

Expressed Sequence Tags from Cephalic Chemosensory Organs of the Northern Walnut Husk Fly, Rhagoletis suavis, Including a Putative Canonical Odorant Receptor

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Source: Journal of Insect Science, 10(51): 1-11

Published By: Entomological Society of America

URL: https://doi.org/10.1673/031.010.5101

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Expressed sequence tags from cephalic chemosensory organs of the northern walnut husk fly, *Rhagoletis suavis*, including a putative canonical odorant receptor

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Abstract

Rhagoletis fruit flies are important both as major agricultural pests and as model organisms for the study of adaptation to new host plants and host race formation. Response to fruit odor plays a critical role in such adaptation. To better understand olfaction in *Rhagoletis*, an expressed sequence tag (EST) study was carried out on the antennae and maxillary palps of *Rhagoletis suavis* (Loew) (Diptera: Tephritidae), a common pest of walnuts in eastern United States. After cDNA cloning and sequencing, 544 ESTs were annotated. Of these, 66% had an open reading frame and could be matched to a previously sequenced gene. Based on BLAST sequence homology, 9% (49 of 544 sequences) were nuclear genes potentially involved in olfaction. The most significant finding is a putative odorant receptor (OR), *RSOr1*, that is homologous to *Drosophila melanogaster Or49a* and *Or85f*. This is the first tephritid OR discovered that might recognize a specific odorant. Other olfactory genes recovered included odorant binding proteins, chemosensory proteins, and putative odorant degrading enzymes.

Keywords: host race, *Juglans nigra*, olfaction, odorant receptor, *Rhagoletis*, Tephritidae, speciation Abbreviations: CSP, chemosensory protein; EST, expressed sequence tag; OBP, odorant binding protein; OR, odorant receptor Correspondence: a ramsdell@life.uiuc.edu, b ssobaski@albion.edu, c hughrobe@uiuc.edu, d k-walden@life.uiuc.edu, a jfeder@nd.edu, f kwanner@life.uiuc.edu, g stewartb@life.uiuc.edu Associate Editor: Zhijian Tu was editor of this paper. Received: 9 June 2008, Accepted: 7 September 2008 Copyright : This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed. ISSN: 1536-2442 | Vol. 10, Number 51 Cite this paper as: Ramsdell KMM, Lyons-Sobaski SA, Robertson HM, Walden KKO, Feder JL, Wanner K, Berlocher SH. 2010. Expressed sequence tags from cephalic chemosensory organs of the northern walnut husk fly, *Rhagoletis suavis*, including a putative canonical odorant receptor. 11 pp. *Journal of Insect Science* 10:51 available online: insectsicence.org/10.51

Introduction

Species of the genus Rhagoletis are important pests of fruits such as apples, cherries, tomatoes, walnuts, and blueberries. They are equally important as the focus of the debate about the possibility of sympatric speciation via the formation of host races on new host plants (Bush 1966; Berlocher and Feder 2002). In the case of the apple host race of Rhagoletis pomonella, two key adaptations arose approximately 150 years ago in the ancestral (and still extant) hawthorn race that allowed colonization of apple. One is alteration of the olfactory response so that both sexes are attracted to the odor of the new host apple (Linn et al. 2003, 2004; Dambroski et al. 2005), and the other is shifting life history phenology to match the fruit ripening time of apple (Filchack et al. 2000). This study is the first attempt to catalog genes involved in olfaction in Rhagoletis by carrying out an expressed sequence tag (EST) project on the antennae and maxillary palps of Rhagoletis suavis (Loew) (Diptera: Tephritidae). This species was used because it can be obtained more easily in the large numbers required for an EST project on olfactory organs than *R. pomonella* can.

