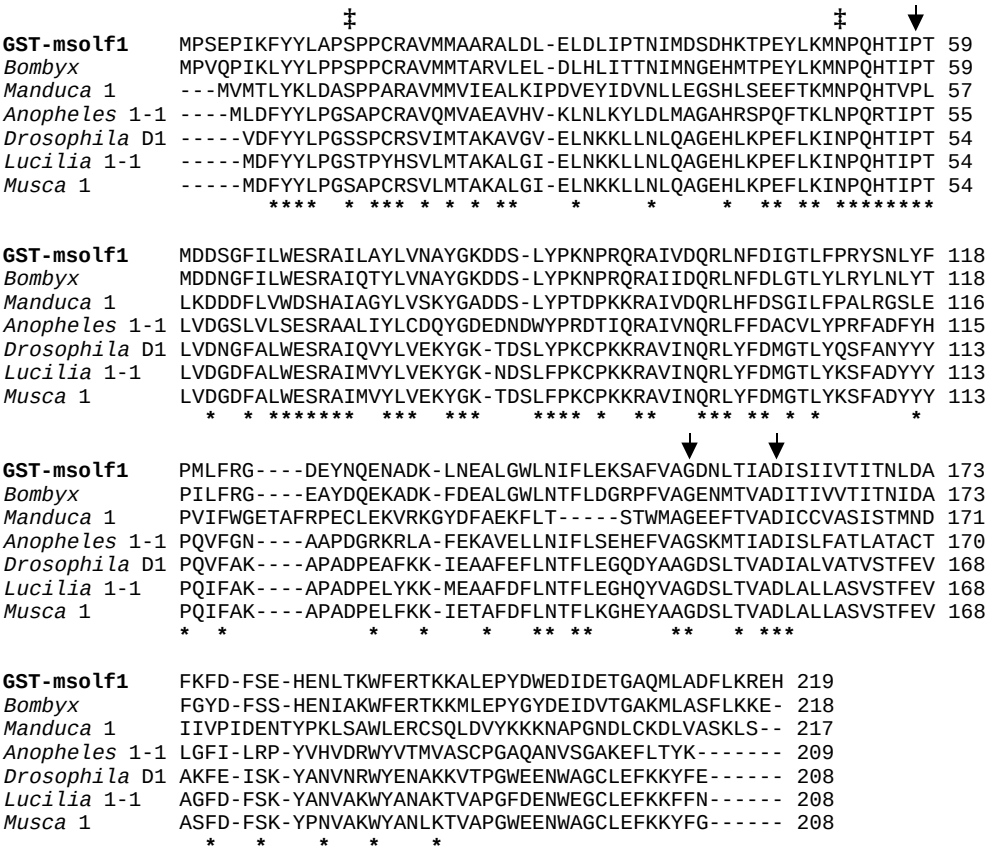


Fig. 4. Amino acid sequence alignment of GST-msolf1 and representative insect Type-2 glutathione-*S*-transferases (GSTs). Numbering is from the first amino acid in mature proteins. Asterisks mark positions at which at least five of the seven amino acids are identical including GST-msolf1. Residues shown previously to distinguish the insect Type-2 proteins from the vertebrate mu, pi, alpha and theta proteins are marked (‡); arrows mark residues determined to be important for the preservation of three-dimentional structure. See Materials and methods for sequence identities.

overlap with GOBP2. This result is consistent with the northern blot analysis comparing male and female antennae; GST-msolf1 expression mirrors PBP expression, which is known to associate specifically with sex-pheromone sensilla.

### *GST activity in the antenna*

Total GST activity in male and female antennae towards CDNB, a highly reactive GST substrate, was measured spectrophotometrically. Conjugation activities for male and female antennae were 53.6±0.43 nmol $min^{-1}$ $antenna^{-1}$ and 43.3±0.83 nmol $min^{-1}$ $antenna^{-1}$, respectively (means ± S.D., $N$=5). Statistical analysis indicates that the difference between male and female mean conjugation activities is significant (*t*-test: two-sample assuming unequal variances; *t* statistic 18.94, $P \leqslant t$=0.0003).

