The Peripheral Olfactory Repertoire of the Lightbrown Apple Moth, *Epiphyas postvittana*

Jacob A. Corcoran[1,2☯¤a], Melissa D. Jordan[2☯¤b], Amali H. Thrimawithana[2], Ross N. Crowhurst[2], Richard D. Newcomb[1,2]*

1 School of Biological Sciences, University of Auckland, Auckland, New Zealand, 2 The New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand

☯ These authors contributed equally to this work.
¤a Current Address: Department of Biology, Lund University, Sölvegatan 37, SE-22362 Lund, Sweden
¤b Current Address: Department of Engineering Science, University of Auckland, Auckland 1142, New Zealand
* Richard.Newcomb@plantandfood.co.nz

Abstract

The lightbrown apple moth, *Epiphyas postvittana* is an increasingly global pest of horticultural crops. Like other moths, *E. postvittana* relies on olfactory cues to locate mates and oviposition sites. To detect these cues, moths have evolved families of genes encoding elements of the peripheral olfactory reception system, including odor carriers, receptors and degrading enzymes. Here we undertake a transcriptomic approach to identify members of these families expressed in the adult antennae of *E. postvittana*, describing open reading frames encoding 34 odorant binding proteins, 13 chemosensory proteins, 70 odorant receptors, 19 ionotropic receptors, nine gustatory receptors, two sensory neuron membrane proteins, 27 carboxylesterases, 20 glutathione-S-transferases, 49 cytochrome p450s and 18 takeout proteins. For the odorant receptors, quantitative RT-PCR corroborated RNAseq count data on steady state transcript levels. Of the eight odorant receptors that group phylogenetically with pheromone receptors from other moths, two displayed significant male-biased expression patterns, one displayed significant female-biased expression pattern and five were expressed equally in the antennae of both sexes. In addition, we found two male-biased odorant receptors that did not group with previously described pheromone receptors. This suite of olfaction-related genes provides a substantial resource for the functional characterization of this signal transduction system and the development of odor-mediated control strategies for horticultural pests.

Introduction

In moths, the ability to detect and respond to odors is essential for finding potential mates, food, and hosts on which to lay their eggs. For moths, odor and pheromone reception predominantly takes place within specialized hairs known as sensilla on their antennae. Each sensillum contains one to three olfactory sensory neurons that extend dendrites into the lymph within

the hair. Odorants enter this lymph through wax-filled pores in the external surface of the sensillum and are transported through the lymph to receptors on the surface of the dendrites, before being broken down to reset the signaling system [1,2].

Proteins involved in these peripheral signaling events have been identified from moths, and include families of proteins involved in binding and transporting odorants, reception and signaling, and system resetting (see [3] for review). Members of all these families have been proposed as targets against which to develop novel, olfactory-mediated pest control strategies.

Odorant Binding Proteins (OBPs) form a large family of small (~15 kDa) hydrophilic carrier proteins that contain two-four disulphide bridges. Within the OBPs are subfamilies specialized in carrying general odorants (General Odorant Binding Proteins, GOBPs; Antennal Binding Protein Xs, ABPXs) and sex pheromone components (Pheromone Binding Proteins, PBPs). Other families of carrier proteins found in the antennae include the smaller Chemosensory Binding Proteins (CSPs) and the larger Takeout proteins (TOs). In the antennae, members of these families are thought to be expressed in the accessory cells and secreted into the sensillum lymph. Structural and binding studies have demonstrated that members of these families are capable of binding small molecules including many odorants and pheromones [4–8].

Three different families of integral membrane proteins have been implicated in the reception of odors. These include members of the odorant receptor (OR) and ionotropic receptor (IRs) families, which are both ligand-gated ion channels [9,10], and the sensory neuron membrane proteins (SNMPs). The ORs and IRs are represented by large families of proteins, whereas only two SNMPs are typically present in moths. ORs are seven-transmembrane-domain non-selective cation channels with intracellular N-termini [11–13]. They include a common obligate co-receptor known as Orco that is required for reception but is not tuned to odors [13]. This co-receptor pairs with tuning receptors to detect a range of different odorants. These OR-Orco complexes are present in the dendritic membrane of sensory neurons housed in sensilla trichodea and basiconica. In comparison, IRs are three-transmembrane-domain proteins that typically detect volatile acids and similar compounds and are found in sensory neurons within sensilla coeloconica [14]. They too form multimeric receptor complexes. SNMPs are members of the CD36 class of membrane proteins and are predicted to contain two-transmembrane regions [15]. The exact role of SNMPs remains unclear.

Pheromone receptors (PRs) form a specialized subset of ORs in moths. They have been identified in many species, mainly from members of the Noctuidae [16,17], Bombycidae [18,19], Saturniidae [20] and Crambidae [21,22]. To date all PRs fall into a single phylogenetic clade within the OR family and generally display higher levels of expression in RNA in the antennae of males than those of females (male-biased expression). Compared with other ORs, receptors from this clade seem to evolve faster, especially compared with Orco [23], with evidence some members are physically clustered within the genome [24].

Members of three families of enzymes have been implicated in the removal of odorant and pheromone compounds in the sensillum lymph to reset the odorant reception system. These odorant degrading enzymes (ODEs) include carboxylesterases (CXEs), glutathione-S-transferases (GSTs) and cytochrome p450s (CYPs). Members of all three families are expressed within moth antennae [25]. In particular, certain CXEs are capable of hydrolyzing acetate sex pheromone components of *Antheraea polyphemus* at an exceptionally rapid rate [26].

The methods used to isolate members of these families have improved significantly with the advent of next generation sequencing technology, both in sequencing cDNA and also directly sequencing genomes. More and more projects are using these technologies to isolate and sequence transcripts from moth antennae, while the complete genomes of an increasing number of species within the Lepidoptera are also being sequenced [27–30].

The lightbrown apple moth, *Epiphyas postvittana*, is a horticultural pest in Australia and New Zealand [31]. *E. postvittana* is a member of the leafroller family Tortricidae, which also contains a number of other horticultural pests including codling moth (*Cydia pomonella*) and the oriental fruit moth (*Grapholita molesta*). Originating from Australia, *E. postvittana* has subsequently become established in New Zealand, Hawaii, California and parts of Europe [31,32]. The moth is primarily a pest of pipfruit, but is also found in high numbers in vineyards [31]. As well as insecticides, odor-mediated strategies such as mating disruption have been employed to control its numbers. The sex pheromone for *E. postvittana* has been identified and is mainly comprised of (*E*)-11-tetradecenyl acetate and (*E,E*)-9,11-tetradecadienyl acetate [33], with further minor components also being identified more recently [34]. Electrophysiological studies have demonstrated that, as well as the major pheromone components [35], antennae of *E. postvittana* can detect many terpenes, esters, alcohols and aldehydes [36].

Some of the elements of the molecular machinery have already been identified and characterized from the periphery of *E. postvittana* antennae [37–40]. These include PBPs, GOBPS, ABPXs and CSP carrier proteins, some ORs, and ODEs from the CXE, GST and CYP families. Pheromone Binding Proteins 1 and 3 from *E. postvittana* (EposPBP1, 3) are more highly expressed in male antennae than in female antennae, whereas EposPBP2 shows the opposite bias in expression [37]. EposPBP1 is capable of binding the major sex pheromone component and displays a high degree of allelic diversity [40]. Also showing male-biased expression in *E. postvittana* antennae is the takeout protein EposTO1 [37]; however, an *in vivo* ligand has yet to be identified for this carrier [7,8]. Three ORs have been isolated from *E. postvittana* [38]. These include the Orco ortholog EposOR2; a receptor, EposOR3, with high affinity for monoterpenes [38,41] that is conserved across the Lepidoptera; and a third (EposOR1) that falls within the pheromone receptor clade.

Here we use next generation sequencing technologies and bioinformatic analyses to isolate further candidate elements of the peripheral olfactory repertoire from *E. postvittana*. We identify large families of carrier proteins, receptors and ODEs. Within the ORs we identify two groups of male-biased receptors, with one found outside the pheromone receptor clade.

Methods

Insect Rearing

E. postvittana were obtained from a colony maintained at the New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand. Larvae were reared on a general all-purpose diet [42]. Moth antennae and bodies (no head or wings) were removed from two- to three-day-old, cold-anesthetized adults using forceps and immediately frozen in liquid nitrogen and stored at -80°C.