Many features of the molecular biology of olfaction in *Rhagoletis* can be anticipated from what is known of olfaction in *Drosophila melanogaster*, which is a key model organism for studying olfaction (Rützler and Zwiebel 2005; Hallem et al. 2006; Vosshall and Stocker 2007). Two major gene families involved are the odorant binding proteins (OBPs) (Hekmat-Scafe et al. 2002) and the odorant receptors (ORs) (Robertson et al. 2003). OBPs are usually highly expressed, which makes detection in antennal EST projects likely (e.g. Robertson et al.1999); whereas ORs are generally expressed at such low levels that they are difficult to obtain with this method. Based on the D. melanogaster genome, it was anticipated that the most important recoveries from this EST project would be key olfactory gene products such as ORs and OBPs. However, other classes of genes have been proposed as having a possible role in olfaction. such as chemosensory proteins (Briand et al. 2002; Lartigue et al. 2002) and odorant degrading enzymes, as well as genes that are of general interest.

Materials and Methods

Flies and collection of antennae and palps

Collection of large numbers of Rhagoletis flies is most easily accomplished by rearing larvae from infested fruit (Rhagoletis life history is described by Boller and Prokopy 1975). In the fall of 2000, approximately 50,000 R. suavis (Loew) larvae were reared from black walnut, Juglans nigra L. (Fagales: Juglandaceae), fruit from sites near White Heath, Illinois (Piatt County). Pupae were placed in a 4° C cold room to break diapause and then removed in batches throughout the spring of 2001. Emerging flies were placed in cages with food and water (Prokopy and Bush 1973) until they could be processed. Processing was carried out as rapidly as possible after eclosion because young adults were assumed to have the highest expression of olfactory receptors. Heads from live flies were removed and accumulated at -80° C. The day before RNA extraction, the frozen heads were shaken on a soil sieve to harvest antennae. Maxillary palps and major head bristles that may also have chemoreceptors were harvested incidentally. Maxillary palps have sensilla used in odor recognition and express gustatory receptors, but mRNA would

not have been obtained from major bristles because the cell bodies are not in the bristles. The shaking and sieving was not severe enough to break the heads, so there was no contamination from brain or eye tissues.

RNA extraction and cDNA library

construction

Total RNA was isolated from antennae and maxillary palps using guanidinium а thiocyanate/phenol-chloroform extraction protocol (RNA Isolation Kit, Stratagene, www.stratagene.com). mRNA was purified from total RNA using a Poly(A) Quik® mRNA Isolation Kit (Stratagene, www.stratagene.com), which utilizes an oligodT cellulose column. A unidirectional plasmid cytomegalovirus-polymerase chain reaction cDNA library primed with oligo-dT was constructed by Stratagene using PCR amplification. The plasmid library was transformed into Stratagene's host strain Epicurian Coli[®] XL-10 Gold[™]. For further details of molecular methods and results see Ramsdell (2004).

Clone sampling & DNA sequencing

Plasmid clones were sampled by plating the library onto LB-kanamycin agar and picking colonies. Colonies were individually transferred to 96-well plates. Each well contained 80 µl of a 30% (v/v) glycerol-LB mixture. Six plates were prepared and submitted to the W.M. Keck Center for Comparative and Functional Genomics (University of Illinois at Urbana-Champaign) for sequencing from the 5' end using ABI automation. Clones of interest were cultured and purified, and the insert was sequenced from both directions when necessary.

Sequence analysis

Sequences were edited with Microsoft Excel®

and BBEdit Lite (Bare Bones Software, Inc., www.barebones.com). DNA Strider v1.1 (Marck 1988) was used for protein translations, to find open reading frames, convert reverse complement sequence reads, and generate Kyte-Doolittle hydropathy plots (Kyte and Doolittle1982). BLAST (Altschul et al., 1990, 1997) was used with networked servers (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov) to find the most similar sequence matches to the R. suavis ESTs in the GenBank databases. Significantly similar matches had E values of 10^{-4} . An initial screen using the tblastn option (translated DNA query searching translated DNA database) was followed with both nucleotide and protein BLAST searches (blastn, blastx, blastp) of the largest open reading frames. Searches were generally restricted to Diptera. Sequences of interest were aligned using Clustal X 2.0 (Larkin et al. 2007) using default settings. EST sequences were deposited in the dbEST EST database at the National Center for Biotechnology Information (Accessions EX453814 EX454354).

