Supporting Information

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SI Materials and Methods

Animal Rearing. Animals were taken from a culture maintained at the Max Planck Institute (MPI) for Chemical Ecology. For breeding, adults were kept in a flight cage containing a *Nicotiana attenuata* plant for oviposition, with artificial flowers providing a sugar solution for nutrition. Three times a week, eggs were collected and transferred to small boxes with artificial diet (46 g of agar, 144 g of wheat germ, 140 g of corn meal, 76 g of soy flour, 75 g of casein, 24 g of salt, 36 g of sugar, 5 g of cholesterol, 12 g of ascorbic acid, 6 g of sorbic acid, 3 g of methylparaben, 9 mL of linseed oil, 60 mL of 37% formalin, 30 mg of nicotinic acid, 15 mg of riboflavin, 7 mg of thiamine, 7 mg of pyridoxine, 7 mg of folic acid, and 0.6 mg of biotin per 1.8 mL of water). Eggs and emerging larvae were kept inside a climate-controlled chamber at 27 °C and 70% humidity. Hatched larvae were transferred into new boxes with artificial diet on metal lattices. On onset of wandering (fifth instar), larvae were allowed to pupate separately in small plastic boxes filled with paper. Pupae were kept inside climate chambers until 1 wk before likely emergence. Animals used for morphological or microarray experiments were separated as pupae and allowed to emerge individually in paper bags in an environmental chamber at 25 °C with 50% relative humidity on a 16-h/8-h photoperiod. For 3D reconstructions, only naïve adult moths 3–5 d posteclosion were used (male, $n = 3$; female, $n = 3$).

Extraction of Total RNA. Tissues were cooled over liquid nitrogen. The frozen tissue was transferred to a liquid nitrogen cooled mortar and ground. The homogenate was covered with 1 mL of TRIzol reagent (Sigma-Aldrich). Further steps were performed according to the manufacturer's instruction, replacing chloroform with 1-bromo-3-chloro-propane. Also, an additional DNase (Turbo DNase, Ambion) treatment was included to eliminate any contaminating DNA. TotalRNA was dissolved in RNA Storage Solution (Ambion), and RNA content and quality was measured photometrically.

Expressed Sequence Tag Generation. RNA extracted from four male and four female animals was unified and further purified by using the RNeasy MinElute Clean up Kit (Qiagen) following the manufacturer's protocol. RNA integrity and quantity was verified on an Agilent 2100 Bioanalyzer by using the RNA Nanochips (Agilent Technologies). RNA quantity was determined on a Nanodrop ND-1000 spectrophotometer. To prevent overrepresentation of the most common transcripts, the reverse-transcribed mRNAs converted to double-stranded cDNAs (see below) were normalized by using the Kamchatka crab duplex-specific nuclease method (1). Normalized, full-length, enriched cDNA libraries were generated by using a combination of the SMART cDNA library construction kit (BD Clontech) and the Trimmer Direct cDNA normalization kit (Evrogen) generally following the manufacturer's protocol but with several important modifications and enzyme replacements essentially as described (2). In brief, 2 μg of total RNA was used, and reverse transcription was performed with a mixture of several reverse-transcription enzymes for 1 h at 42 °C and 90 min at 50 °C. cDNA size fractionation was performed with SizeSep 400 spun columns (GE Healthcare) that resulted in a cutoff at ∼200 bp. Each step of the normalization procedure was carefully monitored to avoid the generation of artifacts and overcycling. The resulting normalized ds-cDNAs were used as a template for NextGen sequencing on a Roche 454 FLX by using standard chemistry at the MPI for Molecular Genetics.