Next Generation Sequencing

Total RNA was extracted from pools of 100 pairs of male and female moth antennae using TRIzol RNA extraction reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. RNA quality and quantity was determined using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA). High quality total RNA isolated from male and female antennae was used to make libraries for paired-end sequencing on an Illumina HiSeq2000 (Axeq Technologies, Rockville, MD).

Read pair quality control check was carried out using FastQC [43]. Sequence data were then pre-processed by the removal of adapters and trimming to a minimum quality threshold of 20 bp and minimum length of 95 bp using fastq-mcf from the ea-utils package [44]. Thereafter, duplicate reads were removed and trimmed by 15 bases from the 5' end and reads containing

Ns or mononucleotides were removed using in-house Perl scripts. *De novo* assembly of the individual libraries was then performed by trans-ABySS (version 1.3.2) [45], where a k-mer series from $k = 31$ to $k = 75$ with an increment of two was used.

Gene Identification and Phylogenetics

BLAST searchable databases consisting of transcriptomic contigs from male and female *E. postvittana* antennae were used to identify candidate chemosensory genes. Tblastn searches were performed with publically available lepidopteran sequences, including previously identified chemosensory genes from *E. postvittana* [37–39]. Identified transcripts were imported into Geneious (version 6.0.6) [46] and annotated for open reading frames, start and stop codons and 5' and 3' untranslated regions. The longest contigs identified for a particular gene from male and female antennal transcriptomes were aligned to confirm sequence accuracy.

Predicted amino acid sequences of *E. postvittana*, OBP, OR and CXE genes were used in multiple sequence alignments and phylogenetic analyses to confirm their annotation and to examine their relatedness to similar genes from other moths. Datasets for each chemosensory gene family were compiled using publically available sequences from the silkworm genome database, and GenBank and accession numbers for these can be found in supplementary file, S1 Dataset. Amino acid sequences were aligned using Muscle as implemented within Geneious. Maximum likelihood trees were constructed using MEGA (version 6.0.5) [47], employing the Jones Taylor-Thornton substitution model, and node support was assessed using 1000 bootstrap replicates. Resulting trees were displayed using FigTree (version 1.4.0) [48] and annotated in Adobe Illustrator. Cellular localization of CXEs was predicted using PSORT [49].

Gene Expression Analyses

Cleaned RNASeq reads were mapped to a known set of olfactory receptor genes using Tophat (version 2.0.13) [50]. The resulting alignments were then used to obtain the Fragments per Kilobase of transcript per Million (FPKM) values using cufflinks (version 2.2.1) [51].

To confirm RNAseq counting results and to compare the expression of candidate ORs in male and female antennae, quantitative RT-PCR (qPCR) was performed using cDNA prepared from three independent biological replicates of pooled male and female antennae and bodies. One of these sets of biological replicates of male and female antennae was the same as that used for the RNAseq experiment described above. RNA extracted from each sample was treated with DNAse I (Life Technologies) and converted to cDNA using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA). cDNA was synthesized from each sample with and without reverse transcriptase to allow for detection of genomic DNA contamination by PCR. cDNA samples that tested negative for genomic DNA contamination were used in qPCR experiments.

Forward and reverse primers for each of the 70 putative *E. postvittana* OR genes, as well as the housekeeping genes *actin*, *α-tubulin* and *elongation factor-1α* (*EF-1α*), were designed using Primer 3 implemented within Geneious v6.0.5. Primer pairs were designed to amplify products of 100–200 bp in length, have TMs of 60°C (± 2°C), a G/C content of 40–60% and to have two to three G or C nucleotides on the 3' end.

Quantitative real-time PCR was conducted on a LightCycler 480 II using Syber Green Master Mix (Roche, Basel, Switzerland) under the following reaction conditions: an initial 2-min incubation at 94°C followed by 45 cycles of 94°C for 15 s, then 60°C for 30 s, then 72°C for 30 s. Each primer pair was tested in triplicate against three biological replicates of each cDNA sample, as well as to a no-template control on a single 384-well microtiter plate. The housekeeping genes *actin*, *α-tubulin* and *EF-1α* were tested in triplicate against each biological replicate of each cDNA sample, as well as a no-template control, on each 384-well microtiter plate. The

efficiency of each primer pair in each cDNA sample and the Cycle Threshold (Ct) value for each PCR was determined using LinRegPCR (version 11.0) software [52,53]. Amplification of single products was verified by melting curve analysis and electrophoresis. Amplification of target sequences was verified by Sanger sequencing of qPCR products.

The relative expression of each gene was calculated using a modified version of the ΔCt method [52,54]. Because the efficiency of a given primer set varied between cDNA samples, resulting Ct values for a particular gene and sample were corrected using the formula (E_{MAX}) $^{Ct}_{corrected} = (E_{sample\ `X'})^{Ct}_{sample\ `X'}$, where E_{MAX} equals the highest efficiency for a primer pair from all samples, $E_{sample\ `X'}$ equals the efficiency of that primer pair in sample 'X', $Ct_{sample\ `X'}$ equals the measured Ct value for sample 'X', and $Ct_{corrected}$ equals the corrected Ct value for sample 'X'. A normalization factor was determined for each sample by averaging the corrected Ct values for the three housekeeping genes from that sample. The relative expression of each gene to the normalization factor for each sample was calculated using the formula $(E_{MAX})^{(\Delta Ct)}$, where E_{MAX} equals the highest efficiency for a particular primer set and ΔCt equals the difference between the Ct of the primer set in that sample and the normalization factor for that sample. The average relative expression was calculated by averaging the relative expression of each gene in three biological replicates. Significant differences between the relative expressions of a particular gene between male and female antennae were determined using a Welch two-sample T-test.

Results

Transcriptome Assembly

Raw read numbers for the female and male transcriptomes were 166,566,716 and 171,208,876, respectively. Individual transcriptome assemblies were generated for female and male antennae. The female antennal assembly generated 270,708 transcripts with an N50 of 1,319 bp and a maximum transcript size of 16,438 bases (GenBank accession SRX456215), whereas the male antennal assembly yielded 266,710 transcripts with an N50 of 1,277 bp and a maximum transcript size of 14,723 bases (GenBank accession SRX456419).

Identification of Chemosensory Genes and Phylogenetics

In this study a total of 19 IRs, nine GRs, two SNMPs, 34 OBPs, 13 CSPs, 27 CXEs, 20 GSTs, 49 CYPs and 18 TOs were identified from male and female *E. postvittana* antennal transcriptomes and their sequences submitted to the Transciptome Shotgun Assembly Sequence Database (TSA) under the accession GCVX00000000. The version described in this paper is the first version, GCVX01000000. The sequences of these genes were confirmed and completed, where possible, using the draft genome of *E. postvittana* (unpublished data). DNA and predicted protein sequences for this set of 191 chemosensory genes can be found in supplementary file, S2 dataset; all but six of these sequences (EposCXE36, EposCYP11, EposCYP38, EposCYP48, EposIR4 and EposIR75p) appear to represent the full-length coding sequence of each gene.