To show the relationships of the *Drosophila melanogaster* OR sequences that are most similar to the *R. suavis* OR, a neighbor-joining tree of corrected distances was built using Clustal X (Larkin et al. 2007). Bootstrapping was performed with Clustal X with 10,000 pseudoreplications.

Results

Recovery of ESTs

A total of 544 clones was sequenced, with an average length $532.02 \pm SE 9.88$ bp (range 14 to 967 bb). A wide variety of gene transcripts was obtained. As expected from a normalized library, 418 (76.8%) of the sequences were unique. The largest number of duplicates was

18 for a sequence similar to DmCG13095 (a peptidase). Of the 544 total sequences, 186 had no obvious ORF and did not produce a significant BLASTx match with a known protein sequence in GenBank. Of the 358 sequences with an ORF, 86 produced either a weak match with a known gene, or a low Evalue match with a sequence of unknown function. Of the 313 sequences with a significant match to a sequence with a known function, 37 were mitochondrial and 276 were nuclear. As expected, protein BLAST searches yielded much smaller E values than did nucleotide searches. The exceptions involved nucleotide matches with sequences from other tephritid flies (R. pomonella and the medfly *Ceratitis capitata*), which usually resulted in the smallest E values, presumably because 3' UTRs retained some sequence similarity.

A representative set of the nuclear matches is shown in 3 1. Given that mRNA was extracted from antennae and maxillary palps, it is not surprising that 48 (9%) of the sequences had a function or putative function relating to chemoreception. Also, 24 of the sequences had a known or possible role in development. This finding is not surprising because the source flies were young adults that were not fully mature. Also included in Table 1 are a few sequences that have been implicated in diapause and life history; such genes were not the target of this study, but they are noted because diapause is critical to host race formation in *Rhagoletis*. Although they do not appear to play a role in diapause initiation, heat shock loci can be up-regulated during diapause (Rinehart et al. 2007).

Chemosensory proteins

Thirteen sequences were recovered that coded for two different chemosensory proteins (CSPs), RsCSP1 and CSP2. The *R. suavis* CSPs matched only chemosensory proteins in the public databases and were identified as belonging to the conserved domain of the CSP family. Proteins from D. melanogaster Antennal Protein 10 (A10 or OS-D) and Ejaculatory Bulb Protein III (PEBme III), were the best matches for RsCSP1 and RsCSP2, respectively. A10 and RsCSP1 had a pairwise amino acid identity of 66%, and RsCSP2 and PEBme III were 82% identical. The R. suavis CSPs have an amino acid identity of 45.7%; the mature forms are 50.9% identical. RsCSP1 was 155 amino acids in length, including a signal peptide of 21 amino acids, and RsCSP2 had a length of 127 with its 18 amino acid signal.

Odorant binding proteins

Nine OBPs, RsObp1 to RsObp9, were recovered. All had top matches to dipteran OBPs in the public databases. The Kyte-Doolittle hydropathy plots of the nine proteins showed typical OBP profiles with hydrophobic peptide signals (Peng and Leal 2001). Including their peptide signals, the OBPs ranged in length from 124 to 164 amino acids. Overall, the R. suavis OBPs were diverse and showed little conservation of amino acid residues. The mature OBPs had mean pairwise amino acid identities of 19.9%, with a range of 7.4 to 55.9%. Signal peptides were 15 to 26 amino acids in length (Ramsdell 2004).

