



Pergamon

Insect Biochemistry and Molecular Biology 30 (2000) 507–514

*Insect  
Biochemistry  
and  
Molecular  
Biology*

www.elsevier.com/locate/ibmb

# High level expression of “male specific” pheromone binding proteins (PBPs) in the antennae of female noctuid moths

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Received 2 November 1999; received in revised form 28 January 2000; accepted 1 February 2000

## Abstract

Pheromone Binding Proteins (PBPs) are one branch of a multigene family of lepidopteran Odorant Binding Proteins (OBPs) that are known for their relatively high levels of expression in male antennae. However, PBP expression has been observed at low levels in female antennae of the Saturniidae, Bombycidae and Lymantriidae, and at relatively high levels in members of the Noctuidae. The function of female PBP expression is unclear, as female lepidoptera are consistently noted for their failure to respond physiologically or behaviorally to sex-pheromone. In this study, the sexual dimorphism of PBP expression was examined in the noctuid moths *Helicoverpa zea*, *Heliothis virescens* and *Spodoptera frugiperda*. A PBP cDNA clone was isolated from female *H. zea*, PBP-*Hzea<sub>f</sub>*. Northern blot analysis indicated relatively high levels of PBP-*Hzea<sub>f</sub>* expression in both male and female antennae, though females consistently expressed about 50% that of males. Western blot analysis of male and female PBP expression supported these relative differences. Immunocytochemical analysis indicates discrete expression localized beneath olfactory sensilla of both male and female antennae. These results suggest female noctuids possess the biochemistry to detect at least components of their sex-pheromone. Alternatively, these results may suggest that PBPs have a more general function in noctuids, possibly reflecting behavioral and life history differences that distinguish this the Noctuidae from other Lepidopteran families. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Olfaction; Pheromone binding protein; Female; cDNA; Noctuidae; Lepidoptera

## 1. Introduction

Interaction between odor molecules and Odorant Binding Proteins (OBP) is the initial biochemical step in odor recognition in many insect species (Vogt and Riddiford, 1981; Vogt et al. 1985, 1999; Prestwich et al., 1995; Pelosi and Maida, 1995; Pelosi, 1996; Steinbrecht, 1996; Carlson, 1996; Breer, 1997; Kaissling, 1998; Wojtasek and Leal, 1999; Krieger and Breer, 1999). OBPs are small, water soluble proteins secreted into the

aqueous lumen of olfactory sensilla. OBPs are generally thought to bind volatile odorants entering the sensillum, transport these odorants through the aqueous lumen, and deliver the odorants to receptor proteins situated in the membranes of olfactory neurons. One group of OBPs include the lepidopteran specific classes of Pheromone Binding Proteins (PBPs) and General Odorant Binding Proteins GOBP1 and GOBP2 (Vogt and Riddiford, 1981; Vogt et al. 1991a, 1999; Breer, 1997; Robertson et al., 1999). While GOBPs are generally noted to be expressed at similar levels in both male and female antennae (e.g. Vogt et al., 1991a,b), PBPs have been noted for strong sexual dimorphism in being expressed primarily or most abundantly in male antennae (Vogt and Riddiford, 1981; Vogt et al., 1991a; Györgyi et al.,

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1988; Raming et al. 1989, 1990; Krieger et al. 1991, 1996; Merritt et al., 1998). Indeed, the sex specific presence of PBP in male antenna of the silk moth *Antheraea polyphemus*, along with its observed interaction with sex-pheromone and localization to the extracellular lumen of sex-pheromone specific sensilla, provided the initial evidence that these proteins had a role in odor detection (Vogt and Riddiford, 1981).

The actual degree of sexual dimorphism regarding the expression of PBP in male vs. female antennae differs among those species studied. In early studies involving N-terminal sequence analysis, Vogt et al. (1991a), noted that the PBPs of *Antheraea polyphemus* and *Hyalophora cecropia* (Saturniidae), *Bombyx mori* (Bombycidae), and *Lymantria dispar* and *Orgyia pseudosugata* (Lymantriidae) were all male specific, undetectable in females. However, while expression of PBP in *Manduca sexta* (Sphingidae) was very strong in male antennae, it was also clearly detectable in female antennae, albeit at relatively low levels (Györgyi et al., 1988; Vogt et al., 1991a). Immunological studies later showed that PBP was also expressed in female antennae of *A. polyphemus*, *A. pernyi* and *B. mori*, and several other related lepidoptera, but always at very low levels compared to that in male antennae (Steinbrecht et al. 1992, 1995). In histological studies of *A. polyphemus*, *B. mori* and *M. sexta*, female expression of PBP was observed to associate with a small number of specific, but otherwise, uncharacterized subgroup of olfactory sensilla (Steinbrecht et al., 1995; Laue and Steinbrecht, 1997; Vogt, unpublished data).