Additionally a total of 60 candidate ORs were identified and assembled from the two transcriptome databases, with an extra ten identified from the draft genome bringing the total number of chemosensory genes identified to 261. Sequences for 55 of the 60 ORs identified from the transcriptome have been deposited into TSA under the accession GCVX00000000; this submission does not include two genes, *EposOR9* and *EposOR20* as they contained internal sequence gaps. Also, the three previously identified EposORs from Jordan et al. [38] have been updated in GenBank. The sequences of the 70 *E. postvittana* OR genes were confirmed, and the coding region completed where necessary, against the draft genome. Only the open reading frame of two OR genes, *EposOR11* and *EposOR20*, are partial because of missing sequence
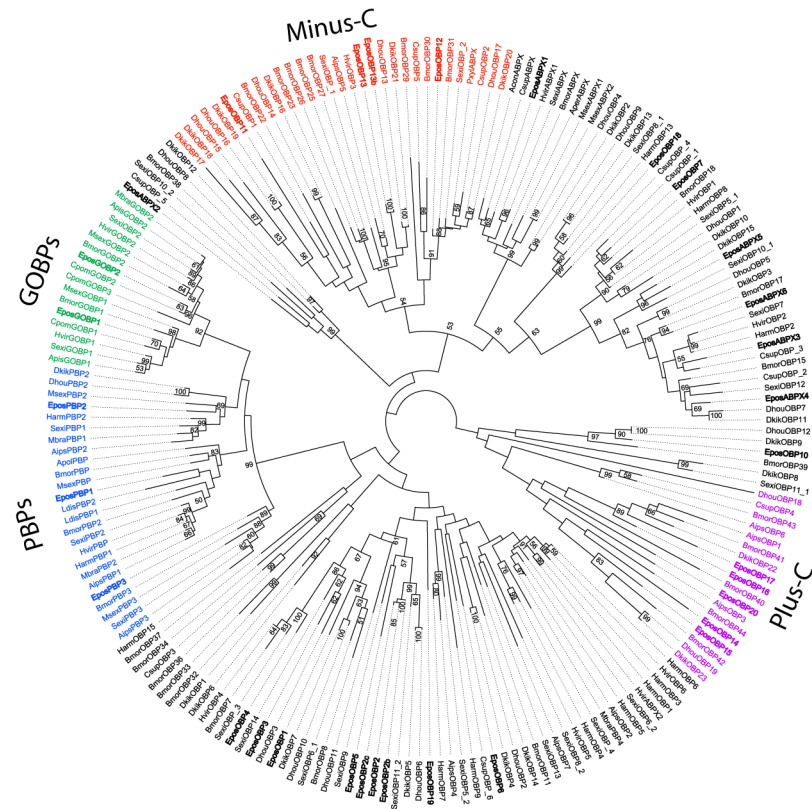
Fig 1. A maximum likelihood phylogenetic tree of odorant binding proteins (OBPs) from moths.
Includes sequences from *Epiphyas postvittana* (Epos), *Bombyx mori* (Bmor), *Cydia pomonella* (Cpom), *Chilo suppressalis* (Csup), *Dendrolimus houi* (Dhou), *Dendrolimus kikuchii* (Dkik), *Helicoverpa armigera* (Harm), *Heliothis virescens* (Hvir), *Lymantria dispar* (Ldis), *Mamastra brassicae* (Mbra), *Manduca sexta* (Msex), *Plutella xylostella* (Pxyl) and *Spodoptera exigua* (Sexi). OBPs identified from *E. postvittana* are in bold. Node support was assessed using bootstrap replicates of 1000 and values greater than 50% are shown. Sub-groups including the PBPs, GOBPs, minus-Cs and plus-Cs are marked.

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relating to predicted exon 1 for *EposOR11* and predicted exon 5 for *EposOR20*. DNA and pre-dicted protein sequence data for all 70 ORs can be found in supplementary file, S3 Dataset.

Phylogenetic analyses of the chemosensory gene families thought to be associated with pheromone reception and degradation in *E. postvittana*, namely the OBP, OR and CXE, multi-gene families from antennae, are provided in Figs 1, 2 and 3. The OBP gene family can be split into different sub-groups according to sequence motifs; the first group are PBPs, GOBPs and OBPS that contain six well-conserved cysteines, the second group are OBPs that are missing two of the conserved cysteines, generally at positions 2 and 5 (minus-C group); and the third group are OBPs that contain extra cysteines and a conserved proline (plus-C group) [55,56]. The EposOBPs were compared against OBPs from 16 other lepidopteran species to construct a phylogenetic tree (Fig 1). Two sequences, EposOBP8 and 9, did not align well with the other OBPs and were not found to exhibit many of the motifs associated with insect OBPs and were therefore not included in any further analyses. In the resulting phylogenetic tree, the three EposPBPs fall in with the other moth PBPs, the two EposGOBPs group with the other moth GOBPs, four EposOBPs fall into the minus-C group (EposOBP11, 12, 13 and 13b), five into the plus-C group (EposOBP14, 15, 16, 17 and 20), and the remaining EposOBPs and
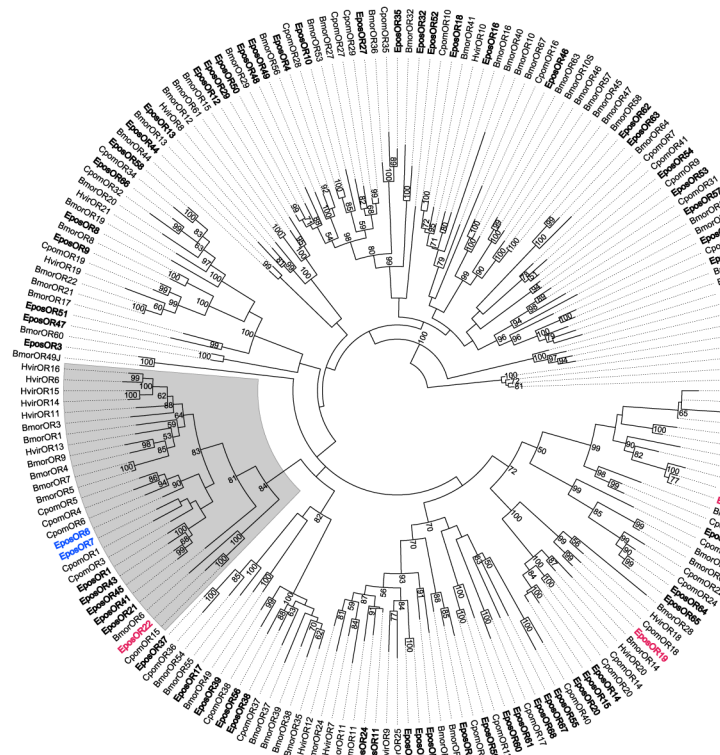
Fig 2. A maximum likelihood phylogenetic tree of odorant receptors (ORs) from moths. Includes sequences from *Epiphyas postvittana* (Epos), *Cydia pomonella* (Cpom), *Heliothis virescens* (Hvir) and *Bombyx mori* (Bmor), and is rooted with the OR co-receptor, Orco. The lepidopteran 'pheromone receptor' clade is shaded. *E. postvittana* ORs are shown in bold font with male-biased *E. postvittana* ORs colored blue and female-biased *E. postvittana* ORs colored pink. Node support was assessed using bootstrap replicates of 1000 and values greater than 50% are shown.

EposABPXs are distributed across the tree. All the EposOBPs form orthologous clusters with one or many OBPs from other species.

Phylogenetic analysis of the EposORs was performed against comprehensive OR datasets from three other lepidopteran species. Of the 70 *E. postvittana* ORs, eight receptors (EposOR1, 6, 7, 21, 22, 41, 43, and 45) fall into a well-supported clade that contains PRs from other moth species, including BmorOR1, BmorOR3, HvirOR6, HvirOR13, HvirOR14 and HvirOR16 [16–18,57,58] (Fig 2). The remaining 62 *E. postvittana* ORs are dispersed throughout the phylogeny, often forming orthologous sets with other moth ORs. There are only a few instances of radiations of ORs within the *E. postvittana* lineage, including one group of five ORs (EposOR30, 31, 33, 34 and 36), one of four ORs (EposOR1, 41, 43 and 45) that falls within the clade containing pheromone receptors from other species, and another of three ORs (EposOR14, 15 and 20) (Fig 2).

