Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 2006;68:75–89
DOI: 10.1159/000093375

Received: July 11, 2005
Returned for revision: September 1, 2005
Accepted after revision: January 17, 2006
Published online: May 16, 2006

Inheritance of Olfactory Preferences II. Olfactory Receptor Neuron Responses from *Heliothis subflexa* × *Heliothis virescens* Hybrid Male Moths

T.C. Baker[a] C. Quero[b] S.A. Ochieng'[c] N.J. Vickers[d]

[a]Department of Entomology, Chemical Ecology Lab, Penn State University, University Park, Pa., USA; [b]Department of Biological Organic Chemistry, IIQAB (CSIC), Barcelona, Spain; [c]Department of Entomology, Kansas State University, Manhattan, Kans., and [d]Department of Biology, University of Utah, Salt Lake City, Utah, USA

Key Words

Heliothis subflexa · *Heliothis virescens* · Hybrid males · Single cell recordings · Sex pheromone · Sex pheromone receptors · Sex pheromone receptor co-expression

Abstract

Single-cell electrophysiological recordings were obtained from olfactory receptor neurons (ORNs) in sensilla trichodea on male antennae of hybrids formed mainly by crossing female *Heliothis subflexa* with male *Heliothis virescens* ('SV hybrids'). We recorded from the A-, B-, and C-type sensilla trichodea, with the latter two types housing ORNs exhibiting response profiles to different pheromone components that we had previously found to be characteristic for each species. For both the B- and the C-type SV hybrid sensilla, most of the ORNs exhibited a spike amplitude and ORN co-compartmentalization within sensilla that more strongly resembled the ORNs of parental *H. subflexa* rather than those of *H. virescens*. The overall mean dose-response profiles of the ORNs in hybrid C- and B-type sensilla were intermediate between those of the *H. virescens* and *H. subflexa* parental type ORNs. However, not all hybrid ORNs were intermediate in their tuning spectra, but rather ranged from those that closely resembled *H. subflexa* or *H. virescens* parental types to those that were intermediate, even on the same antenna. The most noteworthy shift in ORN responsiveness in hybrid males was an overall increase in sensitivity to Z9–14:Ald exhibited by Z9–16:Ald-responsive ORNs. Heightened cross-responsiveness to Z9–14:Ald by hybrid ORNs correlates well with observed behavioral cross-responsiveness of hybrids in which Z9–14:Ald could substitute for Z9–16:Ald in the pheromone blend, a behavior not observed in parental types. The hybrid ORN shifts involving greater sensitivity to Z9–14:Ald also correlate well with studies of hybrid male antennal lobe interneurons that exhibited a shift toward greater cross-responsiveness to Z9–14:Ald and Z9–16:Ald. We propose that the differences between parental *H. virescens*, *H. subflexa*, and SV hybrid male pheromone ORN responsiveness to Z9–16:Ald and Z9–14:Ald are most logically explained by an increased or decreased co-expression of two different odorant receptors for each of these compounds on the same ORN.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/bbe

T.C. Baker
Department of Entomology, Chemical Ecology Lab
Penn State University
University Park, PA 16802 (USA)
Tel +1 814 862 4435, Fax +1 814 863 4439, E-Mail tcb10@psu.edu

Introduction

In studies of moth sex pheromone olfaction, understanding the amount of malleability that olfactory receptor neurons (ORNs) possess from generation to generation is essential to understanding how evolutionary shifts might occur in pheromone blend composition. Male antennal ORN response spectra that can accommodate novel female-emitted pheromone components might facilitate shifts in pheromone blend composition that are due to saltational changes resulting from the sudden expression of previously unexpressed biosynthesis-related pseudogenes [Roelofs et al., 2002; Baker, 2002].

Studies of hybrid male moths, such as *Ostrinia nubilalis* [Roelofs et al., 1987] have been instructive in understanding how the distribution and tuning profiles of ORNs within sensilla can shift and correspond with behavioral phenotypes. The genes controlling ORN spike amplitude were shown not to be closely linked to those controlling behavioral preference for a particular odor mixture [Roelofs et al., 1987; Hansson et al., 1987; Löfstedt et al., 1989; Glover et al., 1989]. Furthermore, in a study of *O. nubilalis* hybrid biotypes Cossé et al. [1995] showed that a significant proportion of males of a particular parental behavioral phenotype possessed antennal ORNs of the opposite parental type.

Much work has now been performed on the sex pheromone olfactory systems of two North American heliothine moth species, *Heliothis virescens* and *Heliothis subflexa*. Matings between *H. subflexa* and *H. virescens* produce viable hybrids [Laster, 1972; Proshold et al., 1983], which provides the material for potentially enlightening neuroethological studies on the evolution of heliothine moth olfaction. The *H. subflexa* pheromone blend is comprised of (*Z*)-11-hexadecenal (Z11–16:Ald) as the major pheromone component [Teal et al., 1981; Klun et al., 1982; Heath et al., 1990; Teal and Tumlinson, 1997], (*Z*)-11-hexadecenol (Z11–16:OH) [Heath et al., 1990; Vickers, 2002], and (*Z*)-9-hexadecenal (Z9–16:Ald) [Vickers, 2002]. The *H. virescens* pheromone blend is comprised of Z11–16:Ald as the major component, with small amounts of (*Z*)-9-tetradecenal (Z9–14:Ald) [Roelofs et al., 1974; Tumlinson et al., 1975; Klun et al., 1979, 1980; Sparks et al., 1979; Vetter and Baker, 1983; Ramaswamy et al., 1985; Teal et al., 1986].

In a previous paper [Baker et al., 2004], we reported the response profile properties of ORNs of *H. subflexa* and *H. virescens* and their organization within antennal sensilla. We found that some of these properties provided definitive characters for identifying sensilla as either the *H. subflexa* type or the *H. virescens* type. Hybrid male olfactory systems could possibly differ from parental type males in ways other than having ORN spike frequency profiles or ORN spike amplitudes that are intermediate between the two parental types [Roelofs et al., 1987; Hansson et al., 1987]. Conceivably this could range from an extreme case of a hybrid male having a mosaic of the two distinctive parental species' sensillar types scattered about on both antennae to a situation in which every ORN spike-size phenotype as well as spike frequency tuning profile within every sensillum is a perfect intermediate to either parental type, such as the case for *Ostrinia nubilalis* hybrids [Hansson et al., 1987; Roelofs et al., 1987]. Knowing how to identify antennal sensilla and the response profiles of ORNs housed within them would provide us with the fine-grained resolution to allow us to determine which of these possibilities is occurring in the inheritance of genes controlling sex pheromone peripheral olfactory pathways. Here we report results of our examination of the trichoid sensilla on the antennae of *H. subflexa* female × *H. virescens* male hybrids, as well as those of males from the reciprocal cross.

Materials and Methods

Insects

Colonies of *H. subflexa* and *H. virescens* were reared on a 14:10 L:D photoperiod at 25°C, 40–50% RH at the University of Utah [Vickers, 2002]. Hybrid colonies were created by mixing approximately equal numbers of *H. subflexa* and *H. virescens*. Pupae of hybrid males resulting from matings between *H. subflexa* females and *H. virescens* males ('SV hybrids'), as well as the reciprocal cross (*H. virescens* females with *H. subflexa* males: 'VS hybrids') were shipped overnight from Utah to the Baker laboratory for electrophysiological investigations on the adult male moths.

Recordings from Single Sensilla Trichodea

We used the cut-sensillum technique [Kaissling, 1974; Van der Pers and Den Otter, 1978], to record from the ORNs within an individual antennal sensillum. The male moth was placed inside a disposable pipette tip with the narrow end cut to allow the head to pass through. The head was immobilized with dental wax, and an Ag/AgCl wire was inserted into the abdomen to serve as a ground connection. The preparation was secured with an alligator clip, making contact with the Ag/AgCl wire, and mounted on a Syntech INR-2 Portable Recording Unit (Syntech, Hilversum, The Netherlands). The antenna was maneuvered with a micromanipulator until a single sensillum trichodeum rested on the sharpened blade of a stationary, vertically positioned tungsten knife, with the sensillum tip hanging over the edge. The tip was cut off using a horizontally oriented maneuverable tungsten knife placed in a second micromanipulator. The cut end was contacted immediately with a saline-filled glass micropipette containing an Ag/AgCl recording electrode.

The AC signal from the recording electrode was connected to the built-in amplifier of the portable recording unit and the AC output fed into a computer. The neural activity (action potentials) was monitored by a loudspeaker and displayed on the computer. We processed the data with a PC-based signal processing software, Syntech AutoSpike version 4.0 (Syntech).

A stream of purified and humidified air continuously blew over the antenna (10 ml/s) through a 14-cm-long glass tube (8 mm ID) whose outlet was positioned 2 cm from the antenna. With a stimulus flow-controller device (SFC-2, Syntech), a 30-ms air pulse at a 15-ml/s flow rate was injected through the odor cartridge and into the air stream flushing the antenna.

Action potential frequency (spikes/s) was calculated by counting the number of spikes occurring during the first 200 ms of the spike train initiated by a stimulus puff. For either non-responding or poorly responding ORNs, spikes were counted during the same 200-ms post-stimulus interval as used for counting spikes of responding ORNs. Action potentials from ORNs were declared to be 'larger' or 'smaller' than the other ORN housed in the same sensillum after measuring and taking a mean of the largest 10 spikes in the spike trains that occurred in response to particular compounds.