Odorant receptor protein

The *R. suavis* OR sequence (EX453813, 634 bp) was identified as an OR because a protein BLAST search of a 450 bp/150 amino acid ORF significantly (2E-04) matched *DmOr49a*. Resequencing of the clone from both ends revealed an unambiguous match with two *D. melanogaster* OR sequences. These were *DmOr49a* (4E-56, amino acid

Bread category Specific type Symbol dbET Accession BP Munch constant Species Locus Chamocarplion receptors R/Oh2 SX43311 / EUXM98 120° GG1313 D. meknogener PhPP1 Chamocarplion receptors R/Oh2 SX43322 - EX43313 73 GG1421 D. meknogener PhP14 Chamocarplion R/Oh2 SX43322 - EX43313 73 GG1421 D. meknogener PhP14 R/Oh2 SX43322 - EX43313 631 GG1431 D. meknogener PhP14 R/OH2 R/Gh2 EX43332 - EX43313 631 GG1431 D. meknogener PhP15 R/GH2 EX43332 - EX43313 631 GG1431 D. meknogener PhP15 R/GH2 EX43332 - EX43313 631 GG1431 D. meknogener PhP15 R/GH2 EX43332 - EX43313 631 GG1431 D. meknogener Ph16 R/GH2 EX43342 EX43342 EX43343 EX4344 EX4344 EX4444 R/GH2 <t< th=""><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></t<>										
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Image: Normalized state Set CG22L0 D. melanogaster awd EX453908 678 CG3260 D. melanogaster Zfrp8 EX453913 596 CG4254 D. melanogaster Zfrp8 EX453914 555 CG7762 D. melanogaster Kpn1 EX453915 611 CG8440 D. melanogaster Lis-1 EX453916 588 CG8567 D. melanogaster Lis-1 EX453916 588 CG8567 D. melanogaster Lis-1 EX453915 511 CG8567 D. melanogaster Lis-1 EX453924 341 CG9635 D. melanogaster Lis-1 Data EX453925 764 CG3644 D. melanogaster bic Inlozyme EX454031 FX45 764 CG3140 D. melanogaster bic Inlozyme EX454045 763 CG11793 D. melanogaster bic Inlozyme EX454046 763 CG3140 D. melanogaster bic <t< td=""><td></td><td></td><td></td><td>EX453905</td><td>541</td><td>CG1780</td><td>D. melanogaster</td><td>ldgf4</td><td>Imaginal disc growth factor 4.</td><td>2.00E-65</td></t<>				EX453905	541	CG1780	D. melanogaster	ldgf4	Imaginal disc growth factor 4.	2.00E-65
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Image: Normal background backgro				EX453915	119	CG8440	D. melanogaster	Lis-I	Lissencephaly-I. Anatomical development.	7.00E-07 E-127
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allozyme EX454045 763 CG11793 D. melanogaster Sod allozyme EX454047 772 CG3140 D. melanogaster Adk2 EX454048 563 CG312031 D. melanogaster Adk2 EX454049 780 CG6058 D. melanogaster Adk2 EX454050 604 CG6058 D. melanogaster Ald genomic sequence EX454051 397 AY930988 R. ponnonella P307 AY930989 R. ponnonella P3072 P3072 P3072	Pheromone production			EX454031 - EX454032	776	CG9747	D. melanogaster	CG9747	Acyl-CoA delta I I-desaturase. Fatty acid biosvnthesis. Also similar to desat2 (CG5925).	2.00E-37
EX454047 772 CG3140 D. melanogaster Adk2 EX454048 563 CG32031 D. melanogaster Argk EX454049 780 CG6058 D. melanogaster Argk EX454049 780 CG6058 D. melanogaster Ald EX454050 604 CG9042 D. melanogaster Ald EX454051 397 Ary930988 R. pomonella P3072	Marker loci	allozyme		EX454045	763	CG11793	D. melanogaster	Sod	Superoxide dismutase.	6.00E-83
EX454048 563 CG32031 D. melanogaster Argk EX454049 780 CG6058 D. melanogaster Ald EX454050 604 CG9042 D. melanogaster Ald EX454051 397 Ary930988 R. pomonella P3072 EX454051 357 Ary930988 R. pomonella P3072				EX454047	772	CG3140	D. melanogaster	Adk2	Adenylate kinase-2.	E-128
EX454047 700 CG0035 D. metamogaster Add EX454050 604 CG9042 D. melanogaster G-3-pdh EX454051 397 AY93098 R. pomonella P3072 CY45A051 350 AX930098 R. pomonella P3072				EX454048	563	CG32031	D. melanogaster	Argk	Arginine kinase.	2.00E-73
EX454051 397 AY930988 R. pomonella P3072 EVAFA053 350 AX031003 B. d. accorded D3072				EX454050	604	CG9042	D. melanogaster	G-3-pdh	Algolase. Glycerol-3-P dehydrogenase.	E-148 3.00E-77
		genomic sequence		EX454051	397	AY930988	R. pomonella	P3072	Rhagoletis EST locus.	7E-55‡
359 AY 91003 K. electromorpha P30/2				EX454052	359	AY931003	R. electromorpha	P3072	Rhagoletis EST locus.	IE-52‡