Comparison of the EposCXEs with other moth antennal esterases revealed a number of orthologous sets and one distinct radiation of *E. postvittana* genes containing eight sequences (Fig 3). One *E. postvittana* CXE, namely EposCXE24, falls into a clade containing pheromone-degrading enzymes (PDEs) from other moths [26]. Eleven EposCXEs, including EposCXE24, were predicted to contain signal peptides and be extracellular in their localization by PSORT, providing evidence for their potential secretion into the sensillum lymph.
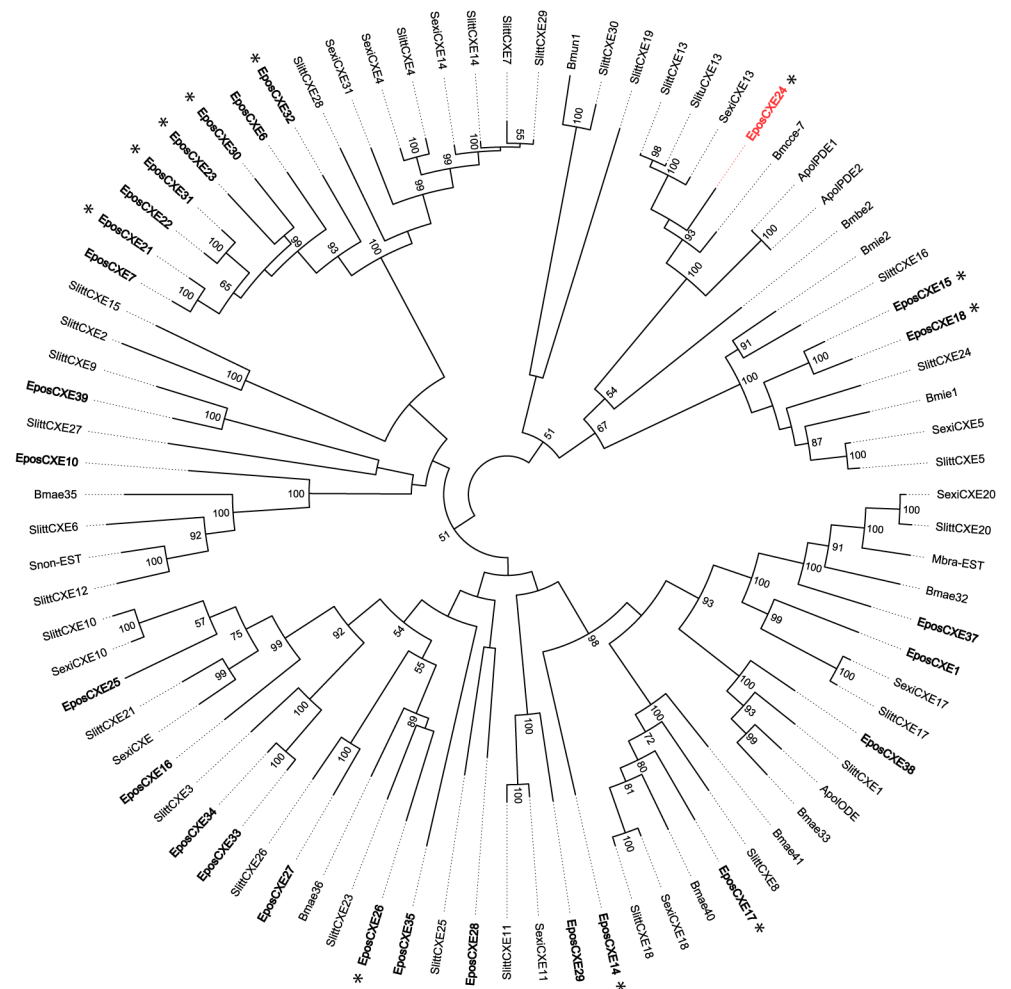
Fig 3. A maximum likelihood phylogenetic tree of antennal carboxylesterases (CXEs) from moths.
Includes sequences from *Epiphyas postvittana* (Epos), *Bombyx mori* (Bm), *Antheraea polyphemus* (Apol),
Mamestra brassicae (Mbra), *Spodoptera littoralis* (Slitt), *Sesamia nonagrioides* (Snon), *Spodoptera litura*
(Slitu) and *Spodoptera exigua* (Sexi). CXEs identified from *E. postvittana* are in bold and those predicted to
be secreted are indicated with an asterisk. The predicted pheromone degrading enzyme is shown in red.
Node support was assessed using bootstrap replicates of 1000 and values greater than 50% are shown.

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Gene Expression Analyses

Of the 191 olfactory-related genes not including ORs, RNAseq count data revealed normalized
transcript counts ranging from 0 to approximately 8.6×10^6 in moth antennae (Table 1). Many
of these genes display sex-biased antennal expression patterns; however, the statistical signifi-
cance of the biased expression could not be assessed because the RNAseq data were conducted
without replication.

Of the 70 *E. postvittana* OR genes identified in the antennal transcriptomes or genome, 65
had normalized RNAseq counts greater than one (Table 2). Transcripts for the *EposOR8, 11,
13, 69* and *70* genes were not detected in the male or female antennal transcriptomes by RNA-
seq analysis. Interestingly, *EposOR8* and *EposOR13* were detectable by PCR using the same
RNA starting material from which the transcriptomes were made, which is likely the conse-
quence of the greater sensitivity of qPCR over RNAseq, given the depth of sequencing

Table 1. Transcript counts in antennal tissue from male and female *Epiphyas postvittana* as determined by RNAseq.

Gene	RNASeq Data (FPKM) female antennae	male antennae	Gene	RNASeq Data (FPKM) female antennae	male antennae	Gene	RNASeq Data (FPKM) female antennae	male antennae
EposABPX1	194673	305620	EposCYP21	34	26	EposIR1	21	15
EposABPX2	27310	27431	EposCYP22	113	66	EposIR3	101	66
EposABPX3	75729	126520	EposCYP23	372	111	EposIR4	72	24
EposABPX4	21668	19058	EposCYP24	240	68	EposIR7d	14	7
EposABPX5	664	327	EposCYP25	4	4	EposIR8a	766	504
EposABPX6	24780	18822	EposCYP26	60	19	EposIR21a	53	27
EposCSP1	30415	26582	EposCYP27	44	24	EposIR25a	185	123
EposCSP2	51286	17848	EposCYP28	42	7	EposIR41a	60	35
EposCSP3	17920	12770	EposCYP29	14	11	EposIR68	51	31
EposCSP4	98800	80082	EposCYP30	88	39	EposIR75b	31	16
EposCSP5	52504	6969	EposCYP31	66	39	EposIR75d	10	9
EposCSP6	87	123	EposCYP32	19	9	EposIR75e	44	27
EposCSP7	88271	41531	EposCYP33	21	12	EposIR75p	31	11
EposCSP8	122	27	EposCYP34	8	13	EposIR75q.1	20	11
EposCSP9	496	90	EposCYP35	4	2	EposIR75q.2	295	225
EposCSP10	102	16	EposCYP36	493	322	EposIR76b	341	277
EposCSP11	105	69	EposCYP37	13	13	EposIR87a	127	77
EposCSP12	79	26	EposCYP38	23	140	EposIR93a	4	1
EposCSP13	64	12	EposCYP39	92	38	EposOBP1	1790	673
EposCXE1	7	6	EposCYP40	6	8	EposOBP2	23	13
EposCXE6	6	3	EposCYP41	38	10	EposOBP2b	35	14
EposCXE7	13	9	EposCYP42	81	30	EposOBP2c	165	78
EposCXE14	17	17	EposCYP43	111	72	EposOBP3	5652	1098
EposCXE15	59	77	EposCYP44	177	87	EposOBP4	632	337
EposCXE16	105	95	EposCYP45	61	34	EposOBP5	2909	716
EposCXE17	207	101	EposCYP46	54	142	EposOBP6	203263	67477
EposCXE18	39	32	EposCYP47	47	12	EposOBP7	159629	45042
EposCXE21	18	7	EposCYP48	119	710	EposOBP8	51	19
EposCXE22	76	47	EposCYP49	491	164	EposOBP9	274	114
EposCXE23	21	8	EposCYP50	15	3	EposOBP10	147	45
EposCXE24	10	5	EposCYP51	40	25	EposOBP11	365	11
EposCXE25	110	83	EposGOBP1	18273	10673	EposOBP12	95	32
EposCXE26	65	80	EposGOBP2	241065	175652	EposOBP13	1302	15
EposCXE27	15	9	EposGR1	8	1	EposOBP13b	6895	36
EposCXE28	68	23	EposGR2	7	5	EposOBP14	173	700
EposCXE29	19	12	EposGR3	3	3	EposOBP15	2048	1051
EposCXE30	22	9	EposGR4	18	8	EposOBP16	259	197
EposCXE31	12	8	EposGR5	18	14	EposOBP17	10	19
EposCXE32	3	1	EposGR6	51	25	EposOBP18	317386	78324
EposCXE33	24	14	EposGR7	64	23	EposOBP19	8234	6170
EposCXE34	14	12	EposGR8	59	32	EposOBP20	2113	1243
EposCXE35	42	21	EposGR9	42	4	EposPBP1	97046	863482
EposCXE36	5	6	EposGST2	332	203	EposPBP2	48920	10709
EposCXE37	98	53	EposGST4	6241	6532	EposPBP3	14081	50553
EposCXE38	65	39	EposGST5	518	372	EposSNMP1	855	364

(Continued)

Table 1. (*Continued*)