Odor Cartridges

Serial dilutions of Z11–16:Ald, Z9–16:Ald, Z11–16:OH, Z11–16:Ac, and Z9–14:Ald were made in HPLC-grade hexane using neat material stored in our laboratory. The purity of the compounds was >98% as determined by capillary gas chromatography-mass spectrometry (GC-MS), and the compounds were free of cross-contamination from the other pheromone components. Serial dilutions of the compounds were made in redistilled HPLC-grade hexane, and the solutions were stored in 4-ml glass vials at –20°C.

For each of the compounds, 10 µl of a diluted solution was pipetted onto a 0.5-F 2.0-cm filter-paper strip held in a Pasteur pipette (15 cm long), hereafter referred to as the odor cartridge. Stimulus doses (loadings on filter paper) tested were 3, 10, 30, 100, and 300 µg, respectively. Solutions were checked by GC-MS to confirm that the amounts of respective compounds at a particular concentration were equal. Antennae were screened for different types of sensilla according to the response profiles of the ORNs housed inside by using the 10-µg cartridges and 30-ms puffs of air pulsed through the cartridge, into the air stream, and over the antenna as described above.

For dose-response profiles, stimulus compounds were selected in random order, beginning with the 3-µg odor cartridges and progressing through the increased dosages up to the 300-µg cartridges. The time period allowed to elapse between every puff was set at 30 s. At the end of each dose-response test, the antenna was stimulated with an additional 100-µg dose of either Z11–16:Ald, Z11–16:Ac, or Z9–14:Ald to verify the initial ORN activities. Response data obtained from ORNs that failed to respond a second time to test stimuli were not included in the final dose-response analyses.

It was essential to construct dose-response profiles for individual hybrid males, due to the many possibilities for how hybrid ORNs could be organized within sensilla, and how their response spectra might differ from the two parental types. These response profiles usually were not replicated due to the working life-time of each male antennal preparation and the long dose-series employed.

Cross-Adaptation Studies

Two differently tuned ORNs co-compartmentalized within the same sensillum can exhibit nondiscriminable impulse amplitudes, as in European corn borer hybrids [Hansson et al., 1987]. Therefore, in order to determine more definitively how many ORNs might be contributing to some of the spike trains that were being evoked by two different compounds, we used a cross-adaptation technique employed in our previous work on *H. subflexa, H. virescens* [Baker et al., 2004] and *H. zea* [Cossé et al., 1998]. Using the stimulus flow controller, a single 50-ms puff was generated followed by an inter-stimulus interval of either 0.3 or 1 s, and then a second 50-ms stimulus from a second cartridge was generated. Depending on the type of sensillum and ORNs, the stimulus regime consisted of: (1) one of the two pheromone compounds emitted from cartridge 1 followed by the same compound from cartridge 2 (self-adaptation using compound 1); (2) the first pheromone compound emitted from cartridge 1, then followed by the second pheromone compound emitted from cartridge 2; (3) same as 2, but with the order of presentation reversed; (4) compound 2 emitted from cartridge 1 followed by this same compound emitted from cartridge 2 (self-adaptation using compound 2).

Results

We recorded from 179 sensilla on 38 SV hybrid antennae, with 155 classified as A-type sensilla, 9 as B-type, and 19 as C-type. Dose-response curves were constructed for ORNs within individual sensilla, using actual emitted amounts that had been analyzed from puffs issuing from these odor cartridges at the previously specified filter paper loadings [Cossé et al., 1998]. Most of our recordings were performed on the 'SV' hybrid (*H. subflexa* females × *H. virescens* males) because crosses in this direction made at the University of Utah were more successful. We did receive some of the 'VS' hybrid (*H. virescens* females × *H. subflexa* males) crosses for comparison, but only 10 sensilla were recorded from these VS hybrid males. These were all B- or C-type sensilla, with A-type ignored.

A-Type Sensilla

Approximately 60% of the 155 SV hybrid A-type sensilla contained a single odorant-responsive ORN that only responded to the major pheromone component, Z11–16:Ald (fig. 1A), typical of ORNs in the A-type sensilla of both parental species (fig. 9) [Baker et al., 2004]. However, in approximately 40% of these SV hybrid sensilla, Z11–16:OH at low emission rates also elicited significant firing from what appeared to be the same ORN, based on a spike amplitude that was similar to that of the ORN during Z11–16:Ald stimulation (8.71 mV ± 1.94 SD for Z11–16:Ald-generated spikes; 9.30 mV ±

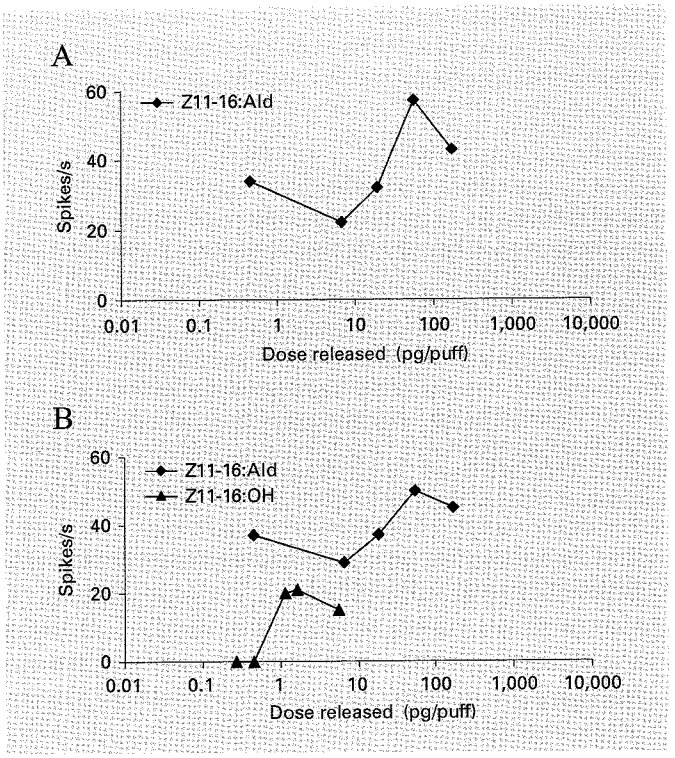


Fig. 1. A Response profile of an olfactory receptor neuron (ORN) from an A-type sensillum from an SV hybrid male exhibiting a typical *H. subflexa* or *H. virescens* profile. **B** Response profile of an ORN from an A-type sensillum with an unusual responsiveness to Z11–16:OH. These two sensilla were on the same antenna of the same SV hybrid male.

1.95 SD for Z11–16:OH-generated spikes; n = 7; fig. 1B). Cross-adaptation was not performed on this sensilla type; however, responses to Z11–16:OH had never been observed in ORNs from A-type sensilla from males of either parental-species type [Baker et al., 2004]. It was clear that the ORNs in individual SV hybrid A-type sensilla exhibited only these two distinct response profiles; there were no intermediate response types. However, both types could occur on the same antenna (fig. 1A, B). The mean SV hybrid A-type sensillar ORN response profile is shown in figure 9 and can be compared with both parental species' A-type sensillar ORN profiles [parental profiles from Baker et al., 2004].

C-Type Sensilla

SV Hybrids. Of the 19 C-type sensilla of SV hybrids that we sampled, 17 of the ORN response profiles with regard to spike amplitude relationships were similar to those of a typical *H. subflexa* C-type sensillum; a larger-spiking ORN responded to both Z11–16:Ac and Z9–14:Ald, and a smaller-spiking ORN responded to Z11–16:OH (fig. 2). In the other 2 sensilla it was the smaller-spiking Z11–16:OH-responsive ORN that also responded to Z9–14:Ald, which is typical of *H. virescens* parental C-type sensilla [Baker et al., 2004]. In these two sensilla the ORN response profile with regard to spike frequency also was similar to a typical *H. virescens* parental C-type profile, having a very low responsiveness to Z9–14:Ald

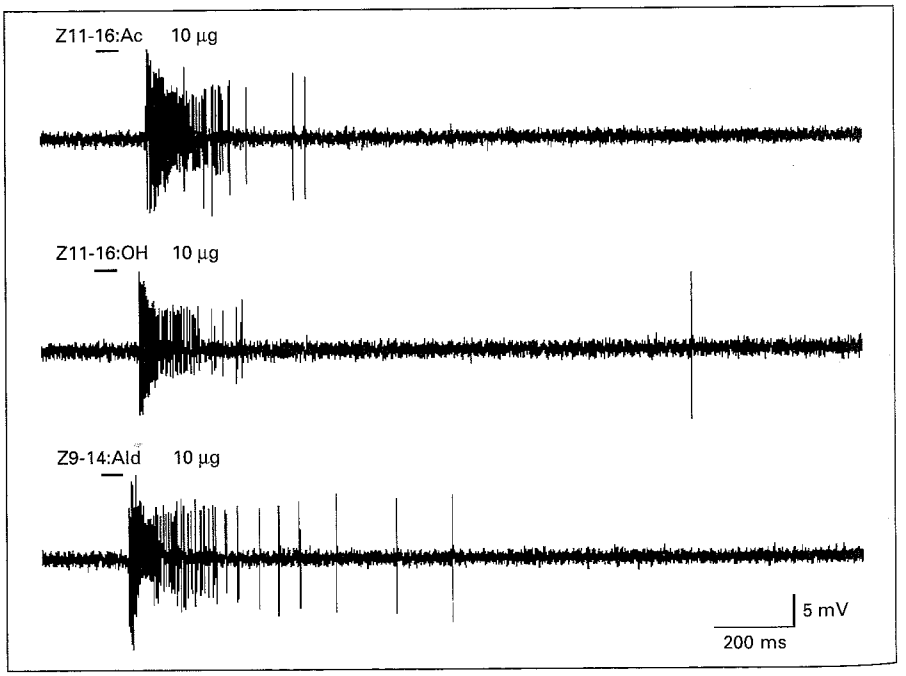


Fig. 2. Spike trains evoked in ORNs in C-type sensilla of SV hybrid male. A large-spiking ORN responds to both Z11–16:Ac (top) and Z9–14:Ald (bottom). A second, smaller-spiking ORN responds to Z11–16:OH. Stimulus bars are 30 ms.