In several studies of species of the Noctuidae, relatively high levels of PBP expression have been noted in female antennae, in the range of 50 to 100% of that seen in male antennae (Steinbrecht et al., 1992; Nagnan-Le Meillour et al., 1996; Maibèche-Coisné et al., 1997). Noctuids differ from other lepidopteran families in which OBPs have been studied in that they have the most complex pheromone composition in terms of the numbers of behaviorally significant components (Klun et al., 1980). It may therefore be that a family specific difference in the expression or function of an odor detection protein such as PBP reflects other olfactory differences such as pheromone composition or complexity of chemosensory based behavior.

In this study, we examined the sexual dimorphism of PBP expression in the noctuid moths *Helicoverpa zea*, *Heliothis virescens* and *Spodoptera frugiperda*. In general, PBP expression was observed to be strong in both males and females, although female antennae of all three species were consistently observed to express about 50% the level of PBP relative to male antennae. These results are consistent with previous observations concerning other noctuid moths. The function of such female expression remains unclear, because female lepidoptera have often been noted for their lack of response to sex-

pheromone (Schweitzer et al., 1976; Boeckh and Boeckh, 1979; Koontz and Schneider, 1987; Hildebrand, 1996; Christensen et al., 1990; Chen et al., 1997). Female expression of PBP may suggest that females are responsive to sex-pheromone in an as yet undetected manner. Alternatively, such expression may suggest that noctuid PBPs are less specialized than the PBPs of other lepidoptera regarding the processing of sex-pheromone signals.

## 2. Materials and methods

### 2.1. Moth tissue

*Helicoverpa zea*, *Heliothis virescens* and *Spodoptera frugiperda* were obtained from laboratory reared colonies each of which were annually infused with field-collected insects and maintained at the USDA-ARS, Crop Science Research Laboratory, Mississippi State, MS (Davis, 1989; Jenkins et al., 1995). Pupae of each species were sexed and placed in separate cages. Emerging adults were allowed to feed on 10% sucrose for about 5 d prior to collection. Moths were plunged into liquid nitrogen then lyophilized (Genesis 25LL, Virtis Co., Gardiner, NY) at shelf temperatures of  $-40^{\circ}\text{C}$  for 24 h,  $0^{\circ}\text{C}$  for 4 h and  $20^{\circ}\text{C}$  for 4 h. Antennae and legs of freeze-dried insects were collected and stored at  $-70^{\circ}\text{C}$  until used for either protein or RNA isolation.

### 2.2. Protein electrophoresis/immunoblotting

Total protein extracts of antennal and leg tissues were prepared as described earlier (Dickens et al., 1995). Native one-dimensional PAGE was performed as described by Laemmli (1970) except that SDS was omitted from gel and running buffers. A 4.5% stacking and 12.5% resolving gel was used for protein separation. Gels were either stained to visualize the protein profiles (Coomassie Blue R-250 or silver) or electroblotted onto nitrocellulose for immuno-detection. Reaction and development of immunoblots with anti-PBP1*Ldispar* were as described previously (Vogt et al., 1989) except blocking time was reduced to 4h and secondary antibody-conjugate complex was anti-rabbit IgG alkaline phosphatase.

### 2.3. Immunocytochemistry

Fresh *S. frugiperda* antennae were opened at both ends and fixed in 2% paraformaldehyde (PFA) in PBS-T (10 mM  $\text{Na}_2\text{HPO}_4$ , 150 mM NaCl, 0.1% Tween-20, pH 7.0) for 1 h at  $20^{\circ}\text{C}$ . Tissue was rinsed twice in PBS-T, then treated in 20% DMSO in methanol overnight at  $4^{\circ}\text{C}$  (or stored at  $-20^{\circ}\text{C}$ ). For further processing, tissue was washed in PBS, dehydrated through a graded series