Since all known *M. sexta* pheromone components are aldehydes (Starratt et al., 1979; Tumlinson et al., 1994), we used a chromatographic assay to determine whether the GST enzymes present in male antennae are capable of modifying the aldehyde odorant *trans*-2-hexenal, which has been shown to generate electroantennogram responses in *M. sexta* males (Schweitzer et al., 1976). Thin-layer chromatography of the soluble antennal fraction incubated alone showed a reproducible pattern of ninhydrin-stained compounds (Fig. 6A, '–GSH'). Soluble fractions containing reduced glutathione (GSH) but no added co-substrate yielded a prominent band with a rate of flow ($R_f$) of 0.31 (Fig. 6A, '+GSH'); this band was absent in the '(–)GSH' lane and is presumably unreacted GSH, which migrates to approximately this position in the absence of other reactants (data not shown). Soluble fraction incubated with GSH plus the co-substrate CDNB yielded a prominent product with an $R_f$ of 0.53 (Fig. 6B, 'CDNB'). The same reaction using the aldehyde odorant *trans*-2-hexenal also produced a prominent product in this region ($R_f$=0.56) (Fig. 6B, 't-2-HEX'), demonstrating the potential for antennal GSTs to conjugate aldehyde odorants.

## Discussion

### *Characterization of GST-msolf1*

Using a technique that favors the identification of tissue-specific mRNAs, we have cloned a GST (GST-msolf1) from the antennae of the sphinx moth *M. sexta*. Northern blot analysis of representative tissue types suggests that GST-msolf1 expression is both antennal-specific and olfactory-specific within the antenna. This protein shares significant similarity with other GST sequences and 34.2–74.8 % sequence identity with the insect Type-2 GSTs. Phylogenetic analysis of several vertebrate and insect GSTs indicates two major divisions, one including the insect Type-1 and a second including the insect Type-2 GSTs. *Manduca sexta* possesses GSTs belonging to both major divisions; two previously identified GSTs from the larval midgut are respectively of the Type-1 and Type-2 divisions. While GST-msolf1 is strongly supported as Type-2 GST, it is only 35 % identical to the *M. sexta* midgut GST of the same division. This distinction is