(fig. 3C; 10). Cross-adaptation studies confirmed the cross-responsiveness of the SV hybrid ORNs to Z11–16:Ac and Z9–14:Ald, and the lack of cross-responsiveness of the Z11–16:OH-sensitive ORN to either Z11–16:Ac or Z9–14:Ald (fig. 4).

Spike frequency profiles of the ORNs in the other 17 C-type sensilla were often quite similar to those of *H. subflexa* parental C-type ORNs, characterized by a sensitivity to Z9–14:Ald that was almost as great as that to Z11–16:Ac and Z11–16:OH (fig. 3A, 10).

However, it is important to recognize that there was substantial variation between ORN spike frequency profiles from sensillum to sensillum for these C-type sensilla, ranging from ones that were clearly more like *H. subflexa* (fig. 3A) to the few that were more like *H. virescens* (fig. 3C), with many having intermediate response properties (fig. 3B). The overall mean spike frequency profile (fig. 10) was intermediate between the two parental species' types [parental profiles from Baker et al., 2004]; however, this mean profile masks the high degree of variation among sensilla and ORNs.

VS Hybrids. We were able to analyze the responses of ORNs housed in 9 C-type sensilla from 9 different VS hybrid males. As in the SV hybrids, 8 of the 9 sensilla contained a large-spiking ORN that was responsive to Z11–16:Ac and a smaller-spiking ORN that was responsive to Z11–16:OH (fig. 5). The larger-spiking ORN was also responsive to Z9–14:Ald, just as in the case of the SV hybrid C-type sensillar ORNs. In the ninth sensillum, the lone exception, the spike sizes were reversed with the larger spiking ORN responsive to Z11–16:OH. In addition, the larger spiking ORN was also sensitive to Z9–14:Ald, whereas the smaller spiking Z11–16:Ac-sensitive ORN was not. The variation between ORN sensitivities of the VS hybrids to Z11–16:OH, Z11–16:Ac and Z9–14:Ald appeared to be similar to that in the SV hybrid C-type ORNs, with perhaps a slightly greater sensitivity to Z9–14:Ald across all the sensilla sampled (fig. 6). The mean action potential frequency profile appeared very similar to that of the SV hybrids for the C-type sensillar ORNs (fig. 6, 10A) with regard to the relative responsiveness of the ORN to Z11–16:Ac and Z9–14:Ald. However, responsiveness of the VS hybrid ORN tuned to Z11–16:OH appeared to be somewhat elevated compared to that of SV hybrids.

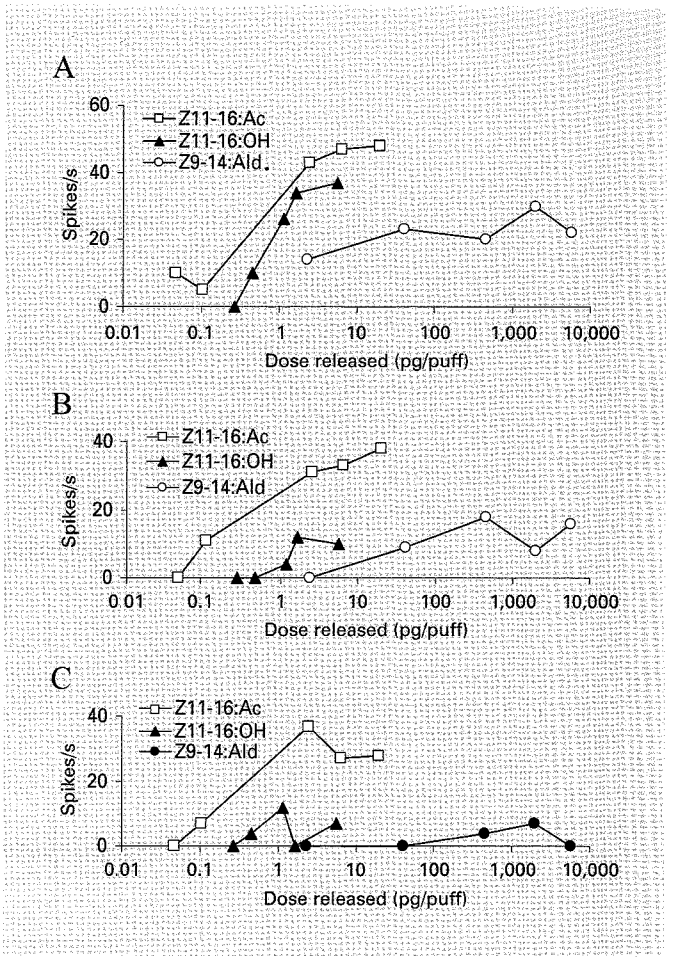


Fig. 3. A Response profiles of ORNs from a C-type SV hybrid sensillum exhibiting a profile close to that of a typical *H. subflexa* male. **B** Response profiles of ORNs from a C-type SV hybrid sensillum exhibiting an intermediate profile between *H. subflexa* and *H. virescens*. In both **A** and **B** a large-spiking ORN responded to Z11–16:Ac and Z9–14:Ald (open symbols), whereas a smaller-spiking ORN responded to Z11–16:OH (solid triangles). **C** ORN response profiles from another C-type sensillum in an SV hybrid male, this time appearing similar to ORNs housed in *H. virescens* C-type sensillum. In this sensillum a larger-spiking ORN responded to Z11–16:Ac (open squares) and a smaller-spiking ORN responded to both Z11–16:OH and Z9–14:Ald (solid symbols).

B-Type Sensilla

When the profiles of B-type ORNs in individual SV hybrid males were examined, 5 out of the 9 sensilla that were recorded had ORNs that exhibited response profiles (fig. 7A) more similar to a typical *H. subflexa* parental B-type (fig. 11C) than to *H. virescens* (fig. 11D). As in *H. subflexa* parental B-type sensilla, these five ORNs responded to both Z9–16:Ald and Z9–14:Ald. However, these hybrid B-type ORNs exhibited equivalent, sometimes greater, sensitivity to Z9–14:Ald compared to Z9–16:Ald (fig. 11A). This differs from the *H. subflexa* parental B-type ORNs that exhibited significantly greater sensitivity

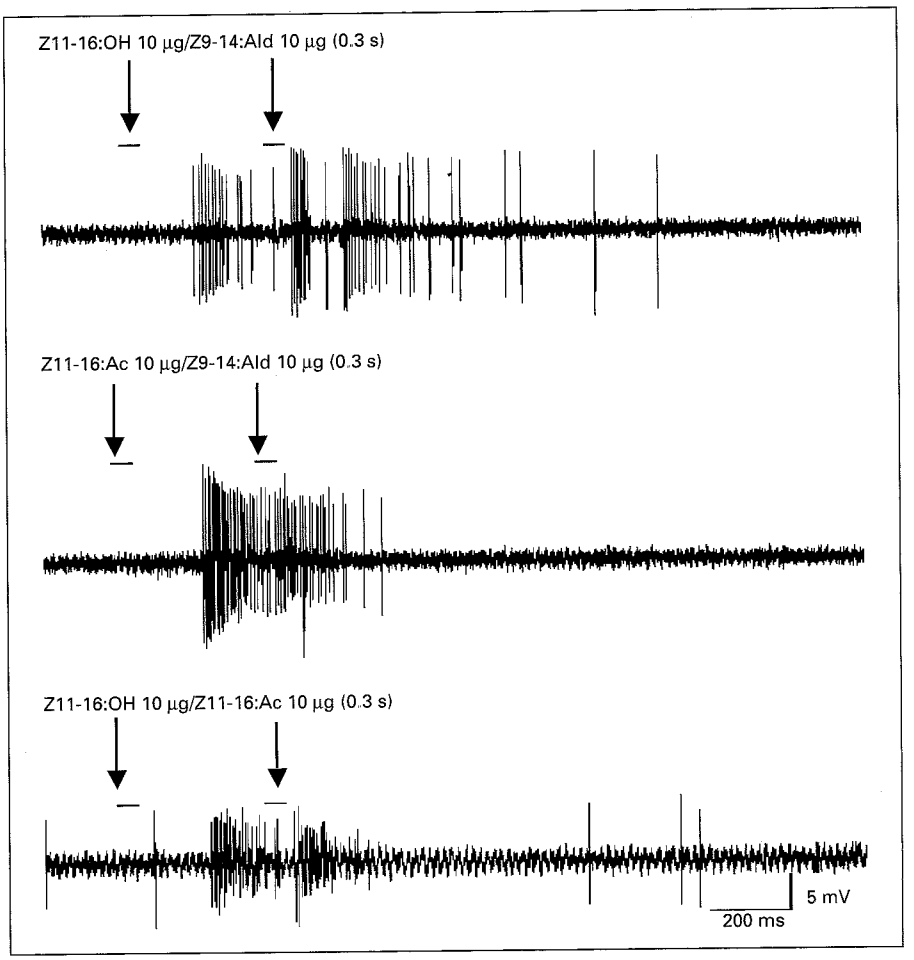


Fig. 4. Cross-adaptation experiments with ORNs in an SV hybrid C-type sensillum. Stimulus bars are 50 ms in duration with a delay of 300 ms between stimuli. The larger-spiking ORN was cross-adapted by Z9–14:Ald and Z11–16:Ac, whereas the smaller-spiking ORN stimulated by Z11–16:OH did not influence the response of the larger spiking ORN to either Z11–16:Ac or Z9–14:Ald.