of ethanol and toluene and incubated in melted paraffin (Periplast +<sup>TM</sup>) for 2 h before embedding in plastic molds. Eight to 10  $\mu\text{m}$  sections were made; ribbons of sections were transferred to water droplets on glass slides coated with albumin (EM Diagnostic) and allowed to dry overnight on a slide warmer at 37°C. Slides were dewaxed with toluene and rehydrated through a graded series of ethanol to PBS. For immunoreaction, sections were reacted in primary antiserum (anti-PBPM<sub>s</sub> sexta (Györgyi et al., 1988), 1:500 dilution and for comparison anti-rGOBP2, 1:500) in 3% non-fat dry milk (NFDM) in PBS overnight at 4°C. Antisera used were anti-PBPM<sub>s</sub> sexta (Györgyi et al., 1988 crude serum at 1:500 dilution) and anti-rGOBP2M<sub>s</sub> sexta (crude serum 1:500 dilution); rGOBP2M<sub>s</sub> sexta was expressed from cDNA (Vogt et al., 1991b; Feng and Prestwich, 1997) and antiserum was generated in a rabbit using rGOBP2M<sub>s</sub> sexta dissolved in 50% Freund's Complete Adjuvant (University of South Carolina Institute for Biological Research Technology Antibody Facility).

Following repeated washing in NFDM-PBS, sections were reacted with secondary antibody [Goat anti-rabbit IgG (H+L) Horseradish Peroxidase Conjugate (ICN), 1:100 dilution, NFDM-PBS] for 2 h at 20°C. Following 4 washes in NFDM-PBS, sections were stained by addition of DAB substrate (Sigma FAST DAB tablets). Finally, sections were washed with PBS and mounted with Aqua Mount Medium (Lerner Laboratories) under coverslips. Sections were photographed using either normal bright field or differential interference contrast optics.

#### 2.4. Library construction/cDNA sequencing

Total RNA was isolated from approximately 3000 female antennae (180 mg dry wt.) of adult *H. zea* by homogenization in guanidinium thiocyanate as described in Technical Bulletin 087 (Promega Inc., Madison, WI). Poly(A)<sup>+</sup>RNA was selected by affinity chromatography on oligo(dT)cellulose (Sambrook et al., 1989). First and second strand cDNAs were synthesized as per directions for the ZAP-cDNA Synthesis Kit from Stratagene (La Jolla, CA) using oligo(dT) linker/primer. Following size selection (>400 bp in length), the cDNAs were ligated to lambda Uni-ZAP XR vector. The amplified library (insert range 0.5–1.4 kb) was plated and duplicate plaque lifts were screened with <sup>32</sup>P-labelled cDNA (959 bp) encoding PBP1 of *H. virescens* (Krieger et al., 1993). Ten clones were selected for 5' sequencing, all of which encoded transcript from the same gene. The longest insert (*Hzea*<sub>f</sub>7a) was fully sequenced using dye primer or dye terminator cycle sequencing FS kits followed by electrophoresis on an Applied Biosystem Model 373 DNA sequencer (Perkin-Elmer Corp., Foster City, CA, USA). The sequences were assembled and analyzed

using the Genetics Computer Group (GCG) software (Madison, WI, USA).

#### 2.5. Northern Blot analysis

Total RNA was isolated as described above from antennae and legs of both sexes of adult *H. zea*, *H. virescens*, and *S. frugiperda* and resolved on denaturing, formaldehyde agarose gels (10  $\mu\text{g}/\text{lane}$ ) (Sambrook et al., 1989). An outer lane containing RNA markers (Promega Inc.) was excised from the gel prior to blotting, stained with ethidium bromide and used for size estimations. The resolved RNA was blotted onto Nytran membranes using a Turbo Blotter (Scheicher and Schuell, Keene, NH) and bound by exposure of the blot to UV light (Stratalinker, Stratagene, La Jolla, CA). The *Hzea*<sub>f</sub>7a insert cDNA was <sup>32</sup>P-labelled by nick translation and used as a probe for overnight hybridization at 45°C in 50% formamide, 5X SSPE, 5X Denhardt's, 0.1% SDS, 150  $\mu\text{g}/\text{ml}$  denatured salmon sperm DNA. Final wash conditions for the RNA blots were 1 h at 52°C in 0.2X SSPE containing 0.1% SDS. Washed blots were exposed to X-ray film at room temperature for 4 h.

### 3. Results

Proteins of male and female antennae and legs (equalized for protein concentration) from *H. zea*, *H. virescens* and *S. frugiperda* were compared under non-denaturing PAGE. Native gels were either stained for total protein (protein profile) or electroblotted onto nitrocellulose for reaction with antibody (anti-PBP1Ldispar) (Fig. 1). The antiserum recognized a single and apparently identical immunoreactive band in both male and female antennal proteins (Fig. 1); no immunoreactive bands were observed among proteins of leg homogenates (data not shown). The electrophoretic migration of the antennal PBP antigen corresponded with identifiable bands in the stained gels (protein profiles, Fig. 1). The level of PBP expression in female antennae for all three species was estimated to be 40 to 50% of that in corresponding males based on densitometry of the original blots, averaged over several experiments.