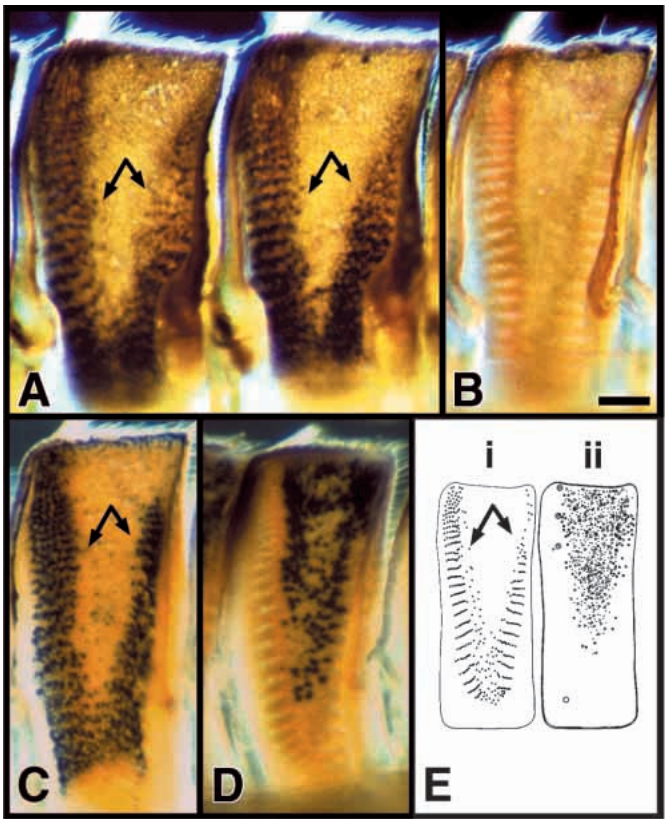


Fig. 5. *In situ* hybridization of GST-msolf1 mRNA expression in whole-mount preparations of male *Manduca sexta* antennae. (A) Hybridization of G7-9 (GST-msolf1) antisense RNA probe to the olfactory epithelium; two contiguous annuli are shown. (B) The negative control using G7-9 sense RNA probe. (C) Hybridization using the pheromone-binding protein (PBP) antisense probe. (D) Hybridization using general-odorant-binding protein 2 (GOBP2) antisense probe. (E) A diagram of the distribution of the different sensillar types in the olfactory epithelium of *M. sexta* (adapted from Lee and Strausfeld, 1990); 'i' identifies the distribution of sex-pheromone-specific sensilla; 'ii' identifies the distributions of several other sensilla types including the general-odorant-sensitive sensilla. In all cases, the olfactory epithelium is visualized from the inner hemolymph side. GST-msolf1 and PBP expression overlap the distribution of the pheromone-specific sensilla (arrows), while GOBP2 expression overlaps the distribution of the general-odorant-sensitive sensilla. Scale bar, 100 μm.

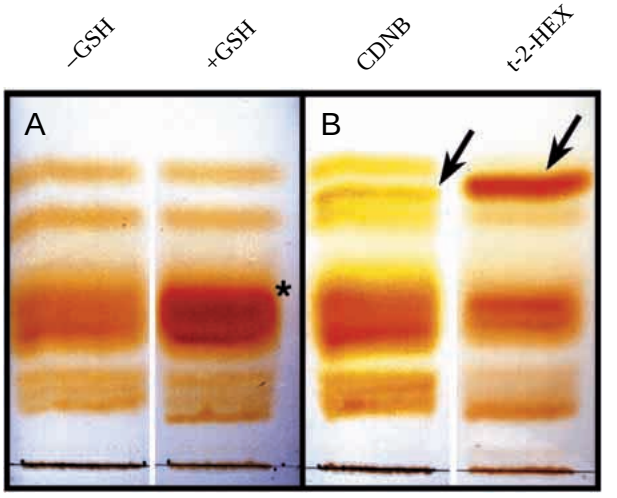


Fig. 6. Chromatographic analysis of antennal glutathione-*S*-transferase (GST) activity against odor and non-odor substrates. (A) GST reactions containing the soluble fractions of male antennal homogenates were performed with (+GSH) or without (–GSH) glutathione but no additional co-substrate. Unreacted GSH is indicated by the asterisk. (B) GST reactions containing glutathione plus either 1-chloro-2,4,-dinitrobenzene (CDNB) or the aldehyde odorant *trans*-2-hexenal (t-2-HEX) as co-substrates. Conjugate reaction products using co-substrates CDNB or the aldehyde odorant *trans*-2-hexenal are indicated by arrows.

supported by the neighbor-joining analysis (Fig. 3), which was based on mean identity differences and suggests that GST-msolf1 is more similar to the dipteran proteins than to either *M. sexta* protein. Phylogenetic separation of the *M. sexta* midgut GST and GST-msolf1 (77 % bootstrap support) and the strong sequence relationship between GST-msolf1 and an antennal homolog from the silk moth *Bombyx mori* (75 % identity, 100 % bootstrap support) suggest that the two olfactory GSTs may represent a distinct group with a unique function that relates to its expression in olfactory tissue.

Characterization of total GST activities in adult male and female antennae demonstrated that antennal GSTs can conjugate GSH to the aldehyde odorant *trans*-2-hexenal, although the concentrations of *trans*-2-hexenal required to demonstrate this activity were quite high. Significantly more GST activity was observed in male antennae than in female antennae, consistent with the male-enriched expression of GST-msolf1 mRNA; however, the differences in activity were much smaller than the difference in the amounts of mRNAs. This discrepancy between GST activity and mRNA expression suggests that additional GSTs are probably expressed in other cells common to the antennae of both sexes, such as the epithelial cells of the tracheae or the hemocytes of the blood (e.g. Franciosa and Berge, 1995). Also, in the light of the restricted expression of GST-msolf1 to sex-pheromone sensilla, additional GSTs might associate with the general-odorant-sensitive sensilla that are also present in both sexes. Whether GST-msolf1 itself can conjugate sex-pheromone components at physiological concentrations remains to be demonstrated. Efforts to use *in vitro* translated GST-msolf1 were unsuccessful as a result of the high endogenous GST activities in both rabbit reticulocyte and wheat germ lysates, and efforts to demonstrate GST conjugation activity towards known *M. sexta* sex-pheromone components (*cis*-9- and *cis*-11-hexadecenal) were unsuccessful as a result of solubility problems at the concentrations required for chromatographic detection.