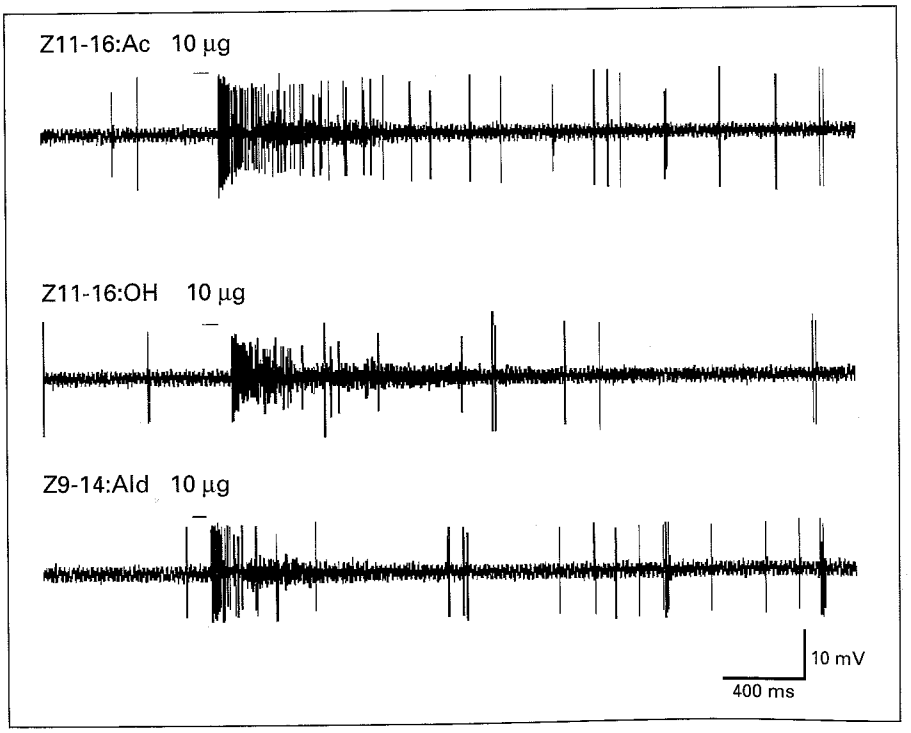
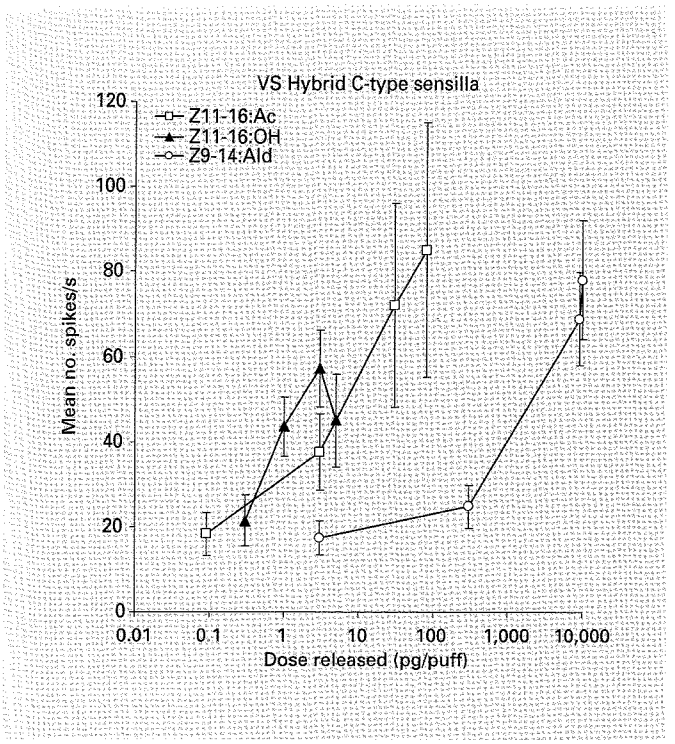
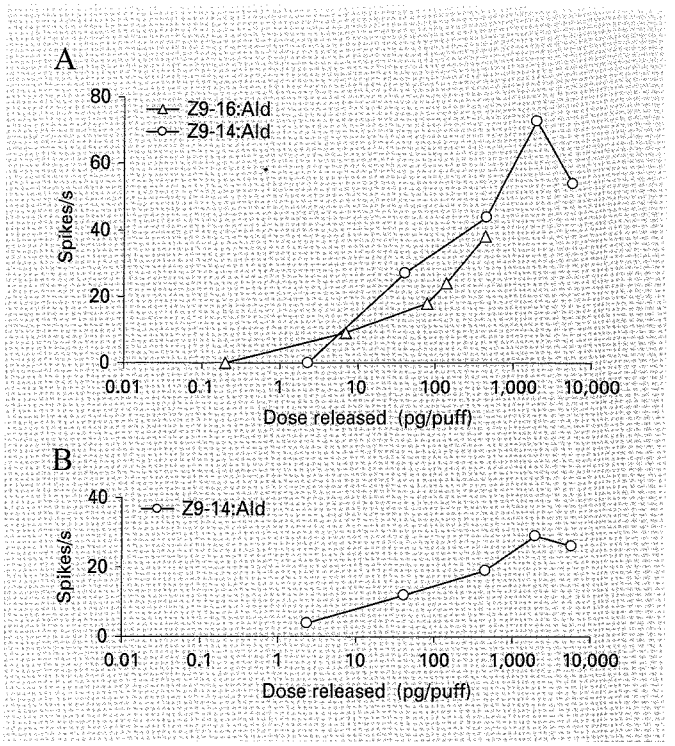


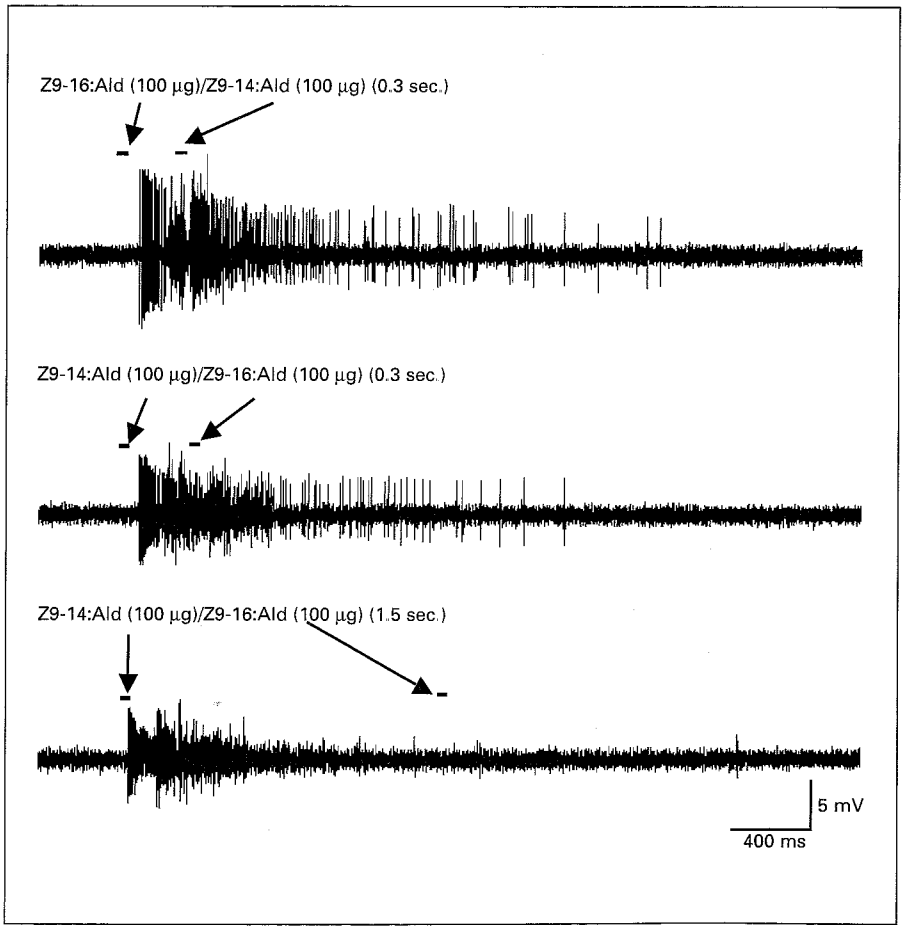
Fig. 5. Spike trains evoked in ORNs in C-type sensilla of a VS hybrid male. A large-spiking ORN responds to both Z11–16:Ac (top) and Z9–14:Ald (bottom). A second, smaller-spiking ORN responds to Z11–16:OH. Stimulus bars are 30 ms.