Microarrays and Data Analysis. The sequences used for microarray probe design included *Manduca* antennal and gut ESTs (3) and all publicly available GenBank sequences for this species, and they were jointly assembled by using Seqman NGen. A resulting list of 25,975 contigs in fasta format was uploaded onto eArray (Agilent Technologies). Two 60-mer oligo probes were designed for each predicted OR, and a single 60-mer oligo probe was designed for the remaining contigs by using eArray tools with standard settings. The microarray was designed with a 4× 44K format with a final number of 37,018 noncontrol probe sets, 1,000 replicate controls, and 1,417 Agilent Technologies built-in controls (structural and spike in).

For sex-specific antennal and head capsule microarray hybridizations, RNA of three individuals of one sex was pooled per preparation, and five larvae each were dissected for gut tissue isolation with four biological replicates per sex (antennae) and tissue (gut and head), respectively. Total RNA was double purified, quality tested, and quantified as mentioned above. Agilent Technologies spike-in RNA was added to 500 ng of total RNA and labeled by using a combination of the QuickAmp Amplification kit (Agilent Technologies) and the Kreatech ULS Fluorescent Labeling Kit with cyanine 3-CTP dye following the manufacturer's instructions. Labeled amplified cRNA samples were purified by using Qiagen RNeasy MinElute columns and analyzed on a Nanodrop ND-1000 spectrophotometer by using the microarray function. Amplified cRNA samples were used for microarray hybridization only if the yield was >825 ng and the specific activity was >8.0 pmol of Cy3 per ug of cRNA. 1,600 ng of cyanine 3-labeled cRNA was used for each array, and hybridization was carried out at 65 °C for 17 h. Slides were washed in GE Wash Buffers according to the manufacturer's instructions (Agilent Technologies). Slides were treated in Stabilization and Drying Solution and scanned with the Agilent Microarray Scanner, and data were extracted from TIFF images with Agilent Feature Extraction software (Version 9.1). Raw data output files were analyzed by using the GeneSpring GX11 and GeneSifter microarray analysis software. The data points were normalized between arrays to the median intensity, and log base 2-transformation of the normalized data was performed. Genes were classified as differentially expressed in GeneSifter for $P < 0.05$ (t test) after correcting for multiple testing by using the Benjamini & Hochberg FDR. Global gene expression patterns were examined by using hierarchical clustering in GeneSpring GX11 (Agilent Technologies). Clustering was performed on both probes and condition (tissues and treatments) by using Pearson Centered distance metric and Centroid linkage rule. Gene expression (presence/absence) in tissues and sexes was determined by using the presence call and threshold criteria implemented in GeneSpring GX11.

Neuroanatomical Procedures. Brains were dissected from the head capsule, transferred to ice-cold 4% formaldehyde in PBS (pH 7.2), and incubated for at least 2 h on a shaker. After fixation, brains were dehydrated in an ascending series of ethanol (50%, 70%, 80%, 90%, 95%, and 3× 100%, at 10 min each) and subsequently rehydrated in a descending series to permeabilize tissue and facilitate homogenous dye penetration. Afterward, brains were incubated in 4% Triton X-100 in PBS containing 3% Lucifer yellow (Sigma-Aldrich) for 3 d on a shaker. Stained brains were dehydrated, cleared with methylsalicylate (M-2047; Sigma-Aldrich) overnight, and finally positioned and sealed in custom aluminum slides. Brains were examined by confocal laser-scanning microscopy using a Zeiss LSM 510 (Carl Zeiss) equipped with a HeNe/Ar

lasermodul and a 10×, 0.45-NA objective lens (C-Apochromat, Zeiss). Optical sections (1,024 × 1,024 pixel) were taken at intervals of 1–1.5 μm for detailed scans of the AL.

Image Segmentation and 3D Reconstruction. 3D reconstructions were carried out by using AMIRA 4.1.2 (Mercury Computer Systems). Individual glomeruli were reconstructed by segmentation of each spherical structure around its center in three focal planes (*xy, xz, yz*). Subsequent use of the wrapping tool allowed us to interpolate 3D shapes. Brain outlines of adjacent neuropil areas serving as orientation guidelines were reconstructed by segmentation, followed by regular interpolation. Total number of all glomeruli was assessed by complete AL reconstructions of three males and three females.