### *Possible function of GST-msolf1 in the antenna of* Manduca sexta

The detoxification of xenobiotic compounds is the primary function attributed to most cytosolic GSTs (for reviews, see

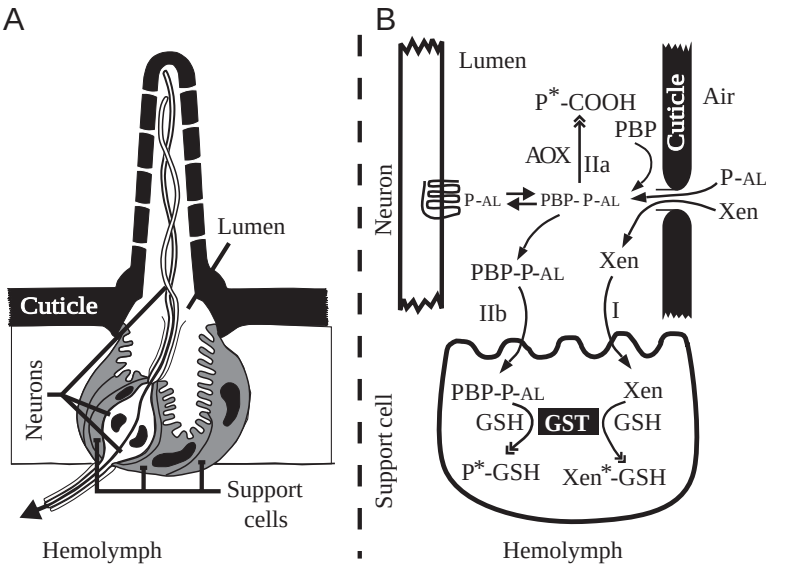


Fig. 7. Possible function of glutathione-*S*-transferases (GSTs) in olfactory sensilla. (A) Diagram of a typical olfactory sensillum showing two olfactory receptor neurons and three support cells. The lumen of the sensillum is isolated from the hemolymph by junctional complexes joining the neurons and support cells (Steinbrecht, 1987). Therefore, odorants, xenobiotics or their metabolites moving from sensillum lumen to hemolymph must pass through the cytosol of these sensillar cells. (B) Model of the possible role of olfactory-specific GSTs in the detoxification of xenobiotics and of the inactivation of *Manduca sexta* aldehyde sex-pheromone odorants. Small holes (pores) penetrate the cuticular wall of a sensillum, permitting entry of both pheromones and xenobiotics (Xen) into the sensillum lymph. Once inside, aldehyde pheromones (P-AL) are bound by pheromone-binding protein (PBP), forming a soluble complex (PBP-P-AL); pheromones are thus transported through the lumen where they can interact with additional PBPs, with aldehyde oxidase (AOX) or with putative receptor proteins located on the dendritic membranes of olfactory receptor cells. Pheromones may be inactivated in the lumen by AOX (pathway IIa) or in the cytosol of support cells by GSTs (pathway IIb). AOX converts the pheromone to an inactive carboxylic acid (P*-COOH); GSTs catalyze the conjugation of pheromone to glutathione (GSH), producing a pheromone–glutathione conjugate (P*-GSH). Xenobiotics entering the lumen may be modified by GSTs in a similar fashion (pathway I).

Pickett and Lu, 1989; Hayes and Pulford, 1995). Considerable research on insect GSTs has focused on the inactivation of synthetic insecticides and the role of GSTs in the development of insecticide resistance (for a review, see Clark, 1990). For example, several studies in flies and mosquitoes have shown elevated levels of GST mRNA and protein in insecticide-resistant strains compared with non-resistant strains (Grant and Matsumara, 1989; Wang et al., 1991; Fournier et al., 1992; Prapanthadara et al., 1993, 1995). In *M. sexta* and several other lepidopteran species, toxic plant compounds have been shown to induce expression of larval midgut GSTs, suggesting that GSTs play a role in the detoxification pathway of plant-derived xenobiotics (Yu, 1983, 1984, 1989; Snyder et al., 1995). Similarly, olfactory-specific GST-msolf1 may function to detoxify xenobiotic compounds attacking the olfactory epithelium (Fig. 7, pathway I).