To gain insights into the regulation and function of the female expressed PBPs, we constructed a female antennal cDNA library of *H. zea* mRNAs and then screened it for sequences cross hybridizable to the previously cloned, male PBP of closely related *H. virescens* (Krieger et al., 1993). We selected and sequenced ten strongly hybridizing clones; the consensus sequence (PBP-*Hzea*<sub>f</sub>) of 912 bp is shown (Fig. 2). The PBP-*Hzea*<sub>f</sub> cDNA sequence contains an open reading frame of 489 nucleotides. Translation and peptide analyses of the open reading frame, alignments with other lepidopteran PBPs, and the presence of an inframe ATG suggested a 20

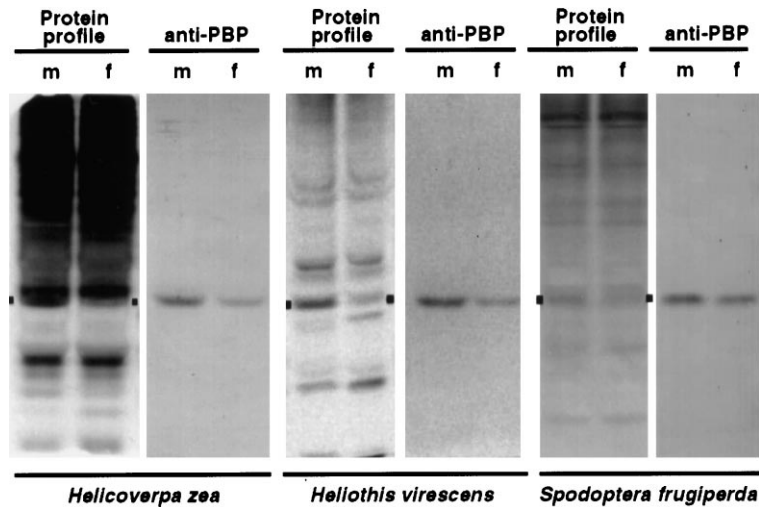


Fig. 1. Relative levels of PBP in male and female antennae of *H. zea*, *H. virescens*, and *S. frugiperda*. Protein profile: native-PAGE gels stained with silver (*H. zea* and *S. frugiperda*) or Coomassie Blue (*H. virescens*). Anti-PBP: immunoblots of duplicate gels reacted with anti-PBP of *L. dispar*. Boxes denote area of stained gel corresponding to immuno-detected PBP. Leg proteins were negative for reaction with anti-PBP (not shown).

amino acid signal peptide followed by a 143 amino acid mature protein terminated by an inframe stop codon. Fig. 3 shows an alignment of PBP-*Hzea<sub>f</sub>* sequence and the PBPs of the male noctuids *H. virescens* and *Mamestra brassicae*, as well as PBPs cloned from the male sphingid *M. sexta* (Györgyi et al., 1988) and saturniid *A. polyphemus* (Raming et al., 1989). The PBP-*Hzea<sub>f</sub>* amino acid sequence shares 92 and 88% identity with the noctuid PBPs of *H. virescens* and *M. brassicae*, respectively. Identity decreases to 68 and 60% for PBPs of *M. sexta* (Sphingidae) and *A. polyphemus* (Saturniidae), respectively. PBP-*Hzea<sub>f</sub>* shares amino acid sequence features that are common in all PBPs and most OBPs; namely, conservation of the six cysteines (marked with asterisks in Fig. 3) and hydrophobicity in the region from amino acids 40 to 60 (Krieger et al., 1993). This hydrophobic region has been proposed to contain the ligand (pheromonal) binding domain (Du and Prestwich, 1995); PBP-*Hzea<sub>f</sub>* identity with the other PBPs in this region is 100, 90, 76 and 52% for *M. brassicae*, *H. virescens*, *M. sexta* and *A. polyphemus*, respectively.

Northern blot analysis was performed to quantify the relative amounts of PBP-specific mRNA in the male and female moths. The PBP-*Hzea<sub>f</sub>* cDNA probe hybridized to antennal RNA of approximately 1.0 kb from all three moth species (Fig. 4). No signal was detectable in the leg tissue. Hybridization was weaker in *S. frugiperda*, perhaps indicating a more divergent PBP as compared to *H. zea* and *H. virescens*. The blot affirmed significant antennal expression of this mRNA in both sexes. Female mRNA expression levels were about 50% to 100% that in males, consistent with the western analyses of protein expression (Fig. 1).