6



7

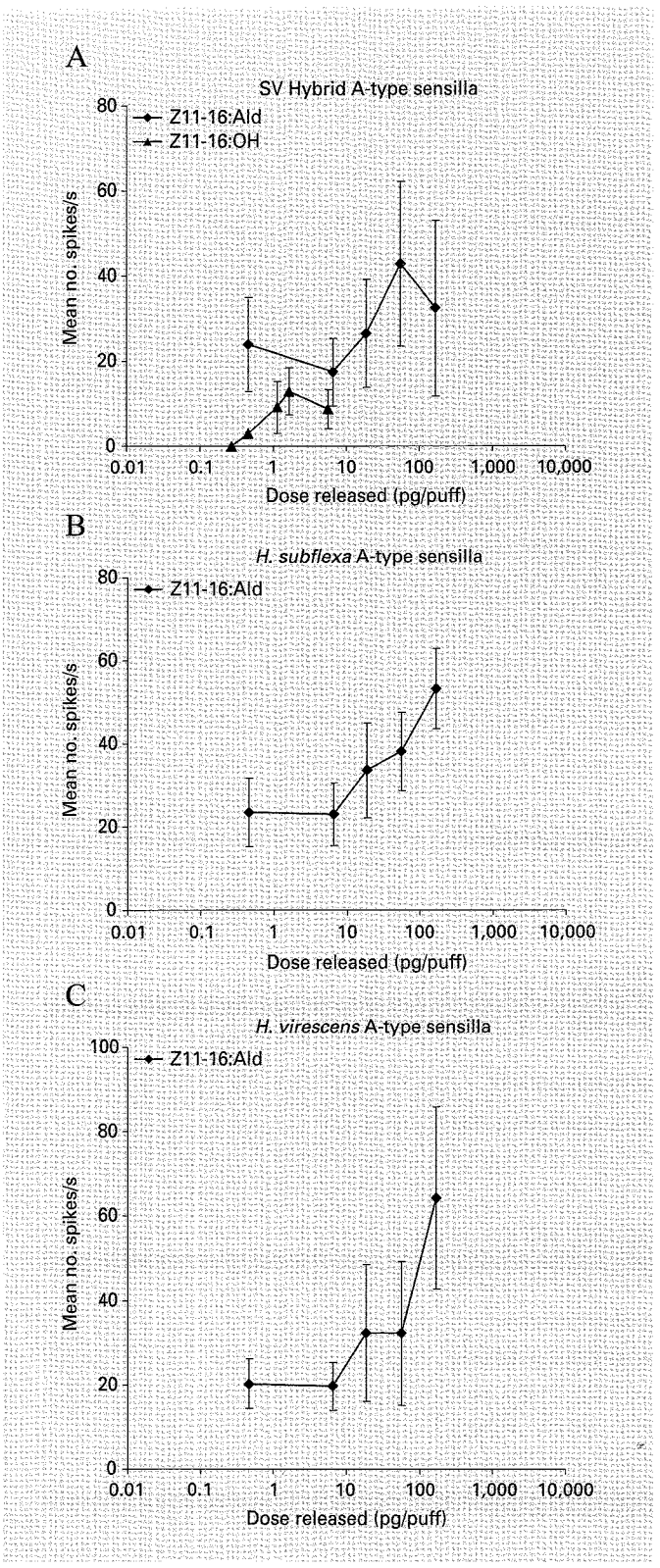


8

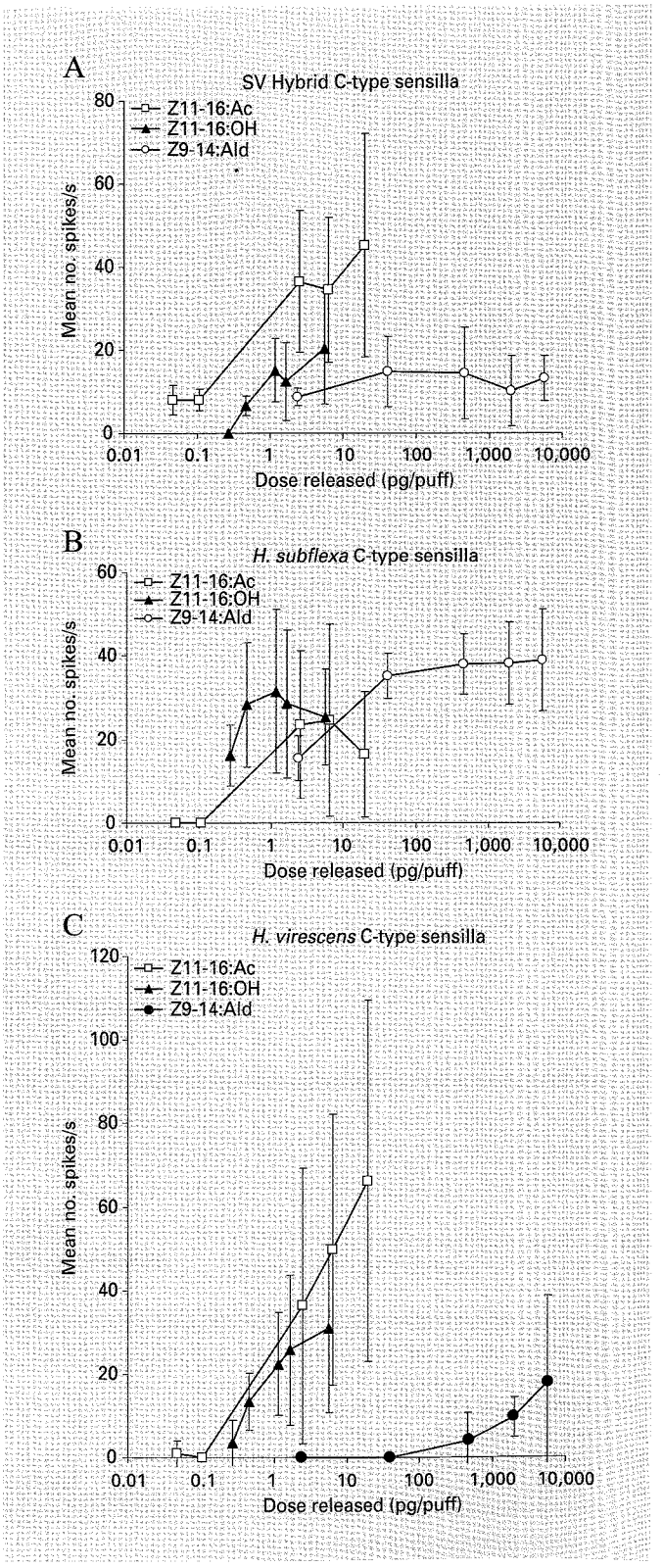
Fig. 6. Mean response profiles of the ORNs from 9 C-type sensilla on VS hybrid males.

Fig. 7. A Response profile of an individual ORN in a B-type hybrid sensillum having an *H. subflexa*-type profile. **B** Profile of a second individual ORN in a B-type hybrid sensillum having an *H. virescens*-type profile. These two sensilla were found on the same antenna of the same SV hybrid male.

Fig. 8. Cross-adaptation experiments with ORNs in an SV hybrid B-type sensillum. Stimulus bars are 50 ms in duration. The ORN was cross-adapted by Z9–14:Ald and Z9–16:Ald.


A
SV Hybrid A-type sensilla
Z11-16:Ald
Z11-16:OH
Mean no. spikes/s
Dose released (pg/puff)
B
H. subflexa A-type sensilla
Z11-16:Ald
Mean no. spikes/s
Dose released (pg/puff)
C
H. virescens A-type sensilla
Z11-16:Ald
Mean no. spikes/s
Dose released (pg/puff)

9


A
SV Hybrid C-type sensilla
Z11-16:Ac
Z11-16:OH
Z9-14:Ald
Mean no. spikes/s
Dose released (pg/puff)
B
H. subflexa C-type sensilla
Z11-16:Ac
Z11-16:OH
Z9-14:Ald
Mean no. spikes/s
Dose released (pg/puff)
C
H. virescens C-type sensilla
Z11-16:Ac
Z11-16:OH
Z9-14:Ald
Mean no. spikes/s
Dose released (pg/puff)