1. Zhulidov PA, et al. (2004) Simple cDNA normalization using kamchatka crab duplex specific nuclease. *Nucleic Acids Res* 32:e37.
2. Vogel H, Heidel AJ, Heckel DG, Groot AT (2010) Transcriptome analysis of the sex pheromone gland of the noctuid moth *Heliothis virescens*. *BMC Genomics* 11:29.
3. Pauchet Y, et al. (2010) Pyrosequencing the Manduca sexta larval midgut transcriptome: Messages for digestion, detoxification and defence. *Insect Mol Biol* 19: 61–75.

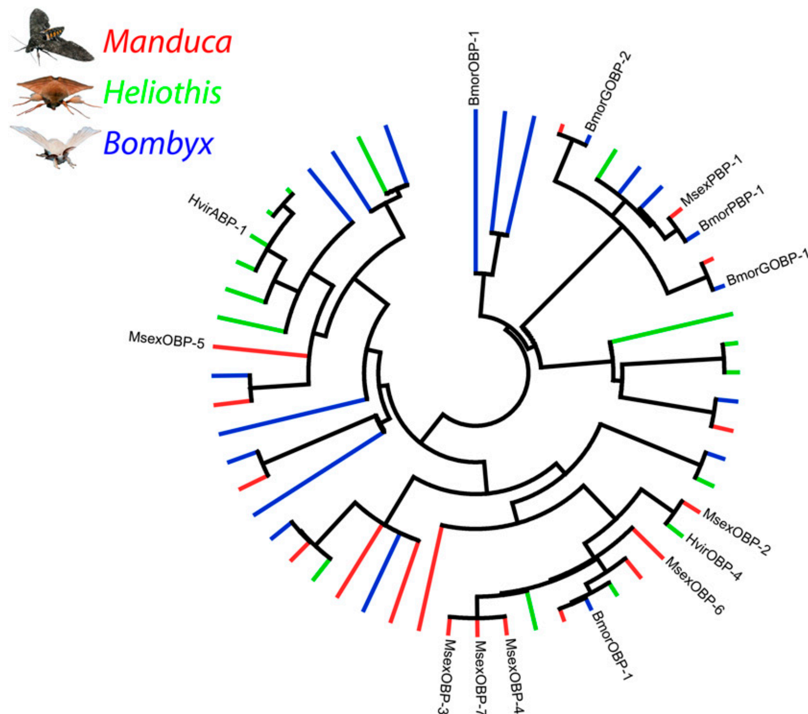

Fig. S1. Lepidopteran OBPs (*Heliothis*, green; *Bombyx*, blue, *Manduca* previously identified, yellow; this study, red; others, black). Linearized dendrogram is based on a maximum-likelihood analysis of a MAFFT alignment of predicted protein sequences. Newly identified OBPs have been indicated by name, as have been single representatives in other species and MsexPBP1 for orientation. The predicted proteins of *Manduca* show high similarity to other lepidopteran OBPs.