To obtain a direct view of the expression pattern of PBPs in male and female antennae, immunohistological

analysis was performed on sections of antennae of male and female *S. frugiperda* using PBP and GOBP2 antisera generated against *M. sexta* proteins. Immunoreactivity to PBP antiserum was abundant but focal; staining cells were localized beneath numerous sensilla of both sexes (Figs. 5A and B). By comparison, patches of immunostaining using anti-GOBP were also observed in both sexes (Figs. 5C and D). In EM-immunohistological studies of the noctuids *Spodoptera littoralis*, *Helicoverpa armigera* and *Autographa gamma*, PBP antigenicity was localized to trichoid sensilla of male antennae, while GOBP antigenicity was localized to basiconic sensilla (Steinbrecht et al., 1996), suggesting the identities of the respective sensilla types stained in Fig. 5.

#### 4. Discussion

These data confirm the relative abundant presence of PBPs in female antennae of noctuid moths. Molecular biological studies suggest the existence of at least two types of OBP in insect antennae. OBP-Type 1 includes the more familiar OBPs including classes characterized by PBP, GOBP1, GOBP2, ABPX, and OS-E; so far, these proteins are known only in the single insect clade of holometabolous (endopterygota) and hemipteran (Hemipteroid Assemblage) insects (see Vogt et al., 1999). A second group of proteins might be termed OBP-Type 2, and includes the OS-D and SAP proteins (e.g. Mameli et al., 1996; Pelosi, 1996; McKenna et al., 1994; Robertson et al., 1999). The OBP Type 2 proteins belong to a gene family apparently independently derived from the OBP-Type 1 proteins; Type 2 proteins have been identified in Orthopteroid as well as the holometabolous insects, suggesting they are widely rep-

1 **CCATGATGTCGGTCAAGCTGGCGCTGGTGGTGGCTGCGTG**  
 M M S V K L A L V V A A W  
 41 **GTGTTTCATCAGGGTGGACCGCTCGCAAGATGTTATTAAG**  
 L F I R V D A S Q D V I K  
 81 AACCTGTCTATGAATTCGCTAAGCCCCTAGAAAGACTGTA  
 N L S M N F A K P L E D C K  
 121 AGAAAGAGATGGATCTCCCAGACTCGGTGACGACAGACTT  
 K E M D L P D S V T T D F  
 161 CTACAACCTTCTGGAAGGAAGGCTACGAGTTCACGAACAGA  
 Y N F W K E G Y E F T N R  
 201 CAGACAGGCTGCGCCATCCTCTGCCTCTCCTCCAAGCTGG  
 Q T G C A I L C L S S K L E  
 241 AGCTGTGGACCAGGAGCTCAAGCTGCATCACGGCAAGGC  
 L L D Q E L K L H H G K A  
 281 GCAGGAGTTCGCCAAGAAACCGGCGCTGACGATGCTATG  
 Q E F A K K H G A D D A M  
 321 GCTAAGCAGCTGGTAGACCTGATCCATGGCTGCGCGCAGT  
 A K Q L V D L I H G C A Q S  
 361 CTACTCCTGAAGTGGTAGATGACCCCTGCATGAAGACCCT  
 T P E V V D D P C M K T L  
 401 CAACGTGGCCAAGTGTCTCAAGGCCAAGATACACGAGCTC  
 N V A K C F K A K I H E L  
 441 AACTGGGCGCCAGCATGGACCTCGTCGTCGAGAAGTCT  
 N W A P S M D L V V G E V L  
 481 TGGCCGAAGTT**TAG**ACTTGCCTGGAGATTTCTGATACCTT  
 A E V  
 521 ACCTCGATTTATTTTTATGTCTATATCTTTTTATTTTTCT  
 561 TACATACCTTTAAAAGCAGGAAGCATAACTATACTTAACT  
 601 GTTCCTTTGTGTGTATTATTTGTCGCTTGCGAAAATTCT  
 641 GTCTGCTTTCGACATTATGCCTACGCATACTTCGTTGCGG  
 681 CCCTTTATAACAATGCTGTGTATAATCTTTGTAGTATCTGA  
 721 TTATTTCTTTATGTTATTTATTTAGTTGATGTTCAAACAG  
 761 CTTGGCATGTTCAACGACCGGATAGAGTAAGTTCGTCG  
 801 AAGTGAATCTCCGCTCGCCAGCGCTCAAGGGAAGGGT  
 841 AGTCTTAAGCTGCAATACTTGATGTGATCTTATTAAGCA  
 881 AATCTACATCAAAAAAAAAAAAAAAAAAAAAA