10

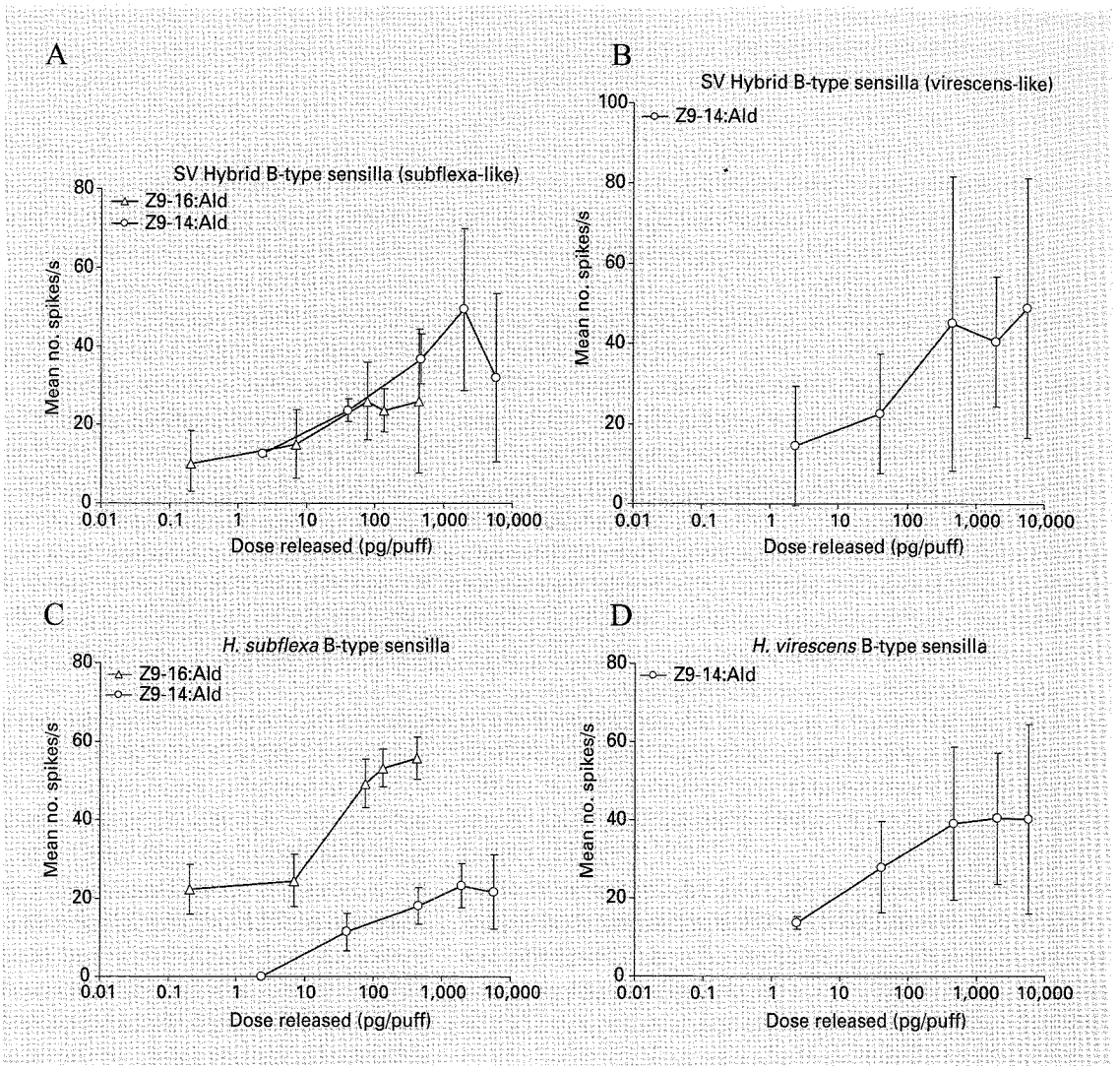


Fig. 11. A Mean response profiles (± SE) of ORNs in the B-type sensilla of SV hybrid males that resemble those of *H. subflexa*, in response to the minor pheromone components of each parental species: Z9–16:Ald for *H. subflexa*, and Z9–14:Ald for *H. virescens*. **B** Mean response profiles of the other type of B-type ORNs in the SV hybrid males that more closely resemble those of *H. virescens*. **C, D**. Mean response profiles of ORNs in the B-type sensilla of *H. subflexa* and *H. virescens*, respectively [data from Baker et al., 2004].

Fig. 9. A Mean response profiles (± SE) of ORNs in the A-type sensilla of SV hybrid males to Z11–16:Ald. **B, C**. Mean response profiles of ORNs of male *H. subflexa* and *H. virescens*, respectively, to Z11–16:Ald [data from Baker et al., 2004].

Fig. 10. A Mean response profiles (± SE) of ORNs in the C-type sensilla of SV hybrid males to the minor pheromone component of *H. subflexa*, Z11–16:OH, to Z9–14:Ald, the minor pheromone component of *H. virescens*, and to Z11–16:Ac, an antagonist to the upwind flight of *H. virescens*. **B, C**. Mean response profiles of ORNs in the C-type sensilla of male *H. subflexa* and *H. virescens*, respectively [data from Baker et al., 2004].

to Z9–16:Ald than they did to Z9–14:Ald (fig. 11C). Cross-adaptation studies of the hybrid B-type ORNs (fig. 8) confirmed that there was only one ORN responding to both Z9–16:Ald and Z9–14:Ald, just as in a typical *H. subflexa* parental B-type sensillum.

The response profiles of the ORNs in the other four SV hybrid B-type sensilla we sampled were quite similar to those from *H. virescens* parental B-type sensilla, responding only to Z9–14:Ald (fig. 7B, 11B). We once found these two distinct types of hybrid B-type ORNs on the

Table 1. Summary of hybrid- and parental-type olfactory receptor neuron (ORN) spike size and response characteristics

Sensillum type	ORN[a]	Spike amplitude	SV hybrid	*H. virescens*	*H. subflexa*
A[a]	1	Large	Z11–16:Ald	Z11–16:Ald	Z11–16:Ald
	1[b]	Large	Z11–16:Ald > Z11–16:OH (>1.5×)		
B[a]	1	Large	Z9–16:Ald & Z9–14:Ald (1×)	–	Z9–16:Ald > Z9–14:Ald (>30×)
	1	Large	Z9–14:Ald	Z9–14:Ald	–
C	1	Large	Z11–16:Ac & Z9–14:Ald (range from *Hv* to *Hs*)	Z11–16:Ac	Z11–16:Ac > Z9–14:Ald (>3×)
	2	Small	Z11–16:OH	Z11–16:OH[c] (>1,000×)	Z11–16:OH

An ORN that is responsive to more than one odorant will have two odorants listed. These ORNs' relative sensitivities to two compounds are shown in parentheses, where the value indicates how much more of the second compound needs to be emitted from an odor cartridge to produce the same action potential frequency as the first compound.

[a] A- and B-type sensilla housed one odorant-responsive ORN.

[b] Based on spike amplitude this ORN likely responded to both Z11–16:Ald and Z11–16:OH.

[c] This ORN also responded to Z9–14:Ald but only at the very high dosages indicated.

same SV hybrid antenna (fig. 7A, B). The mean profiles for these two classes of B-type ORNs from the SV hybrids are shown in figure 11A and B, as compared with the two parental types in figure 11C and D [from Baker et al., 2004].

Discussion

The alterations in ORN response profiles of SV hybrid males compared to the profiles of parental type ORNs occurred with varying degrees of severity within sensilla. The effect of hybridization on ORN physiology appeared to create a combination of both a mosaic of more or less 'parental type' ORNs residing in some of the sensilla across the antenna, and an array of highly ameliorated ORNs within single sensilla having spike frequency profiles that were intermediate between both parental profiles. Nevertheless, the compartmentalization of ORNs into different sensilla remained essentially the same as the parental types with recognizable A-, B- and C-type sensilla (table 1).

The main kinds of hybrid ORN tuning shifts compared to either parental type were as follows. The A-type hybrid sensilla, in addition to an ORN having the usual response to Z11–16:Ald, often had an unusual ORN response to Z11–16:OH that had never been observed in either species. In B-type hybrid sensilla, we found ORNs tuned to Z9–16:Ald that exhibited equal sensitivity to Z9–14:Ald, intermediate to the profiles of *H. virescens* and *H. subflexa*, which previously had never been seen in the B-type ORNs of either species [Berg et al., 1995; Baker et al., 2004]. Finally, there were many hybrid C-type sensilla in which the Z11–16:Ac-responsive ORNs' cross-responsiveness (sensitivity) to Z9–14:Ald was reduced compared to that of the *H. subflexa* parental type ORNs (table 1).

Hybrid ORN Architecture and Tuning Related to Hybrid Behavior and Projection Interneuron Functional Morphology

Preservation of the Z11–16:OH Pathway in Hybrids

Overall, the within-sensillum spike sizes (architecture) and response profiles (tuning) of hybrid male ORNs appeared to be more similar to *H. subflexa* than *H. virescens*, which is consistent with the dominance of *H. subflexa* behavior and physiology of olfactory interneurons also noted in SV hybrids [Vickers, 2006a, b]. In the present

study, the large-spiking ORN in the C-type hybrid sensillum tuned to Z11–16:Ac was cross-responsive to Z9–14:Ald, just as it is in *H. subflexa*. In *H. virescens* it is the small-spiking Z11–16:OH-tuned ORN that exhibits weak sensitivity to Z9–14:Ald. Therefore, the hybrid C-type ORN sensillar architecture preserves the Z11–16:OH-tuned ORN for agonistic input to its own glomerulus in the MGC, just as in *H. subflexa* [Vickers, 2006b]. This dedicated pathway would appear to explain the observed retention in hybrids of the *H. subflexa* parental-type behavioral requirement that Z11–16:OH be present in pheromone component blends in order to evoke significant levels of upwind flight [Vickers, 2002, 2006a].