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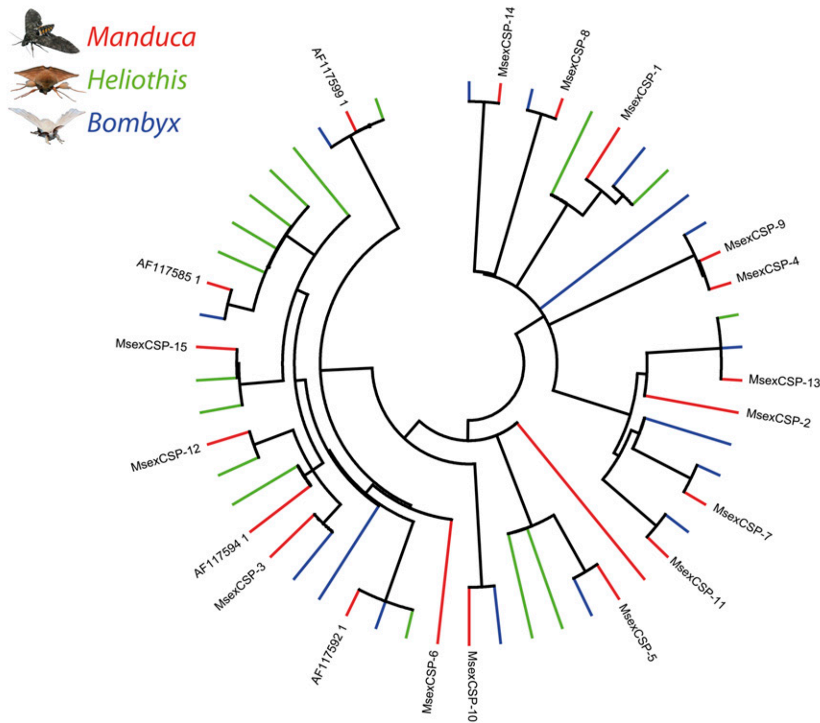

Fig. S2. Lepidopteran CSPs and *Manduca* CSP candidate transcripts, dendrogram calculated, and colored as described in Fig. 1. The number of predicted CSPs *Manduca* (red) is comparable to *Bombyx* (blue) and *Heliothis* (green). *Manduca* CSPs are indicated by name or accession number.

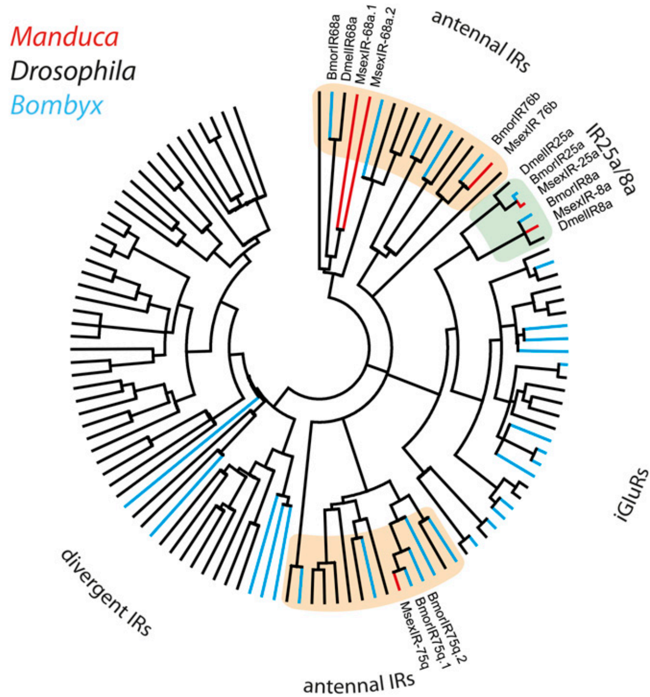

Fig. S3. Similarity of *Manduca* IR candidates (red) to IRs/IGluRs of *Drosophila* (black) and *Bombyx* (blue). Depicted is a maximum-likelihood dendrogram, calculated based on the alignment of predicted amino acid sequences. Colored boxes indicate the antennal IR (orange) and IR25a/8a subgroups (green).