Fig. 2. PBP-*Hzea*<sub>f</sub> cDNA sequence and deduced amino acid sequence. The positions of start ATG and stop TAG codons are in bold. A typical leader (signal) sequence is underlined. (GenBank accession number AF 090191).

resented at least among Neopterous insects (see Vogt et al., 1999). Among the Type 1 OBPs, the PBPs and GOBPs appear to be lepidopteran specific. Multiple PBPs are known in several lepidopteran species (Vogt et al., 1989; Krieger et al., 1991; Maibèche-Coisné et al., 1998; Robertson et al., 1999). While PBPs appear widespread among moth families, species diversity is thought to have occurred within lineages relatively specific to a given species (Merritt et al., 1998). In our current study, a PBP was identified by sequence only in females. The presence of this PBP in males of *H. zea*, *H. virescens* and *S. frugiperda* was suggested by Western blot using antiserum against the PBPs of *L. dispar* and by Northern blot using the female *H. zea* derived probe. Similarly, PBP was localized in the antennae of *S. frugiperda* using antiserum against the PBP1 of *M. sexta*. It may be that these probes detected multiple PBPs displaying differential expression patterns, given the

		*		
<i>H. zea</i> <sub>f</sub>	SQDVIK	NLSMNF	AKPLEDC	CKEMDLPDSVTTDFYNF
<i>H. vir.</i>	-----M-----	-----	-----	-----
<i>M. bra.</i>	---EIM---	-----	-----	-----
<i>M. sexta</i>	-P--M--CL--G-A-DE--A--N-S--IKD--A--V--	-----	-----	-----
<i>A. poly.</i>	-PEIM---N--G-AMDQ--D-LS-----VA-L-----D	-----	-----	-----

		* *		
<i>H. zea</i> <sub>f</sub>	YEFTNR	QTGC	AILCL	SSKLELLDQELKLH
<i>H. vir.</i>	-----H-----S-----	-----	-----	-----M-----
<i>M. bra.</i>	-----	-----	-----	-----Q--
<i>M. sexta</i>	--VS--D--K--DMI-PDG--N-M--	-----	-----	-----
<i>A. poly.</i>	-VM-D-LA---N--AT--DVV-PDGN---N-KD--M--	-----	-----	-----

		* * *		
<i>H. zea</i> <sub>f</sub>	GADDAMA	KQLVDL	IHGCAQ	STPEVDDPCMKTLN
<i>H. vir.</i>	-----M-----S-----DAT-----A-----	-----	-----	-----
<i>M. bra.</i>	---E-----T---D-AA---A---M-S-	-----	-----	-----
<i>M. sexta</i>	---E-----L-I--N-EN--P#N--A-L--DI----	-----	-----	-----
<i>A. poly.</i>	---ET--Q---I---EK-A-P#N--K---ID--M---	-----	-----	-----

<i>H. zea</i> <sub>f</sub>	AKIHEL				NWPMSMDLVVGEVLA				143
<i>H. vir.</i>	-----E-----								
<i>M. bra.</i>	T-V-	VE-I-							
<i>M. sexta</i>	KE--K--	N-----							
<i>A. poly.</i>	KE--K--	V-N----		I-----					

Fig. 3. PBP-*Hzea*<sub>f</sub> alignment with male PBPs identified from other moth species. Bars designate identical amino acids relative to PBP-*Hzea*<sub>f</sub>. The male sequences are from *Heliothis virescens* (Krieger et al., 1993), *Mamestra brassicae* (Maibèche-Coisné et al., 1998), *Manduca sexta* (Györgyi et al., 1988) and *Antheraea polyphemus* (Raming et al., 1989). Asterisks denote six conserved cysteines, a common feature of moth OBPs. The shaded box indicates a hydrophobic region (amino acids 40 to 60) proposed to contain the ligand-binding domain (Du and Prestwich, 1995).