Gene	RNASeq Data (FPKM)		Gene	RNASeq Data (FPKM)		Gene	RNASeq Data (FPKM)	
	female antennae	male antennae		female antennae	male antennae		female antennae	male antennae
EposCXE39	22	15	EposGST6	130	81	EposSNMP2	501	344
EposCYP1	132	87	EposGST7	1000	1800	EposTO1	103597	50124
EposCYP3	61	20	EposGST8	1267	472	EposTO2	950	834
EposCYP4	162	114	EposGST9	5264	3256	EposTO3	3735	2115
EposCYP5	113	65	EposGST10	346	202	EposTO4	3774	3826
EposCYP6	1532	956	EposGST11	17621	17056	EposTO5	59	49
EposCYP7	118	42	EposGST12	245	134	EposTO6	48	13
EposCYP9	535	164	EposGST13	708	259	EposTO7	60	13
EposCYP10	360	107	EposGST14	337	412	EposTO8	322	38
EposCYP11	13	15	EposGST15	48	48	EposTO9	342	228
EposCYP12	223	116	EposGST16	190	94	EposTO10	402	444
EposCYP13	4	6	EposGST17	755	552	EposTO11	33	7
EposCYP14	9519	5579	EposGST18	149	85	EposTO12	200	83
EposCYP15	73	39	EposGST19	123	77	EposTO13	263	33
EposCYP16	60	10	EposGST20	406	197	EposTO14	2668	2416
EposCYP17	44	22	EposGST21	59	11	EposTO15	74	18
EposCYP18	6592	3419	EposGST22	69	68	EposTO16	14	6
EposCYP19	82	17	EposIgluR	14	6	EposTO17	8	3
EposCYP20	1315	732				EposTO18	11	7

Antennal binding protein X (APBX), chemosensory protein (CSP), carboxylesterase (CXE), cytochrome p450 (CYP), general odorant binding protein (GOBP), gustatory receptor (GR), glutathione-S-transferase (GST), ionotropic receptor (IR), odorant binding protein (OBP), pheromone binding protein (PBP), sensory neuron membrane protein (SNMP), and take out protein (TO). Data represent transcript counts from a single transcriptome made from male or female antennal mRNA.

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achieved. This sensitivity difference also likely explains why some gene transcripts were detected in both sexes by qPCR but in only one by RNAseq.

Sixty-seven of the 70 *E. postvittana* OR genes were detected by qPCR for expression in adult male or female antennae or bodies. Transcripts for *EposOR11*, *69* and *70* were not detected in any tissues despite attempts with multiple primer pairs. Of the 67 OR transcripts that were detected, 64 showed enriched expression in antennae compared to bodies, with 30 detected exclusively in male and/or female antennae and not in male or female bodies. Four genes, *EposOR6*, *7*, *30* and *34* had significantly greater levels of mRNA expression in male than in female antennae ($P = 0.046$, $P = 0.009$, $P = 0.004$ and $P = 0.002$, respectively) ([Fig 4A](#)). Five receptors, *EposOR19*, *22*, *31*, *36* and *40* had significantly greater levels of mRNA expression in female than in male antennae ($P = 0.023$, $P = 0.032$, $P = 0.031$, $P = 0.012$ and $P = 0.018$, respectively) ([Fig 4B](#)). We note that the EposOR genes that showed significant sex-biased mRNA expression by qPCR also showed biased expression patterns by RNAseq, demonstrating that despite the fact that we could not conduct statistical analyses on RNAseq data, it was a good predictor of sex-biased expression for these genes.

Discussion

Transcriptome sequencing has become a popular approach to identify genes involved in tissue-specific functions. Here we have used this approach to identify genes involved in the peripheral

Table 2. Odorant receptor (OR) mRNA expression in antennal and body tissue of female and male *Epiphyas postvittana* as determined by quantitative RT-PCR and OR transcript counts in antennal tissue as determined by RNAseq.

Gene	qPCR data (fold change to reference genes)				RNASeq Data (FPKM)	
	female antennae	female body	male antennae	male body	female antennae	male antennae
EposOR1	0.03263	0.00024	0.07130	BLD	191	104
EposOrco	2.62100	BLD	3.55188	BLD	2676	1397
EposOR3	0.00768	BLD	0.00663	BLD	35	30
EposOR4	0.03408	BLD	0.03337	0.00011	72	53
EposOR5	0.01009	0.00002	0.00138	BLD	319	37
EposOR6	0.00073	BLD	0.04183	0.00600	0	12
EposOR7	0.00315	0.00010	0.01561	0.00138	0	90
EposOR8	0.00053	BLD	0.00006	0.00002	0	0
EposOR9	0.00145	BLD	0.00127	BLD	18	6
EposOR10	0.00791	0.00007	0.00624	0.00115	37	9
EposOR11	BLD	BLD	BLD	BLD	0	0
EposOR12	0.00143	BLD	0.00192	0.00001	74	74
EposOR13	0.00028	0.00004	0.00071	0.00059	0	0
EposOR14	0.01350	0.00003	0.00650	0.00028	35	12
EposOR15	0.03509	0.00502	0.02606	0.02465	73	34
EposOR16	0.00265	BLD	0.00726	0.00023	3	1
EposOR17	0.00560	0.00493	0.01428	0.00325	2	1
EposOR18	0.03153	0.00029	0.02091	BLD	63	28
EposOR19	0.07996	0.00007	0.03561	BLD	288	57
EposOR20	0.01524	0.00139	0.01714	0.00381	74	23
EposOR21	0.00729	0.00002	0.00099	0.00003	66	6
EposOR22	0.07837	0.00007	0.02709	BLD	108	43
EposOR23	0.00058	0.00025	0.00145	0.00108	1	1
EposOR24	0.01978	BLD	0.02527	BLD	20	9
EposOR25	0.03330	0.00034	0.03440	0.00260	46	15
EposOR26	0.05425	0.01337	0.11274	0.01719	21	15
EposOR27	0.05022	BLD	0.04528	0.00128	104	46
EposOR28	0.01670	BLD	0.00903	0.00009	22	20
EposOR29	0.04849	0.00020	0.02772	0.00027	36	18
EposOR30	0.02020	0.00398	0.69629	0.02740	0	536
EposOR31	0.00616	BLD	0.00003	BLD	189	0
EposOR32	0.04552	BLD	0.03881	BLD	18	6
EposOR33	0.00640	BLD	0.00047	0.00004	60	0
EposOR34	0.01131	0.00043	0.32708	0.00233	1	779
EposOR35	0.00408	BLD	0.00543	BLD	26	14
EposOR36	0.02528	BLD	0.00014	0.00004	57	0
EposOR37	0.00393	0.00008	0.01092	0.00008	20	17
EposOR38	0.04631	0.00006	0.11024	0.00197	50	19
EposOR39	0.09887	BLD	0.19689	BLD	50	24
EposOR40	0.32466	0.00007	0.00507	0.00135	383	1
EposOR41	0.00446	0.00019	0.00911	BLD	29	19
EposOR42	0.05713	BLD	0.09239	BLD	70	47
EposOR43	0.09436	0.00068	0.04906	0.00181	21	17
EposOR44	0.00199	BLD	0.00317	BLD	44	19
EposOR45	0.01907	0.00015	0.02347	0.00011	147	75

(Continued)

Table 2. (*Continued*)

Gene	qPCR data (fold change to reference genes)				RNASeq Data (FPKM)	
	female antennae	female body	male antennae	male body	female antennae	male antennae
EposOR46	0.00932	0.00015	0.01168	0.00079	28	16
EposOR47	0.08845	0.00096	0.07746	0.00430	52	31
EposOR48	0.06465	0.00007	0.07733	0.00034	102	75
EposOR49	0.00271	0.00022	0.00522	0.00008	6	5
EposOR50	0.00054	0.00008	0.00144	0.00010	3	1
EposOR51	0.05788	0.00038	0.06611	0.00031	163	84
EposOR52	0.00986	BLD	0.00955	BLD	24	9
EposOR53	0.01144	0.00003	0.01077	0.00011	128	92
EposOR54	0.02487	0.00003	0.02158	0.00011	47	23
EposOR55	0.00098	0.00001	0.00569	0.00026	37	28
EposOR56	0.00100	BLD	0.00071	BLD	10	4
EposOR57	0.00114	BLD	0.00610	0.00001	8	3
EposOR58	0.00405	0.00001	0.00339	0.00003	81	40
EposOR59	0.01240	0.00061	0.00962	0.00044	11	3
EposOR60	0.00464	0.00010	0.01035	BLD	38	32
EposOR61	0.01409	0.00006	0.01719	BLD	160	50
EposOR62	0.00589	BLD	0.00451	BLD	30	16
EposOR63	0.00017	BLD	0.00011	BLD	7	3
EposOR64	0.05393	BLD	0.01088	BLD	227	56
EposOR65	0.01115	0.00005	0.00184	0.00004	108	17
EposOR66	0.00506	0.00020	0.00296	BLD	14	4
EposOR67	0.02124	0.00001	0.01068	0.00007	128	43
EposOR68	0.02752	0.00027	0.02945	BLD	55	32
EposOR69	BLD	BLD	BLD	BLD	0	0
EposOR70	BLD	BLD	BLD	BLD	1	0

Quantitative RT-PCR data represent the mean fold change to reference genes from three biological replicates. RNAseq data represent transcript counts from a single transcriptome made from male or female antennal mRNA. BLD = Below Limit of Detection.