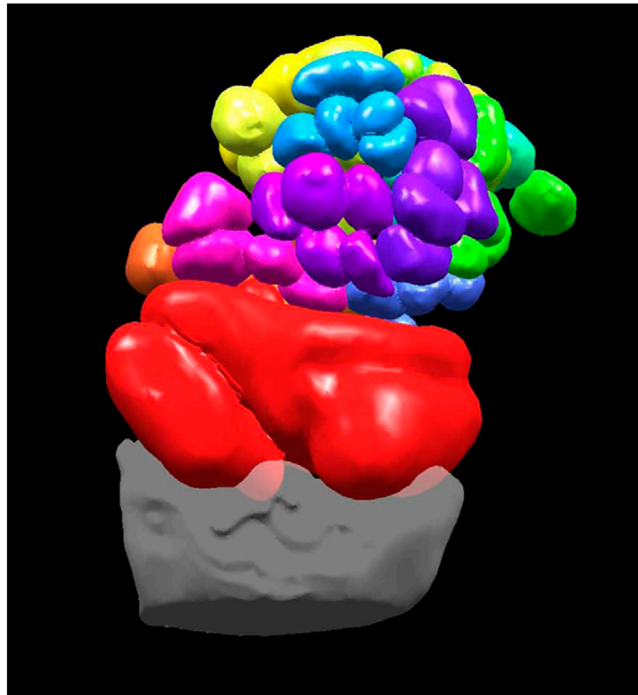

Fig. S4. Reconstruction of the male antennal lobe. The data are presented as 3D data. Glomeruli are presented as schematics, with colors indicating groupings used as basis for nomenclature.

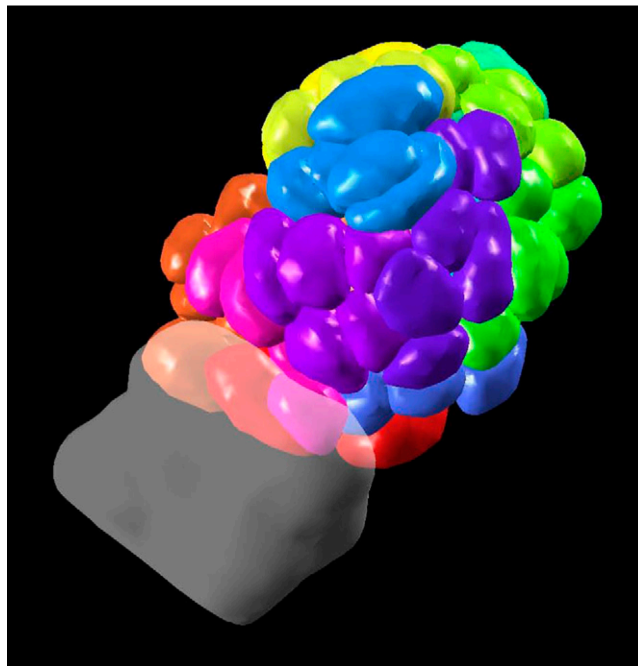

Fig. S5. Reconstruction of the female antennal lobe. The data are presented as 3D data. Glomeruli are presented as schematics, with colors indicating groupings used as basis for nomenclature.