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identity = 31%) and *DmOr85f* (1E-37, amino acid identity = 26%). The alignment of these three sequences is shown in Figure 1. Based on the alignment, it is likely that a few amino acids were missing at the N-terminus of the R. suavis sequence. To increase the likelihood that the nearest known homolog of the R. suavis receptor was found, the nine Drosophila OR sequences were included in the neighbor-joining tree analysis, ranked in order of decreasing E value, between DmOr49a and an Anopheles gambiae receptor (AGAP001912, 8E-28). The resulting neighbor-joining tree (Figure 2, shows only the relevant part of tree, including Or85f from

Drosophila pseuodoobscura supports the conclusion that the *D. melanogaster* homolog of the R. suavis odorant receptor sequence, henceforth RsOr1, was DmOr49a. The RsOr1 sequence clearly showed the characteristic hydropathy plot of a 7-transmembrane protein, with alternating hydrophobic and hydrophilic regions (Figure 3).

Discussion

Chemosensory Proteins

The function of CSPs is not clear at this point. They are highly expressed in insect antennae, and some work supports a role as olfactory

DM49a	-MEKLRSYEDFIFMANMMFKTLGYDLFHTPKPWWRYLLVRGYFVLCTISNFYEASMVTTR	59
RHAG	QVFWGPNALFRAVGYDFQRLPRPYWRQILMKAVLIFMILSAICIRIYMFMS	51
DM85f	MEPVQYSYEDFARLPTTVFWIMGYDMLGVPKTRSRRILYWIYRFLCLASHGVCVGVMVFR	60
	: :* :***: *:. * :* .: * :	
DM49a	IIEWESLAGSPSKIMRQGLHFFYMLSSQLKFITFMINRKRLLQLSHRLKELYPHKEQNQR	119
RHAG	LRELIIRDDILN-SFRLGAFIAYGVDSNVKFAYFIFKAHRLRKIYDFLAAEYPQTSSEQK	110
DM85f	MVEAKTID-NVSLIMRYATLVTYIINSDTKFATVLQRS-AIQSLNSKLAELYPKTTLDRI	118
	: * * * * * **:. ::	
DM49a	KYEVNKYYLSCSTRNVLYVYYFVMVVMALEPLVQSCIMYLIGFGKADFTYKRIFP	174
RHAG	LYKIDIYGFQRAP-VMICAYMAVVASIMLSPLLQSIVTYIIDIYRFGYDAAEYPYLHPIP	169
DM85f	YHRVNDHYWTKSFVYLVIIYIGSSIMVVIGPIITSIIAYFTHNVFTYMHCYP	170
	···· · · · · · · · · · · · · · · · · ·	
DM49a	-TRLTFDSEKPLGYVLAYVIDFTYSOFIVNVSLGTDLWMMCVSSOISMHLGYLANMLASI	233
RHAG	- MPYNFDYYTPRYYIPVYMVESLNGHFSSTTNLGTDLFISIFSGQLCMQLEYLGYSLETY	228
DM85f	YFLYDPEKDPVWIYISIYALEWLHSTQMVISNIGADIWLLYFQVQINLHFRGIIRSLADH	230
	: *: * :::*:*:: *: ::: : *	
DM49a	RPSPETEQQDCDFLASIIKRHQLMIRLQKDVNYVFGLLLASNLFTTSCLLCCMAYYTVVE	293
RHAG	EPSMEKSEDDCEFLRKWIRKHQLMLGLCADLDEVFGTTLLCKLITNCTYFCIIVAQLMLE	288
DM85f	KPSVKHDQEDRKFIAKIVDKQVHLVSLQNDLNGIFGKSLLLSLLTTAAVICTVAVYTLIQ	290
	.** : .::* .*: . : : * *:: :** * .*:*. :* :. :::	
DM49a	GFNWEGISYMMLFASVAAQFYVVSSHGQMLIDLSTNLAKAAFESKWYEGSLRYKKEILIL	353
RHAG	GYGYGFLNFGSFFFLTVAQFFMVCQYGQNLITISEHLSFSAYKNRWYNGSKAYKKMILTI	348
DM85f	GPTLEGFTYVIFIGTSVMQVYLVCYYGQQVLDLSGEVAHAVYNHDFHDASIAYKRYLLII	350
	* *** *** .:* :	
DM49a	MAQAQRPLEISARGVIIISLDTFKILMTITYRFFAVIRQTVEK 396	
RHAG	ITRAQTPANLTAKGFQPISLLTFQIVMSVTYRVFAVLQQVFD- 390	
DM85f	IIRAQQPVELNAMGYLSISLDTFKQLMSVSYRVITMLMQMIQ- 392	
DHOJI		