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events of odorant and pheromone reception in the lepidopteran pest, *E. postvittana*. In total, 266,710 and 270,708 putative transcripts were produced from male and female antennae, respectively. From these we inferred the presence of 251 predicted proteins that are potentially involved in odorant and pheromone binding, reception and degradation. The average transcript length of our male (N50 = 1,277 bp) and female (N50 = 1,319 bp) antennal transcriptomes aided the determination of the full length coding sequences of most of these genes through examination of untranslated regions and open reading frames of single contigs. To date we have verified the full length sequence of 36 genes from the dataset, including 30 ORs, three OBPs, two SNMPs and one CXE by PCR from antennal cDNA.

The set of olfactory-related genes that have been identified from the antennae of *E. postvittana* has been substantially extended by this study. Previously through micro-sequencing of purified protein and degenerate PCR, four OBPs, EposPBP1, EposPBP2, EposGOBP1 and EposGOBP2, were identified [40]. EST libraries from male antennae were also generated, enabling the identification of sequences for 12 more candidate binding proteins, one takeout protein, three ORs, two SNMPs and 25 odor-degrading enzymes [37]. With this study we have expanded the total number of identified olfactory-related genes from *E. postvittana* that are in
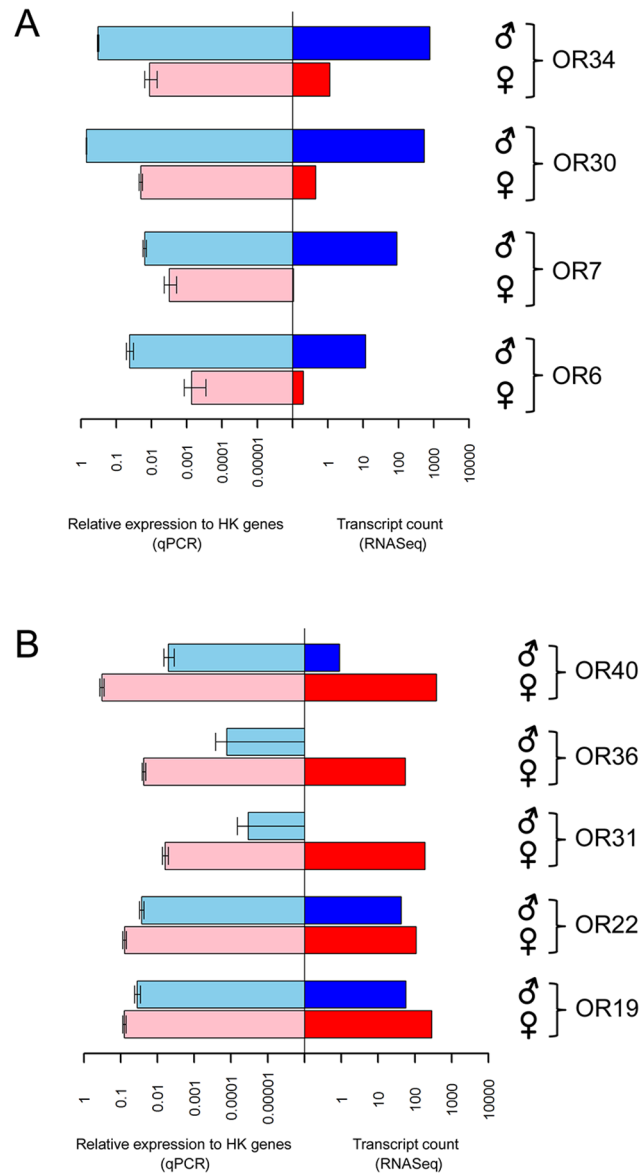
Fig 4. Male-biased (A) and female-biased (B) antennal odorant receptor mRNA expression in
***Epiphyas postvittana*.** Comparison of OR mRNA expression in male (♂) and female (♀) antennae as
determined by quantitative real-time PCR (qPCR; left panel) and RNASeq (right panel). For qPCR data, bars
represent mean (± SEM) of OR mRNA expression relative to reference genes from three biological replicates.
For RNAseq data, bars represent transcript counts from a single transcriptome made from male or female
antennal mRNA.

the public domain to: 34 OBPs, 13 CSPs, 70 ORs, 19 IRs, two SNMPs, 38 CXEs, 22 GSTs, 51
CYPs and 18 TOs. Within the Tortricidae there are few transcriptome datasets from olfactory
tissues, with the only other comparable dataset from the antennae of codling moth, *Cydia
pomonella* [59]. This *E. postvittana* antennal dataset therefore makes an important contribu-
tion to the growing datasets of gene sequences from pest tortricids and, in particular genes en-
coding proteins predicted to be involved in peripheral olfactory signal transduction.

We then focused on genes likely to encode elements of the sex pheromone signal transduc-
tion pathway, namely members of the specialized odorant-binding protein family, odorant

receptor family, sensory neuron membrane protein family, and carboxylesterase family, since the predominant sex pheromone components of *E. postvittana* are acetate esters.

Three pheromone-binding proteins have previously been identified in *E. postvittana* [38], with one shown to bind the major sex pheromone component [40]. In this current study no further potential pheromone binding proteins were identified.

Seventy ORs were identified from *E. postvittana* antennae in this study, which correlates well with the number of glomeruli (50–70) observed in the antennal lobe of other tortricid moths [60,61]. Odorant receptors that may play a role in pheromone detection are typically identified through either being members of the so-called pheromone receptor clade or by showing male-biased expression in adult antennae. Of the 70 *E. postvittana* ORs, eight were identified as being part of the phylogenetic clade associated with a role in pheromone reception in other moths. Seven of the eight EposORs that fall within this 'PR clade' contain the characteristic 'PWE' amino acid motif within the final transmembrane domain that is seen in members of this group. One receptor, EposOR21, does not contain this motif and phylogenetic analysis suggests this receptor is basal to other receptors in the clade. EposORs 1, 41, 43 and 45 share relatively high (~61%) identity at the protein level and form their own group within the clade, and may have arisen by recent gene duplication events.

The two male-biased EposORs within the PR clade, EposOR6 and EposOR7, are most closely related to ORs from *C. pomonella*, CpomOR6 and CpomOR1, respectively. The other receptor that falls in the pheromone receptor clade, EposOR22, is female-biased and is closely related to CpomOR15 [59]. In terms of male-biased expression, EposOR6 and EposOR7 from within the PR clade and EposOR30 and EposOR34 from a more distant part of the phylogenetic tree all showed significantly greater levels of expression in male than in female antennae. This is the first case we are aware of where receptors from outside the PR clade display male-biased expression. These two groups of male-biased receptors (EposOR6 and EposOR7; EposOR30 and EposOR34) form clusters of genes physically within the genome (unpublished draft genome and BAC analysis).

One possible explanation for the observed male-biased expression profiles of EposOR6,7,30 and 34 is that male *E. postvittana* have large numbers of tricoid sensilla present on their antennae that are not found on the female [37]. Single sensillum recordings have shown that these tricoid sensilla are responsive to components of the female-produced sex pheromone and therefore are likely to contain olfactory sensory neurons that express PRs [35]. Aside from simple numerical differences in the number of pheromone sensitive sensilla, it is also possible that there are absolute differences in steady state transcript levels of PRs between the sexes in certain classes of olfactory sensory neurons tuned for pheromone. It is difficult to ascertain the relative contribution of these different hypotheses from the methods we employed to assess sex-biased expression from RNA extractions of whole antennae.