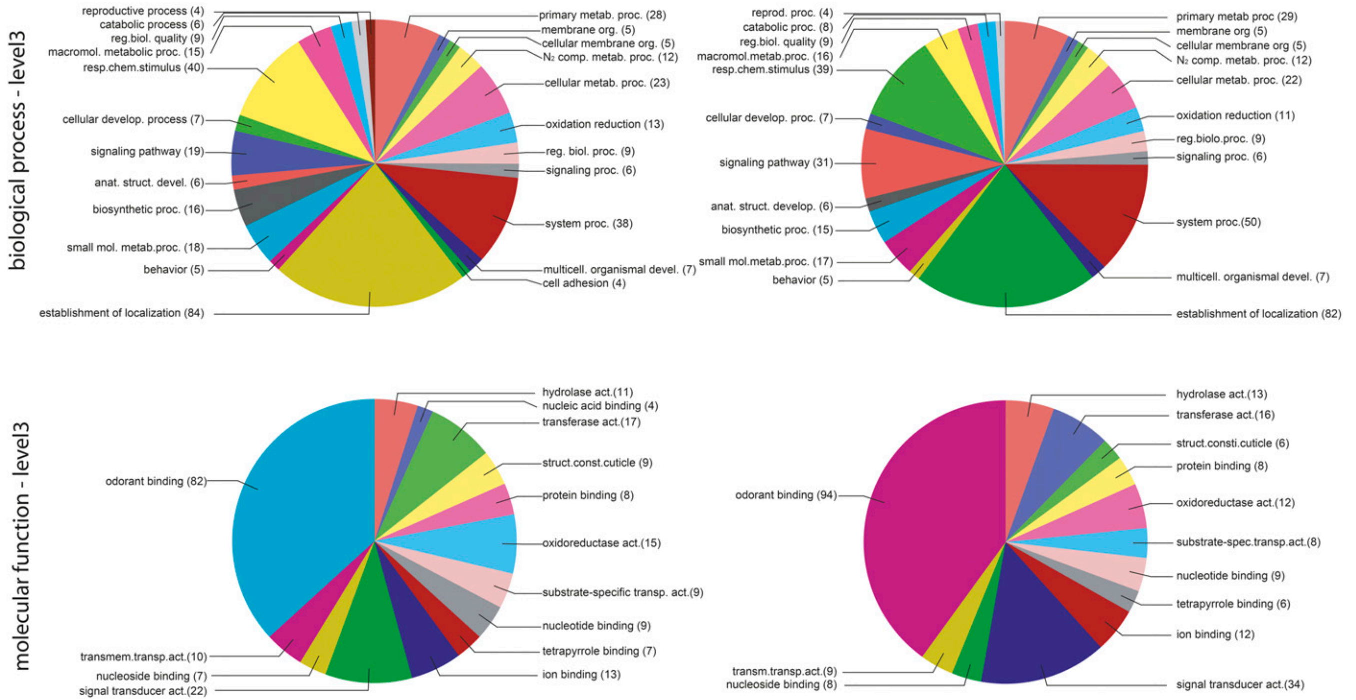

Fig. S6. Comparison of male and female gene representation with the larval midgut, using microarray data and GO categorization for biological processes (*Upper*) and molecular function (*Lower*; both level 3). Depicted are transcripts with presence calls in each respective antennal tissue (male, left; female, right), but no presence calls in the larval midgut. Total number of gene objects are presented in brackets.