substitutions. High quality figures are available online.

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ligand transporters (Briand et al. 2002; Lartigue et al. 2002). Recent work in *Bombyx mori*, however, indicates that they are commonly expressed in many parts of the body in addition to antennae (Conga et al. 2007). The fact that two different CSPs were recovered in this small study of 544 ESTs indicates that, consistent with other work, CSPs are highly expressed in antennae, but their possible role in *Rhagoletis* olfaction remains uncertain.

Odorant binding proteins

Drosophila melanogaster has 51 OBPs (Hallem et al. 2006, Hallem and Carlson 2006). Thus the recovery of nine different *R. suavis* OBP sequences, all with *D. melanogaster* orthologues, (Table 1) from only 544 ESTs suggests that most, if not all, of the *R. suavis* OBPs could be recovered by a modestly more extensive EST study. The exact role that OBPs could play in host

specificity remains unknown; however, it is quite likely that they play a significant part. Recent work on *Drosophila* pheromone reception demonstrates both that OBPs are necessary for chemoreception and that some are highly specific for particular odorants (Xu et al. 2005).

The *R. suavis* OBPs have a mean pairwise amino acid identity of about 20%, which is typical for phylogenetically distant members of the OBP gene family (Robertson et al. 1999). Their diversity, coupled with their apparent homology to *D. melanogaster* OBPs, make them good candidates for use as genetic tools in studies of acalypteran and other dipteran lineages.

The odorant receptor sequence

Odorant receptors are believed to play a critical role in the host finding behavior in insects, yet they are difficult to obtain without



Figure 2. Neighbor-joining tree of sequences similar to the *Rhagoletis suavis* odorant receptor (*Drosophila pseuodooscura* Or85f, and *D. melanogaster* Or85f and Or49a). Distances are percent dissimilarities corrected for multiple replacements. Values under the distances are bootstrap values. High quality figures are available online.



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completely sequenced genomes. Only a few odorant receptor sequences have been discovered in insect EST projects (and none of these are published), suggesting a low rate of expression. Indeed Vosshall et al. (1999) noted that *D. melanogaster* ORs were present in fewer than 1 in 500,000 clones in an antennal library. This is lower than this rate of 1 OR in 544 clones, but it is likely that a *Rhagoletis* genome will be necessary to obtain a complete set of OR genes.