In addition to male-biased receptors, we also identified receptors that showed female-biased expression that could potentially be involved in host location or male pheromone recognition. These five female-biased receptors (EposOR19, 22, 31, 36, 40) include two (EposOR31 and EposOR36) that are closely related to the two male-biased ORs that are not part of the PR clade. None of the female-biased receptors was closely related to those found to be female-biased in their expression in *Bombyx mori* (BmorOR19, 30, 45, 46, 47) [62,63]. Functional characterization of these male- and female-biased ORs, as well as members of the PR clade, remains to be conducted.

Two SNMPs were identified among the antennal transcripts of *E. postvittana*. In other insects SNMP1 has been implicated in pheromone reception, through its requirement for pheromone reception in *Drosophila* [64] and its ability to enhance the response of ectopically expressed moth pheromone receptors *in vivo* [65]. SNMPs are members of the CD36-like

family and may have roles in presentation of the pheromone to the receptor or signal resetting through transporting the ligand inside the cell after receptor activation [66,67].

Twenty-four CXEs were found among the antennal transcripts, with thirteen of them predicted to be secreted. Only one CXE seems a good candidate for being involved in the degradation of *E. postvittana* acetate pheromone components based on phylogenetic relatedness to other pheromone-degrading enzymes [26,68], namely EposCXE24. Other PDEs display high affinity for pheromone components and rapid hydrolytic properties [26,69,70] and we would expect similar characteristics for a PDE in *E. postvittana* to allow rapid resetting of the pheromone transduction system.

Together this set of genes potentially involved in pheromone and odorant signal transduction in *E. postvittana* comprises an important dataset from which to study chemoreception in this organism, as well as a potential set of targets against which to develop novel olfactory-based control strategies for horticultural pests.

Supporting Information

S1 Dataset. Datasets used in the phylogenetic analysis for OBP, OR and CXE gene families.
(XLSX)

S2 Dataset. DNA and predicted protein sequence for *Epiphyas postvittana* non-OR chemosensory genes.
(TXT)

S3 Dataset. DNA and predicted protein sequence for *Epiphyas postvittana* OR genes.
(TXT)

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Author Contributions

Conceived and designed the experiments: JAC MDJ RDN. Performed the experiments: JAC MDJ. Analyzed the data: JAC MDJ AHT. Contributed reagents/materials/analysis tools: RNC RDN. Wrote the paper: JAC MDJ AHT RNC RDN.

References

1. Kanaujia S, Kaissling KE (1985) Interactions of Pheromone with Moth Antennae—Adsorption, Desorption and Transport. J Insect Physiol 31: 71–81.

2. Pelosi P (1996) Perireceptor events in olfaction. J Neurobiol 30: 3–19. PMID: 8727979

3. Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol 58: 373–391. doi: 10.1146/annurev-ento-120811-153635 PMID: 23020622

4. Pelosi P, Maida R (1995) Odorant-binding proteins in insects. Comp Biochem Physiology B, Biochem Mol Biol 111: 503–514. PMID: 7613772

5. Xu P, Atkinson R, Jones DN, Smith DP (2005) *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. Neuron 45: 193–200. PMID: 15664171

6. Damberger FF, Michel E, Ishida Y, Leal WS, Wuthrich K (2013) Pheromone discrimination by a pH-tuned polymorphism of the *Bombyx mori* pheromone-binding protein. Proc Natl Acad Sci U S A 110: 18680–18685. doi: 10.1073/pnas.1317706110 PMID: 24158483

7. Hamiaux C, Basten L, Greenwood DR, Baker EN, Newcomb RD (2013) Ligand promiscuity within the internal cavity of *Epiphyas postvittana* Takeout 1 protein. J Structl Biol 182: 259–263. doi: 10.1016/j.jsb.2013.03.013 PMID: 23563188

8. Hamiaux C, Stanley D, Greenwood DR, Baker EN, Newcomb RD (2009) Crystal structure of *Epiphyas postvittana* takeout 1 with bound ubiquinone supports a role as ligand carriers for takeout proteins in insects. J Biol Chem 284: 3496–3503. doi: 10.1074/jbc.M807467200 PMID: 19073605

9. Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB (2009) Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136: 149–162. doi: 10.1016/j.cell.2008.12.001 PMID: 19135896

10. Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K (2008) Insect Olfactory Receptors are Heteromeric Ligand-Gated Ion Channels. Chem Senses 33: S74–S74.

11. Smart R, Kiely A, Beale M, Vargas E, Carraher C, Kralicek AV et al. (2008) *Drosophila* odorant receptors are novel seven transmembrane domain proteins that can signal independently of heterotrimeric G proteins. Insect Biochem Mol Biol 38: 770–780. doi: 10.1016/j.ibmb.2008.05.002 PMID: 18625400

12. Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH et al. (2008) *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452: 1007–U1010. doi: 10.1038/nature06861 PMID: 18408711

13. Benton R, Sachse S, Michnick S, Vosshall L (2006) Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. Chem Senses 31: A5–A5.

14. Rytz R, Croset V, Benton R (2013) Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. Insect biochem Mol Biol 43: 888–897. doi: 10.1016/j.ibmb.2013.02.007 PMID: 23459169

15. Rogers ME, Krieger J, Vogt RG (2001) Antennal SNMPs (sensor neuron membrane proteins) of lepidoptera define a unique family of invertebrate CD36-like proteins. Journal of Neurobiology 49: 47–61. PMID: 11536197

16. Wang G, Vasquez GM, Schal C, Zwiebel LJ, Gould F (2011) Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. Insect Mol Biol 20: 125–133. doi: 10.1111/j.1365-2583.2010.01045.x PMID: 20946532

17. Groβe-Wilde E, Gohl T, Bouche E, Breer H, Krieger J (2007) Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. Eur J Neurosci 25: 2364–2373. PMID: 17445234

18. Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, Tanoue S et al. (2004) Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc Natl Acad Sci U S A 101: 16653–16658. PMID: 15545611

19. Groβe-Wilde E, Svatos A, Krieger J (2006) A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem Senses 31: 547–555. PMID: 16679489

20. Forstner M, Breer H, Krieger J (2009) A receptor and binding protein interplay in the detection of a distinct pheromone component in the silkmoth *Antheraea polyphemus*. Int J Biol Sci 5: 745–757. PMID: 20011135

21. Wanner KW, Nichols AS, Allen JE, Bunger PL, Garczynski SF, Linn CE et al. (2010) Sex Pheromone Receptor Specificity in the European Corn Borer Moth, *Ostrinia nubilalis*. PLoS One 5.

22. Miura N, Nakagawa T, Touhara K, Ishikawa Y (2010) Broadly and narrowly tuned odorant receptors are involved in female sex pheromone reception in *Ostrinia* moths. Insect Biochem Mol Biol 40: 64–73. doi: 10.1016/j.ibmb.2009.12.011 PMID: 20044000

23. Carraher C, Authier A, Steinwender B, Newcomb RD (2012) Sequence comparisons of odorant receptors among tortricid moths reveal different rates of molecular evolution among family members. PLoS One 7: e38391. doi: 10.1371/journal.pone.0038391 PMID: 22701634

24. Yasukochi Y, Miura N, Nakano R, Sahara K, Ishikawa Y (2011) Sex-Linked Pheromone Receptor Genes of the European Corn Borer, *Ostrinia nubilalis*, Are in Tandem Arrays. PLoS One 6.

25. Vogt RG (2005) Molecular basis of pheromone detection in insects.; Gilbert LI, Iatro K., Gill S., editor. London: Elsevier.