Heightened Hybrid ORN Sensitivity to Z9–14:Ald Related to Hybrid Behavior. In hybrid B-type sensillar ORNs as well there was a retention of *H. subflexa* parental characteristics, most obviously the cross-responsiveness to Z9–14:Ald and Z9–16:Ald in the majority of the ORNs sampled. Notably, the tuning curves in response to Z9–14:Ald of these hybrid ORNs were shifted upward to become as sensitive to Z9–14:Ald as they are to Z9–16:Ald. Another form of elevated sensitivity to Z9–14:Ald also occurred compared to *H. subflexa*, in that the remaining SV hybrid B-type ORNs sampled only responded to Z9–14:Ald and gave no response to Z9–16:Ald. This profile appears to be similar, if not identical, to that of the B-type ORNs of *H. virescens*. The presence of these two hybrid ORN types in B-type sensilla having heightened sensitivity to Z9–14:Ald could explain a distinctive feature of hybrid male behavior: the capacity to substitute Z9–14:Ald for Z9–16:Ald in upwind flight tests [Vickers, 2006a]. This substitutive ability was not possible in either *H. subflexa* or *H. virescens* [Vickers et al., 1991; Vickers, 2002]. Vickers [2006b] found that projection neurons (PNs) arborizing in the DM glomerulus of the macroglomerular complex (MGC) of SV hybrid males exhibited almost equal sensitivity to Z9–14:Ald as they did to Z9–16:Ald. Neurons with such equivalent cross-sensitivity to these two compounds and arborizing in this DM glomerulus had not been found before in *H. virescens* or in *H. subflexa*. In *H. virescens* all PNs arborizing in the DM glomerulus were tuned to Z9–14:Ald [Berg et al., 1998; Vickers et al., 1998], and in *H. subflexa* they were all tuned to Z9–16:Ald with the exception of one interneuron that also showed a slight, but lower, cross-responsiveness to Z9–14:Ald than to Z9–16:Ald [Vickers and Christensen, 2003].

It would appear from our current results that the heightened cross-reactivity of the hybrid Z9–16:Ald-responsive antennal lobe PNs to Z9–14:Ald [Vickers, 2006b] is dictated to a large degree by the heightened cross-reactivity of the B-type ORNs to Z9–14:Ald. Five out of the 9 B-type ORNs from which we recorded were equally sensitive to Z9–14:Ald and Z9–16:Ald, whereas in all of the 34 B-type ORNs of *H. subflexa* parental males sampled, the B-type ORNs always were significantly less sensitive to Z9–14:Ald than to Z9–16:Ald [Baker et al., 2004; S.G. Lee et al., unpubl. observ.].

Odorant Receptor Expression Dictates ORN Tuning Profiles

What factors could produce these hybridization-generated shifts in ORN response profiles? It is clear from recent landmark studies of *Drosophila* ORNs [Dobritsa et al., 2003; Hallem et al., 2004; Hallem and Carlson, 2004] that odorant receptor (OR) expression is the major factor determining the response profile of an ORN. The mechanisms by which ORNs express different ORs, especially sister ORNs that are co-compartmentalized in the same sensillum, is not very well understood [Hallem and Carlson, 2004]. In Lepidoptera, the sensillum trichodeum plus an entourage of support cells is created starting from one mother cell. After four mitotic divisions 2 or 3 sister ORNs plus their mitotically-related support cells are produced [Keil and Steiner, 1990, 1991; Keil, 1992; Steinbrecht, 1999].

In *Drosophila*, the final two sister ORNs within one sensillum basiconicum usually express different ORs in a stereotypical way. Corresponding to the ORs expressed on them, the two ORNs respond in characteristic fashion to different suites of non-pheromonal odorants [Dobritsa et al., 2003; Hallem and Carlson, 2004]. In *Heliothis* or *Helicoverpa* species, as in *Drosophila*, the response profiles of pheromone-sensitive sister ORNs co-compartmentalized within a single sensillum [e.g., Baker et al., 2004], show that each pair of ORNs responds optimally and predictably to different pheromone-related ligands. In some classes of sensilla (such as A-type), one of these co-compartmentalized ORNs is always completely silent to any tested odorants, whereas the other responds readily to a pheromone component. The presence of silent ORNs has been revealed by cobalt staining of pairs of ORNs in A-type sensilla of *H. zea* [Lee et al., 2005], *H. subflexa*, and *H. virescens* [Berg et al., 1998]. Cobalt staining also revealed that a silent ORN is also present in the B-type sensilla of *H. subflexa* [S.G. Lee et al., unpubl. observ.].

Co-Expression of Two Odorant Receptors on the Same ORN Explains the Tuning Shifts of SV Hybrid ORNs

'One-Gene-One-Receptor' in Vertebrates. The broad yet specific tuning of certain ORN types in SV hybrid (and parental-type) males suggests that multiple ORs are expressed in the dendritic membranes of these neurons. Is it possible for two or more ORs to be expressed on the same ORN? The expression of vertebrate ORs on olfactory sensory neurons is monoallelic [Mombaerts, 2004a] with only one gene expressed per neuron with some exceptions [c.f., Rawson et al., 2000]. The 'one-gene-one-receptor' (per-ORN) end-product might occur through a selection process on the population of developing ORNs that culls the ORNs that express no, or else more than one, OR [Mombaerts, 2004 a, b].

In Insects, the One-Receptor-per-ORN Rule Does Not Apply. Goldman et al. [2005] recently showed that in the *D. melanogaster* maxillary palp ORN (ORN pb2A), two odorant receptors, *Or33c* and *Or85e*, are naturally coexpressed and functional. These same two receptors are also co-expressed in the homologous ORN in *D. pseudoobscura* [Goldman et al., 2005]. Earlier results had shown that two odorant receptors, *Or22a* and *Or22b*, were naturally expressed on one type of *D. melanogaster* antennal ORN named ab3A [Dobritsa et al., 2003; Hallem and Carlson, 2004; Hallem et al., 2004]. Moreover, in wild-type ab3A ORNs that were made to ectopically express a different OR, *Or47b*, in addition to this ORNs' native ORs, *Or22a* and *Or22b*, the ORNs functioned perfectly well. They exhibited spike frequency profiles in response to the suite of odorants tested that were intermediate to those observed in ab3A ORNs expressing only one or the other OR [Dobritsa et al., 2003].

Other evidence that at least some pairs of different *Drosophila* ORs might be expressed together on the same ORNs comes from a study in which all the known *Drosophila* ORs were ectopically expressed one at a time in mutant ab3A ORNs in which expression of the *Or22a/b* ORs had been eliminated by the *reaper* gene, and then these various receptor response profiles were compared to all of the known *Drosophila* antennal ORN profiles in an attempt to find a match [Hallem et al., 2004]. Although most of the new ectopic-expression receptor profiles could be matched to those of known ORNs on known locations on the antenna, there were eleven cases in which there was no match, raising the possibility that pairs of some of these ORs normally are expressed on a single ORN to create an unexpected, intermediate profile [Hallem et al., 2004; Hallem and Carlson, 2004].

Possible Co-Expression of Pheromone Receptor Genes on Single ORNs in Heliothine Moths. It follows, then, that one possible and fairly simple explanation for the shifts that we saw in the hybrid ORN response profiles compared to either parental type is that two different pheromone receptors can be simultaneously expressed on a single ORN in heliothine moths. Variations in the degree of expression of different pheromone receptor alleles from either parental type would explain the individual variation in profiles among the sister ORNs in individual heliothine sensilla. For instance, the variation in B-type sensilla ORN response profiles in SV hybrids could reflect a differential co-expression of alleles for a Z9–16:Ald receptor and a Z9–14:Ald receptor, with an equal co-expression of the Z9–14:Ald OR allele with the Z9–16:Ald OR allele in one-half of the B-type sensilla. In the other half of the B-type sensilla, the Z9–14:Ald OR allele is expressed alone, as it apparently is in *H. virescens*. Another possibility is that the Z9–16:Ald and Z9–14:Ald ORs are coded by different genes, as in the *Drosophila* genes for *Or33e* and *Or85e* that are co-expressed on the same ORN [Goldman et al., 2005]. Krieger et al. [2004] recently identified candidate pheromone receptor genes in *H. virescens* and demonstrated 4 clones that in situ hybridization studies showed to be intimately associated with the long sensilla trichoidea that are known to house pheromone-responsive ORNs. Currently, however, no experiments have been conducted to test whether two or more of these putative pheromone receptors are expressed in the same ORN.

Co-Expression of Receptors on the Same ORN Related to Shifts in Sex Pheromone Communication. A mutation in a *Drosophila* POU-domain transcription factor implicated in expressing ORs resulted in profound changes in ORN response profiles [Clyne et al., 1999]. Some of these changes involved the complete silencing of an ORN that had previously been responsive to odorants, and in some other types of ORNs a shifting responsiveness occurred which created an entirely novel profile to a different set of odorants [Clyne et al., 1999]. The interplay between transcription factors and OR genes might account for the diversity of ORN profiles observed in the current experiments, such as observed in the C-type VS hybrid sensilla. Alterations in ORN tuning can possibly facilitate shifts in behavioral responsiveness that could affect males' success in selecting appropriate mates and perhaps influence speciation. A broadening of responsiveness in ORNs might account for the first stages of sex pheromone mutation-driven saltational speciation [Baker, 2002; Roelofs et al., 2002], in which the sudden expression of a pseudo-

gene in females allows for an alternative pheromone blend to be emitted that is tracked by males over generations in a process called 'asymmetric tracking' [Phelan, 1992, 1997]. A broader responsiveness to a variety of blends that includes the new females' blend would not only provide the new females with mates, but would put such males at a selective advantage in locating available mates. A second stage of asymmetric tracking would occur when a linkage disequilibrium develops and assortative mating of males with females emitting the new blend increases. The old pheromone component is selected against. This reinforcement stage of the process could involve the development of behavioral antagonism to the old compound [Baker, 2002]. We suggest that this could occur by means of enhanced expression of the gene for the OR tuned to the old compound on an ORN involved in antagonistic pathways. It may or may not continue to be co-expressed at significant levels along with the gene for the receptor tuned to the new compound on the ORN involved in attraction.