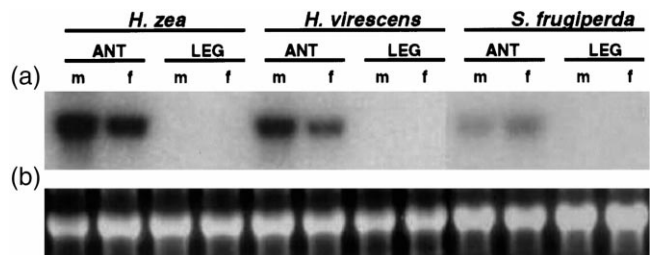


Fig. 4. Relative levels of mRNA encoding PBP in males and females of *H. zea*, *H. virescens*, and *S. frugiperda*. (A) total RNA from antennae and legs of each sex were resolved on formaldehyde-agarose gels, blotted, and probed with the full-length cDNA isolated from female *H. zea* (Fig. 2). The hybridization signal is approximately 1.0 kb; (B) portion of duplicate gel stained with ethidium bromide to show relative loading of RNA on gel as indicated by the major ribosomal RNA band.

possible heterogeneity of PBPs within these species. Nevertheless, PBPs define a distinct class of OBP which has been consistently shown to associate with sex-pheromone specific sensilla (e.g. Vogt et al., 1989; Laue and Steinbrecht, 1997; Rogers et al., 1999). The expression of a PBP in females strongly suggests its association with sensilla with functions more specialized, for example, than the generic detection of plant volatiles.

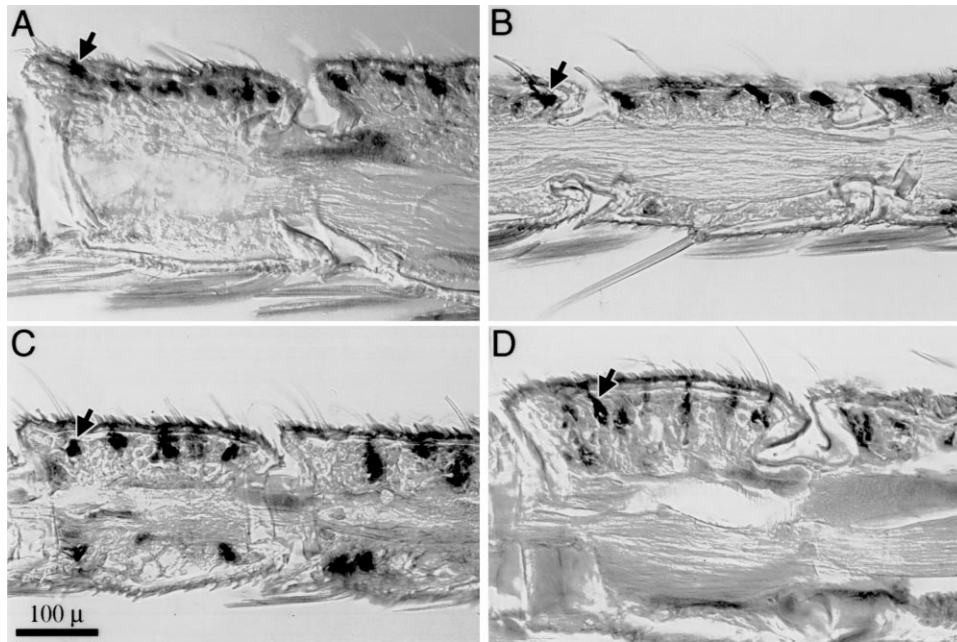


Fig. 5. Immunocytochemical localization of PBP and GOBP within antennae of male and female *Spodoptera frugiperda*. Longitudinal section of male (A) and female (B) antennal segments revealing focussed immunostaining of PBP in epidermal cells below long olfactory sensilla. Immunostaining with anti-GOBP is focussed in discrete patches in antennal epidermis beneath shorter olfactory sensilla of males (C) and females (D). Arrows indicate representative staining in each panel; size bar applies to all panels.

Although PBP-*Hzea*<sub>f</sub> is the first reported full-length cDNA for a female PBP, previous work has provided evidence of female expression of PBPs. Györgyi et al. (1988) observed low levels of PBP expression by both Northern and Western blot analyses in *M. sexta*. Vogt et al. (1991a) surveyed antennal specific proteins from six lepidopteran species: in *M. sexta*, a low abundance female antennal protein was identified as PBP by N-terminal sequence analysis; however, no PBP was detected in female antennal homogenates of *A. polyphemus*, *H. cecropia*, *B. mori*, *L. dispar* or *O. pseudosugat*. Nevertheless, EM-immunocytochemistry on male and female antennae of both *A. polyphemus* and *B. mori* revealed a small population of PBP immunoreactive sensilla basiconica in female antennae (Laue et al., 1994; Steinbrecht et al., 1995; Laue and Steinbrecht, 1997); the same has been observed in histological in situ hybridization studies in *M. sexta* (Vogt, unpublished). Immunoblots of the noctuid *Autographa gama* using anti-PBP<sub>*A. polyphemus*</sub> suggested equivalent levels in male and female antennae (Steinbrecht et al., 1992). Finally, N-terminal sequence analyses of antennal proteins of the noctuid *Mamestra brassicae* revealed identical PBP sequences in both male and female antennae (Nagnan-Le Meillour et al., 1996; Maibèche-Coisné et al., 1997).