The restricted expression of GST-msolf1 in olfactory tissue suggests that GST activity is important for olfactory function. Such enzymatic protection against volatile xenobiotics seems to be advantageous in a tissue that has evolved to capture effectively the volatile compounds important for the initiation of reproduction and feeding behaviors. However, the restricted expression to a functionally defined population of olfactory sensilla also suggests an alternative or additional role of GST-msolf1, that of signal termination of the sex-pheromone molecules (Fig. 7, pathways IIa, IIb).

Odorant signal termination in the Lepidoptera has previously been discussed in the context of the soluble odorant-degrading enzymes (ODEs) that reside in the aqueous lumen of the olfactory sensilla. The sex pheromone of *M. sexta* contains a specific ratio of at least eight different 16-carbon unsaturated aldehydes (Starratt et al., 1979; Tumlinson et al., 1994). Rybczynski et al. (1989) characterized an antennal-specific aldehyde oxidase (AOX) from the olfactory sensilla of *M. sexta*. The *M. sexta* AOX was localized within the olfactory sensilla and was shown rapidly to degrade a major component of the pheromone to the corresponding carboxylic acid with a half-life of approximately 0.5 ms. Antennal-specific AOXs were subsequently identified in the silk moths *Antheraea polyphemus* and *Bombyx mori*, which also use aldehyde compounds as components of their sex pheromones (Rybczynski et al., 1990). In *A. polyphemus*, the AOX complemented an antennal-specific esterase of similar properties but which presumably targeted the acetate ester component of the sex pheromone of that species (Vogt et al., 1985).

If GST-msolf1 contributes to the signal termination of aldehydes (e.g. pheromones), it probably does so in a very different manner from the previously identified AOX. Typical of other GSTs and consistent with the apparent lack of a secretory signal peptide, GST-msolf1 is presumably localized within the cytosol of the sensilla support cells, while the odorant-degrading enzymes (ODEs) are secreted proteins localized within the extracellular sensilla lumen. In contrast to the extracellular ODEs, cytosolic GSTs can presumably act on odorants only after they have entered the support cells (Fig. 7). One possible mechanism for odorant internalization may involve the odorant-binding proteins (OBPs). *In vivo* labeling studies in the gypsy moth *Lymantria dispar* (Vogt et al., 1989) and immunological studies localizing *A. polyphemus* PBP in olfactory sensilla (Steinbrecht et al., 1992) have suggested that the OBPs are internalized by the support cells. Internalization of OBPs may also facilitate the removal of odorants from the sensillum lumen. Alternatively, because the sex-pheromone

molecules are lipophilic, they may easily enter the support cells across the apical membranes independent of PBP transport. Either situation may allow some pheromone to enter the support cells, avoiding degradation by AOX. Pheromone termination within the support cells would contribute to the overall maintenance of low levels of background signal and thus contribute to the sensitivity of the sex-pheromone detection system.

The findings presented here suggest that GST-msolf1 may play a dual role in the antenna by contributing to the detoxification of compounds that might interfere with sex-pheromone detection and to the signal termination of sex-pheromone odorants. Assuming that the odor receptors expressed in the olfactory neurons are distributed along the entire length of the sensillum, it seems awkward to localize an odor-termination process to the base of the sensillum. However, such localization has been demonstrated for the signal-termination enzymes of purinergic odorants in the spiny lobster *Panulirus argus* (Gleeson et al., 1991, 1992). Furthermore, it seems exceptional for a specific signal-termination mechanism to be localized in the support cell cytosol, in a compartment separated by the support cell membrane from the extracellular compartment of pheromone recognition. Nevertheless, in *M. sexta*, the antennal-specific expression of GST-msolf1 and its localization to a subdomain of the olfactory epithelium argue most strongly that GST-msolf1 is involved, in a very specific way, with the sex-pheromone detection pathway. Detailed functional studies with expressed GST-msolf1 protein should help clarify this role.

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