26. Ishida Y, Leal WS (2005) Rapid inactivation of a moth pheromone. Proc Natl Acad Sci U S A 102: 14075–14079. PMID: 16172410

27. You M, Yue Z, He W, Yang X, Yang G, Xie M et al. (2013) A heterozygous moth genome provides insights into herbivory and detoxification. Nat Genet 45: 220–225. doi: 10.1038/ng.2524 PMID: 23313953

28. Zhan S, Merlin C, Boore JL, Reppert SM (2011) The monarch butterfly genome yields insights into long-distance migration. Cell 147: 1171–1185. doi: 10.1016/j.cell.2011.09.052 PMID: 22118469

29. Heliconius Genome Consortium (2012) Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487: 94–98. doi: 10.1038/nature11041 PMID: 22722851

30. International Silkworm Genome C (2008) The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. Insect biochem Mol Biol 38: 1036–1045. doi: 10.1016/j.ibmb.2008.11.004 PMID: 19121390

31. Suckling DM, Brockerhoff EG (2010) Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). Annu Rev Entomol 55: 285–306. doi: 10.1146/annurev-ento-112408-085311 PMID: 19728834

32. Tooman LK, Rose CJ, Carraher C, Suckling DM, Paquette SR, Ledezma LA et al. (2011) Patterns of Mitochondrial Haplotype Diversity in the Invasive Pest *Epiphyas postvittana* (Lepidoptera: Tortricidae). J Econ Entomol 104: 920–932. PMID: 21735912

33. Bellas TE, Bartell RJ, Hill A (1983) Identification of two components of the sex pheromone of the moth, *Epiphyas postvittana* (Lepidoptera, Tortricidae). J Chem Ecol 9: 503–512. doi: 10.1007/BF00990222 PMID: 24407457

34. El-Sayed AM, Mitchell VJ, Manning LAM, Suckling DM (2011) New Sex Pheromone Blend for the Lightbrown Apple Moth, *Epiphyas postvittana*. J Chem Ecol 37: 640–646. doi: 10.1007/s10886-011-9964-x PMID: 21573864

35. Rumbo ER (1983) Differences between Single Cell Responses to Different Components of the Sex-Pheromone in Males of the Lightbrown Apple Moth (*Epiphyas-Postvittana*). Physiol Entomol 8: 195–201.

36. Suckling DM, Karg G, Gibb AR, Bradley SJ (1996) Electroantennogram and oviposition responses of *Epiphyas postvittana* (Lepidoptera: Tortricidae) to plant volatiles. New Zeal J Crop Hort 24: 323–333.

37. Jordan MD, Stanley D, Marshall SDG, De Silva D, Crowhurst RN, Gleave AP et al. (2008) Expressed sequence tags and proteomics of antennae from the tortricid moth, *Epiphyas postvittana*. Insect Mol Biol 17: 361–373. doi: 10.1111/j.1365-2583.2008.00812.x PMID: 18651918

38. Jordan MD, Anderson A, Begum D, Carraher C, Authier A, Marshall SDG et al. (2009) Odorant Receptors from the Light brown Apple Moth (*Epiphyas postvittana*) Recognize Important Volatile Compounds Produced by Plants. Chem Senses 34: 383–394. doi: 10.1093/chemse/bjp010 PMID: 19293399

39. Begum D (2011) Identification and analysis of olfactory receptors from the light brown apple moth, (*Epiphyas postvittana*). PhD Thesis, School of Biological Sciences, University of Auckland, Auckland, New Zealand.

40. Newcomb RD, Sirey TM, Rassam M, Greenwood DR (2002) Pheromone binding proteins of *Epiphyas postvittana* (Lepidoptera: Tortricidae) are encoded at a single locus. Insect Biochem Mol Biol 32: 1543–1554. PMID: 12530222

41. Corcoran JA, Jordan MD, Carraher C, Newcomb RD (2014) A novel method to study insect olfactory receptor function using HEK293 cells. Insect Biochem Mol Biol 54C: 22–32.

42. Singh P (1974) A chemically defined medium for rearing *Epiphyas postvittana* (Lepidoptera: Tortricidae). New Zeal J Zool 1: 241–243.

43. Andrews S. FastQC. A quality control tool for high throughput sequence data. Available: http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc. Accessed 7th October 2014.

44. Aronesty E. ea-utils: "Command-line tools for processing biological sequencing data". Available: http://code.google.com/p/ea-utils/wiki/FastqMcf. Accessed 7th October 2014.

45. Robertson G, Schein J, Chiu R, Corbett R, Field M, Jackman SD et al. (2010) De novo assembly and analysis of RNA-seq data. Nat Methods 7: 909–912. doi: 10.1038/nmeth.1517 PMID: 20935650

46. Biomatters. Geneious v6.0.6. Available: http://www.geneious.com. Accessed 6th October 2014.

47. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725–2729. doi: 10.1093/molbev/mst197 PMID: 24132122

48. Rambaut A. FigTree v1.4.0. Available: http://tree.bio.ed.ac.uk/software/figtree. Accessed 6th October 2014.

49. Nakai K, Horton P (1999) PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. Trends Biochem Sci 24: 34–36. PMID: 10087920

50. Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25: 1105–1111. doi: 10.1093/bioinformatics/btp120 PMID: 19289445

51. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ et al. (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28: 511–515. doi: 10.1038/nbt.1621 PMID: 20436464

52. Ramakers C, Ruijter JM, Deprez RHL, Moorman AFM (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett 339: 62–66. PMID: 12618301

53. LinRegPCR v11. Available: http://www.LinRegPCR.nl. Accessed 6th October 2014.

54. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29.

55. Fan J, Francis F, Liu Y, Chen JL, Cheng DF (2011) An overview of odorant-binding protein functions in insect peripheral olfactory reception. Genet Mol Res: GMR 10: 3056–3069. doi: 10.4238/2011. December.8.2 PMID: 22180039

56. Hekmat-Scafe DS, Scafe CR, McKinney AJ, Tanouye MA (2002) Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. Genome Res 12: 1357–1369. PMID: 12213773

57. Nakagawa T, Sakurai T, Nishioka T, Touhara K (2005) Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307: 1638–1642. PMID: 15692016

58. Krieger J, Groβe-Wilde E, Gohl T, Dewer YME, Raming K, Breer H (2004) Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). Proc Natl Acad Sci U S A 101: 11845–11850. PMID: 15289611

59. Bengtsson JM, Trona F, Montagne N, Anfora G, Ignell R, Witzgall P et al. (2012) Putative Chemosensory Receptors of the Codling Moth, *Cydia pomonella*, Identified by Antennal Transcriptome Analysis. PLoS One 7: e31620. doi: 10.1371/journal.pone.0031620 PMID: 22363688

60. Masante-Roca I, Gadenne C, Anton S (2005) Three-dimensional antennal lobe atlas of male and female moths, *Lobesia botrana* (Lepidoptera: Tortricidae) and glomerular representation of plant volatiles in females. J Exp Biol 208: 1147–1159. PMID: 15767314

61. Varela N, Couton L, Gemeno C, Avilla J, Rospars JP, Anton S (2009) Three-dimensional antennal lobe atlas of the oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae): comparison of male and female glomerular organization. Cell Tissue Res 337: 513–526. doi: 10.1007/s00441-009-0839-1 PMID: 19649654

62. Wanner KW, Anderson AR, Trowell SC, Theilmann DA, Robertson HM, Newcomb RD (2007) Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. Insect Mol Biol 16: 107–119. PMID: 17257213

63. Anderson AR, Wanner KW, Trowell SC, Warr CG, Jaquin-Joly E, Zagatti P et al. (2009) Molecular basis of female-specific odorant responses in *Bombyx mori*. Insect Biochem Mol Biol 39: 189–197. doi: 10.1016/j.ibmb.2008.11.002 PMID: 19100833

64. Benton R, Vannice KS, Vosshall LB (2007) An essential role for a CD36-related receptor in pheromone detection in *Drosophila*. Nature 450: 289–U213. PMID: 17943085

65. Syed Z, Kopp A, Kimbrell DA, Leal WS (2010) Bombykol receptors in the silkworm moth and the fruit fly. Proc Natl Acad Sci U S A 107: 9436–9439. doi: 10.1073/pnas.1003881107 PMID: 20439725

66. Rogers ME, Steinbrecht RA, Vogt RG (2001) Expression of SNMP-1 in olfactory neurons and sensilla of male and female antennae of the silkmoth *Antheraea polyphemus*. Cell Tissue Res 303: 433–446. PMID: 11320659

67. Vogt RG (2003) Biochemical diversity of odor detection: OBPs, ODEs and SNMPs. In: Blomquist GJaV, R. G., editor. Insect Pheromone Biochemistry and Molecular Biology: Elsevier Academic Press. pp. 391–445.

68. Vogt RG, Riddiford LM (1981) Pheromone binding and inactivation by moth antennae. Nature 293: 161–163. PMID: 18074618

69. Rybczynski R, Vogt RG, Lerner MR (1990) Antennal-specific pheromone-degrading aldehyde oxidases from the moths *Antheraea polyphemus* and *Bombyx mori*. J Biol Chem 265: 19712–19715. PMID: 2246254

70. Ishida Y, Leal WS (2008) Chiral discrimination of the Japanese beetle sex pheromone and a behavioral antagonist by a pheromone-degrading enzyme. Proc Natl Acad Sci U S A 105: 9076–9080. doi: 10.1073/pnas.0802610105 PMID: 18579770