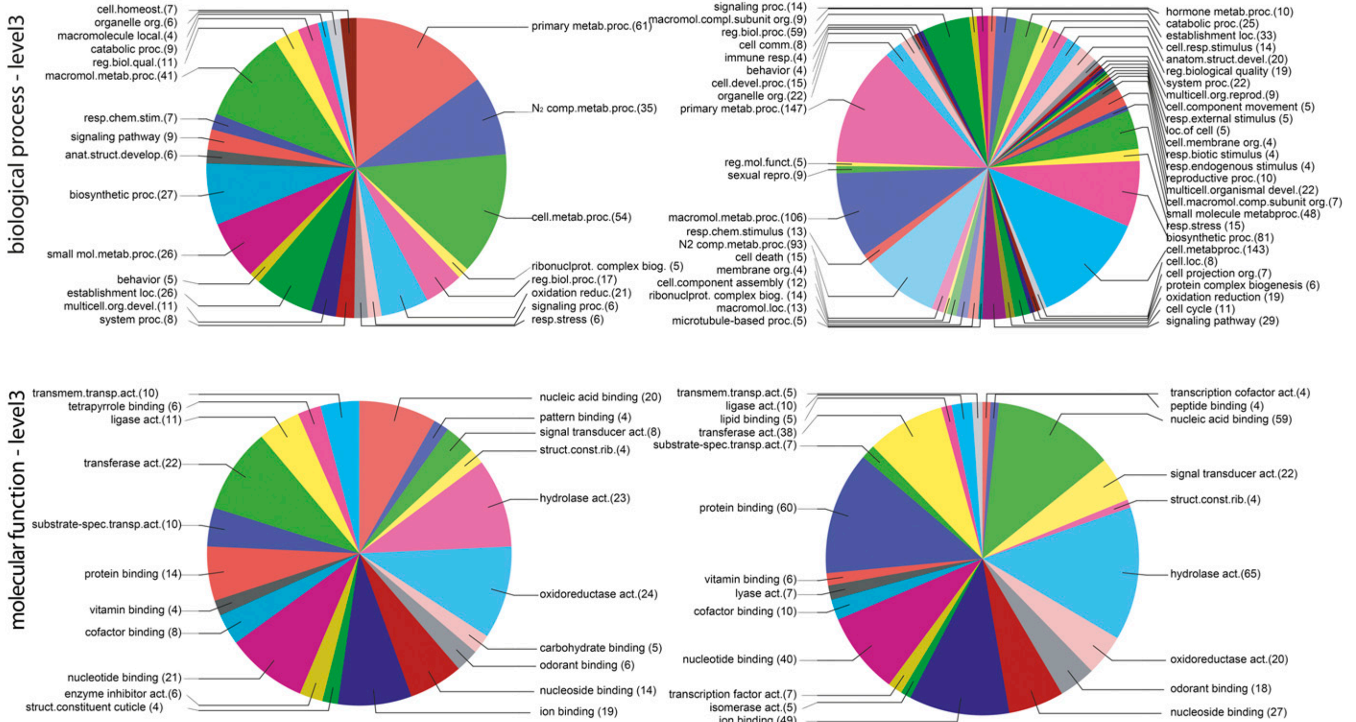

Fig. S7. Comparison of male and female gene expression using GO categorization for biological processes (*Upper*) and molecular function (*Lower*; both level 3) based on microarray presence calls. Total number of gene objects presented in brackets. The total number of categories for biological processes is higher in the female antenna, as is the total number of gene objects.