The function of PBPs has, from the start, been implicated in the detection of sex-pheromone molecules (e.g. Vogt and Riddiford, 1981; Vogt et al. 1985, 1999; Prestwich et al., 1995; Pelosi and Maida, 1995; Pelosi, 1996; Steinbrecht, 1996; Breer, 1997; Kaissling, 1998; Wojtasek and Leal, 1999; Krieger and Breer, 1999). In

this context, the presence of PBPs in olfactory sensilla of female antennae suggests that females may detect either their own sex-pheromone, or at least certain components of their sex-pheromone. Indeed, female autodetection of sex-pheromones has been demonstrated for several species (reviewed in Schneider et al., 1998), including several species of Tortricidae (Ross et al., 1979; Den Otter et al. 1978, 1996), the arctiid *Panaxia quadripunctaria* (Schneider et al., 1998) and the noctuids *Spodoptera littoralis* (Ljüngberg et al., 1993; Ochieng et al., 1995) and *Trichoplusia ni* (Seabrook et al., 1987).

An alternative view is that PBPs have a dual function, transporting pheromone odorants in the sex-pheromone specific sensilla, but possibly other odorants in a subset of plant volatile sensitive sensilla of both male and female antennae. Such a view would suggest a more complicated set of selective pressures at work in the evolutionary design of PBPs than has previously been suggested (Vogt, 1987; Vogt et al. 1985, 1999). Furthermore, the varying degrees of male–female difference with respect to the amount of PBP expression observed between the Saturniidae, Sphingidae, Lymantriidae and Noctuidae may indicate that the PBPs of saturniids, sphingids, and lymantriids are far more specialized than the PBPs of noctuids, a difference which would likely be reflected in behavioral and life history differences which define these Lepidopteran families. Although, it is also possible that family based expression differences are due to comparative degrees of morphological sexual dimorphism; male antennae of Saturniidae, Sphingidae

and Lymantriidae may have a much greater elaboration of sex-pheromone specific sensilla (along with gene products related to pheromone detection) than do those of the Noctuidae.

It is well established that there are multiple OBPs present in any given lepidopteran antennae, including several PBPs, GOBP1, GOBP2 as well as other OBP-related proteins (e.g. Vogt and Lerner, 1989; Breer et al., 1990; Vogt et al. 1991a, 1999; Laue and Steinbrecht, 1997; Robertson et al., 1999). A pattern emerging from histological studies (e.g. Laue and Steinbrecht, 1997) may be that PBPs are the predominant if not only OBP expressing in the long sensilla trichoidea, which are male and sex-pheromone specific. However, all OBPs appear to be expressed among the other classes of olfactory sensilla on both male and female antennae, but in combinatorial patterns which may contribute to diverse functional roles for different classes and subclasses of olfactory sensilla. In this context, PBPs may be under relatively strict selection for interacting with pheromonal odorants, and the gene regulatory processes controlling PBP expression have been evolutionarily manipulated to express this capability in diverse ways, possibly allowing the animals to process pheromonal information in multiple contexts.

## Acknowledgements

The authors thank Drs. Frank M. Davis and Johnie N. Jenkins, USDA-ARS, Mississippi State, MS for graciously supplying insects used in this study, and Mr. Douglas Dollar for technical assistance. We also thank Prof. Heinz Breer and Dr. Jurgen Krieger, University Stuttgart-Hohenheim, Stuttgart, Germany for sharing the cDNA for PBP of male *H. virescens*, Prof. Glenn Prestwich for supplying rGOBP2 for antiserum production, and Ms. Ming Sun for histological assistance. Support for RGV was from National Institutes of Health (NIDCD DC-00588) and United States Department of Agriculture (CGRP 94-37302-0615) and the National Science Foundation (IBN 9731005). Mention of a trademark, product, or vendor does not constitute a guarantee or warranty of the product by USDA, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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