RsOr1 is significant as the first reported putative ligand-binding receptor from a tephritid fly. It is not the first tephritid receptor; that distinction belongs to a receptor recovered from C. capitata by Larrson et al. (2004). However, the C. capitata OR was homologous to the atypical, "non-canonical" Or83b, which plays a role in localizing conventional or "canonical" receptors to the membrane and is highly conserved across insects (Jones et al. 2005). But Or83b does not bind odorant ligands, and, thus, its homologs are unlikely to play a direct part in host plant adaptation. RsOr1, on the other hand, was clearly homologous to the canonical DmOr49a. Unfortunately, it is not possible, at this point, to speculate on the volatile, or volatiles, which elicits a response from *RsOr1*, as the ligands of *DmOr49a* have not yet been determined (Hallem and Carlson 2006).

However, it is probable that the OR sequences, or their expression patterns, or both, differ substantially between *R. suavis* and the apple maggot *R. pomonella*. The fruit volatiles of apples are characterized by high concentrations of esters (Linn et al. 2003; Souleyre et al. 2005), while those of the ancestral host of *R. pomonella*, hawthorns, are characterized by ethyl acetate, long-chain alcohols, and various aldehydes (Linn et al.

2003). But a completely different spectrum of volatiles. dominated bv terpenes and walnut terpenoids, occurs in fruits (Hennemanm et al. 2002). Moreover, many of the walnut terpenoids, such as β -pinene, limonene, β -caryophylene, and α -humulene (Hennemanm et al. 2002) did not elicit any responses from the (incomplete) set of ORs tested by Hallem and Carlson (2006). Thus R. suavis may provide insights into insect olfaction that are not possible with Drosophila.

R. suavis may be a good species with which to study the various roles of odorant degrading enzymes in olfaction. As pointed out by Rützler and Zwiebel (2005),odorant degrading enzymes are necessary to remove the signaling molecule after a cell response been initiated and also has because chemosensory systems must be open to the environment. Odorant degrading enzymes may have a secondary role of degrading toxic odorants before they can cause cellular damage. One of the major components of walnut fruit odor is limonene, which is used as an insecticide, and also causes "spontaneous stimulation of sensory nerves" (Weinzierl 1998, p. 106; mechanism not known). Detoxification of limonene in the cutworm Spodoptera is reported to be similar to mammalian detoxification (Miyazawa et al. 1998), where oxidative degradation by cytochrome P450s appears to be the most important pathway (e.g., Miyazawa et al. 2002). No cyt P450 sequences were recovered in this study, but they represent one of several pathways that should be studied in olfaction in phytophagous insects.

While tremendous strides have been made in understanding the molecular biology of chemosensation in recent years (Rützler M and, Zwiebel LJ. 2005, Hallem et al. 2006,

Vosshall and Stocker 2007), we are still very far from being able to understand the relative importance for host adaptation of peripheral vs. central processes, sequence vs. expression differences, or even the relative importance of the different classes of genes involved. Koop et al. (2008) have recently demonstrated that expression differences for both Ors ORs and OBPs have been involved in the adaptation of Drosophila sechellia to its food plant Morinda citrifolia. But more classes of molecules will need to be included in future such studies. ODEs and CSPs will certainly need to be added. But even genes that seemingly have little to do with olfaction may be important. For example, Hsp70 genes could affect receptor function in chemosensory cells because of their role of in guiding the folding of proteins (Bukau et al. 2006).

Acknowledgments

We thank Steve Ramsdell for the many hours he spent collecting and processing black walnuts. Without his assistance, obtaining the 50,000 flies necessary for this study would not have been possible. We thank an anonymous reviewer for the suggestion that Hsp70 genes could play a role in olfaction. NSF DEB-99-77011, NSF DEB 06-14528, and AG 2007-35604-17886 provided support.

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