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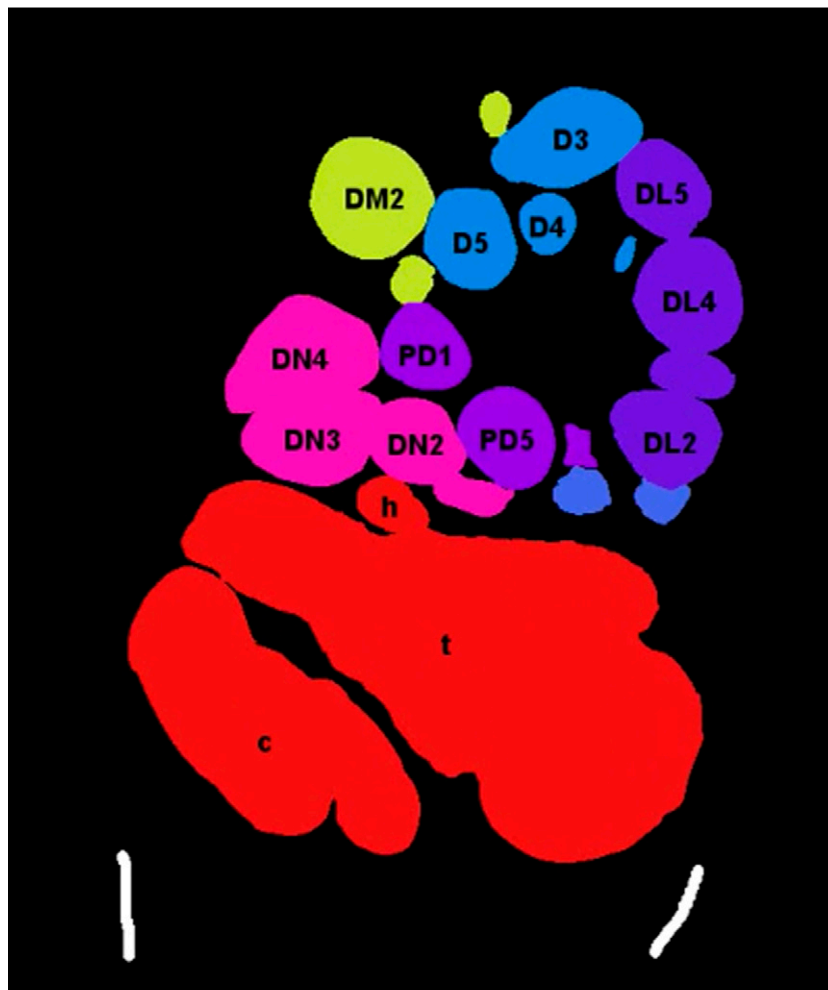
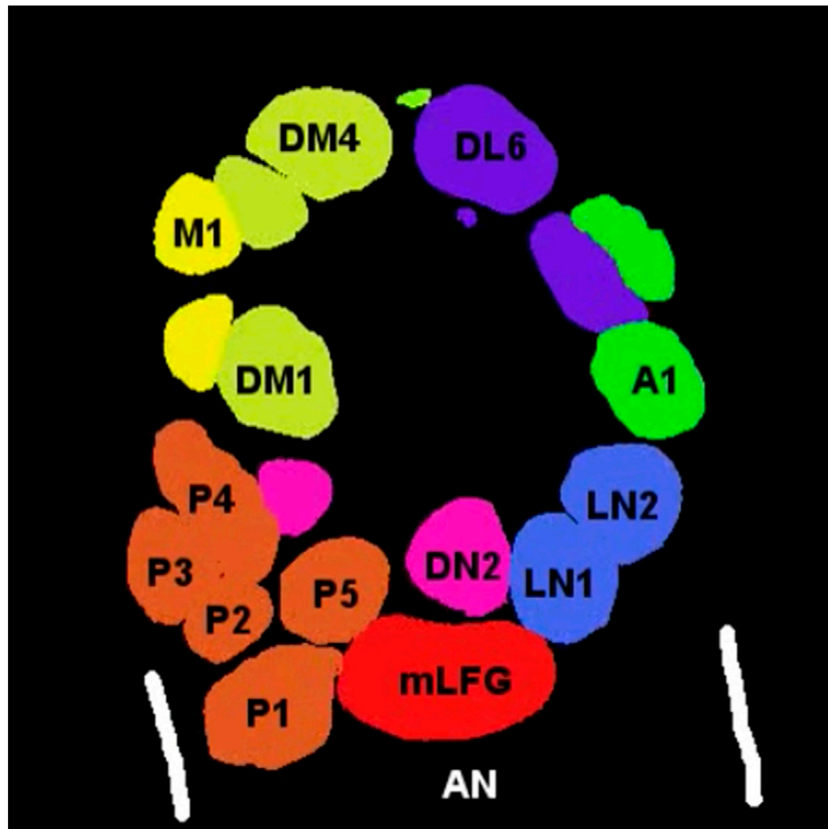


Movie S1. Reconstruction of the male antennal lobe. The data are presented as movie file, following the *z* axis. Glomeruli are presented as schematics, with colors indicating groupings used as basis for nomenclature.

Movie S1



Movie S2. Reconstruction of the female antennal lobe. The data are presented as movie file, following the *z* axis. Glomeruli are presented as schematics, with colors indicating groupings used as basis for nomenclature.

Movie S2

Dataset S1. Olfactory genes identified in this study, FASTA formatted file

Dataset S1

Sequences have been named according to existing nomenclature or closest homolog where possible.

Dataset S2. Files in fasta format containing the sequences used for the generation of Fig. 2

Dataset S2

Dataset S3. **Files in fasta format containing the sequences used for the generation of Fig. S1**

Dataset S3

Dataset S4. **Files in fasta format containing the sequences used for the generation of Fig. S2**

Dataset S4

Dataset S5. **Files in fasta format containing the sequences used for the generation of Fig. S3**

Dataset S5