A Change in Receptor Expression on an Insect ORN Does Not Change the Glomerular Target The fact that insect ORNs have been shown to target the same glomerulus independent of OR gene expression [Dobritsa et al., 2003; Goldman et al., 2005] argues that pheromone blend shifts can occur in which compounds such as Z9–14:Ald, previously involved in agonistic pathways, can subsequently become involved in antagonistic pathways. It is the glomeruli and the interconnectivity and activity patterns of local interneurons plus patterns of the output PNs that are read at higher centers such as the mushroom bodies [MacLeod et al., 1998; Stopfer et al., 2003] that seem to be more significant in regulating agonistic and antagonistic behavioral effects in insects [Vickers and Christensen, 2003]. If expression patterns of OR genes can be changed without changing the target glomeruli, then much more flexibility can occur in the evolution of pheromone component usage. Expression of ORs on existing ORNs can be enhanced or repressed to change their tuning profiles to suites of compounds, and the existing agonistic or antagonistic pathways' sensitivities can be made broader or narrower.

The very broadly tuned ORN in the C-type sensilla on *H. zea* antennae [Cossé et al., 1998] responding to three different behaviorally antagonistic ligands sends its axon to the antero-medial antagonist-related glomerulus of the MGC [Lee et al., 2005]. This type of ORN serves as a catch-all for three different behaviorally antagonistic pheromone-related compounds, including Z9–14:Ald, emitted by several non-conspecifics. At the same time, the second ORN in the *H. zea* C-type sensilla responsive to Z9–16:Ald and involved in attraction, also responds well to Z9–14:Ald and sends its axon to the dorso-medial glomerulus of the MGC involved in attraction [Lee et al., 2005]. Thus it may be that the same receptor is co-expressed with a different receptor on two different types of ORNs and could modulate male behavior in ways important to the evolution of sex pheromone blends by broadening the responsiveness of ORNs. It will be interesting to see whether the OR genes identified in *H. virescens* by Krieger et al. [2004] are the same ones that are expressed to different degrees on the ORNs of other heliothine moths such as *H. subflexa* and *H. zea*. The SV hybrids of our current study have given us new insight into the possible ways that the heliothine ORN system can modulate behavioral responsiveness to pheromone-component-related odorants.

Acknowledgements

This research was funded by NSF grants IBN-9910783 to T.C.B. and IBN-9905683 to N.J.V. We thank Drs. Fred Gould, Amy Sheck, Sara Oppenheim and Mr. Mark Sisterson of North Carolina State University for their assistance in establishing a colony of *H. subflexa* at the University of Utah. We thank Keri Swearingen, Dave Kelly, and Matt Pond for assisting with the rearing and maintenance of moth colonies, and Bryan Banks for revising the figures.

References

Baker TC (2002) Mechanism for saltational shifts in pheromone communication systems. Proc Nat Acad Sci USA 99:13368–13370.

Baker TC, Ochieng SA, Cosse AA, Lee SG, Todd JL, Quero C, Vickers NJ (2004) A comparison of responses from olfactory receptor neurons of *Heliothis subflexa* and *Heliothis virescens* to components of their sex pheromone. J Comp Physiol A 190:155–165.

Berg BG, Tumlinson J, Mustaparta H (1995) Chemical communication in heliothine moths. IV. Receptor neuron responses to pheromone compounds and formate analogues in the male tobacco budworm moth *Heliothis virescens*. J Comp Physiol A 177:527–534.

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H (1998) The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. J Comp Physiol A 183:669–682.

Clyne PJ, Certel SJ, de Bruyne M, Zaslavsky L, Johnson WA, Carlson JR (1999) The odor specificities of a subset of olfactory receptor neurons are governed by Acj6, a POU-domain transcription factor. Neuron 22:339–347.

Cossé AA, Campbell MG, Glover TJ, Linn CE Jr, Todd JL, Baker TC, Roelofs WL (1995) Pheromone behavioral responses in unusual male European corn borer hybrid progeny not correlated to electrophysiological phenotypes of their pheromone-specific antennal neurons. Experientia 51:809–816.

Cossé AA, Todd JL, Baker TC (1998) Neurons discovered in male *Helicoverpa zea* antennae that correlate with pheromone-mediated attraction and interspecific antagonism. J Comp Physiol A 182:585–594.

Dobritsa AA, van der Goes van Naters W, Warr CG, Steinbrecht RA, Carlson JR (2003) Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 37: 827–841.

Glover TJ, Perez N, Roelofs WL (1989) Comparative analysis of sex pheromone-response antagonists in three races of European corn borer. J Chem Ecol 15:863–873.

Goldman AL, Van der Goes van Naters W, Lessing D, Warr CG, Carlson JR (2005) Coexpression of two functional odor receptors in one neuron. Neuron 45:661–666.

Hallem EA, Ho MG, Carlson JR (2004) The molecular basis of odor coding in the *Drosophila* antenna. Cell 117:965–979.

Hallem EA, Carlson JR (2004) The odor coding system of *Drosophila*. Trends Gen 20:453–459.

Hansson BS, Löfstedt C, Roelofs WL (1987) Inheritance of olfactory response to sex pheromone components in *Ostrinia nubilalis*. Naturwissenschaften 74:497–499.

Heath RR, Mitchell ER Cibrian-Tovar J (1990) Effect of release rate and ratio of (Z)-11-hexadecen-1-ol from synthetic pheromone blends on trap capture of *Heliothis subflexa* (Lepidoptera: Noctuidae). J Chem Ecol 16:1269–1268.

Kaissling K-E (1974) Sensory transduction in insect olfactory receptors. In: Biochemistry of Sensory Functions (Jaenicke L, ed), pp 243–273. Berlin: Springer-Verlag.

Keil TA (1992) Fine structure of a developing insect olfactory organ: morphogenesis of the silkmoth antenna. Micro Res Tech 22:351–371.

Keil TA, Steiner C (1990) Morphogenesis of the antenna of the male silkmoth, *Antheraea polyphemus*. II. Differential mitoses of 'dark' precursor cells create the anlagen of sensilla. Tissue Cell 22:705–720.

Keil TA, Steiner C (1991) Morphogenesis of the antenna of the male silkmoth, *Antheraea polyphemus*. III. Development of olfactory sensilla and the properties of hair-forming cells. Tissue Cell 23:821–851.

Klun JA, Plimmer JR, Bierl-Leonhardt BA, Sparks AN, Chapman OL (1979) Trace chemicals: the essence of sexual communication systems in *Heliothis* species. Science 204:1328–1330.

Klun JA, Plimmer JR, Bierl-Leonhardt BA, Sparks AN, Primiani M, Chapman OL, Lepone G, Lee GH (1980) Sex pheromone chemistry of female tobacco budworm moth, *Heliothis virescens*. J Chem Ecol 6:177–183.

Klun JA, Leonhardt BA, Lopez JD Jr, Lachance LE (1982) Female *Heliothis subflexa* (Lepidoptera: Noctuidae) sex pheromone: Chemistry and congeneric comparisons. Environ Entomol 11:1084–1090.

Krieger J, Grosse-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 USA 101: 11845–11850.

Laster ML (1972) Interspecific hybridization of *Heliothis virescens* and *Heliothis subflexa*. Environ Entomol 1:682–687.

Lee S-G, Carlsson MA, Hansson BS, Todd JL, Baker TC (2005) Antennal lobe projection destinations of *Helicoverpa zea* male olfactory receptor neurons responsive to heliothine sex pheromone components. J Comp Physiol A (in press).

Löfstedt C, Hansson BS, Roelofs WL, Bengtsson BO (1989) No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer *Ostrinia nubilalis* Huebner (Lepidoptera: Pyralidae). Genetics 123:553–556.

MacLeod K, Bäcker A, Laurent G (1998) Who reads temporal information contained across synchronized and oscillatory spike trains? Nature 395:693–698.

Mombaerts P (2004a) Genes and ligands for odorant, vomeronasal and taste receptors. Nature Neurosci Rev April 2004:263–278.

Mombaerts P (2004b) Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. Curr Opin Neurobiol 14:31–36.

Phelan PL (1992) Evolution of sex pheromones and the role of asymmetric tracking. In: Insect Chemical Ecology (Roitberg BD, Isman MB, eds), pp 265–314. New York: Chapman and Hall.

Phelan PL (1997) Genetic and phylogenetics in the evolution of sex pheromones. In: Insect Pheromone Research (Cardé RT, Minks AK, eds), pp 563–579. New York: Chapman and Hall.

Proshold FI, Martin DF, Laster ML, Raulston JR, Sparks AN (1983) Release of backcross insects on St. Croix to suppress the tobacco budworm (Lepidoptera: Noctuidae): methodology and dispersal of insects. J Econ Entomol 76:885–891.

Ramaswamy SB, Randle SA, Ma WK (1985) Field evaluation of the sex pheromone components of *Heliothis virescens* (Lepidoptera: Noctuidae) in cone traps. Environ Entomol 14:293–296.

Rawson NE, Eberwine J, Dotson, R, Jackson J, Ulrich P, Retrepo D (2000) Expression of mRNAs encoding for two different olfactory receptors in a subset of olfactory receptor neurons. J Neurochem 75:185–195.

Roelofs WL, Hill AS, Cardé RT, Baker TC (1974) Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. Life Sci 14:1555–1562.

Roelofs WL, Liu W, Hao G, Jiao H, Rooney AP, Linn CE Jr (2002) Evolution of moth sex pheromones via ancestral genes. Proc Natl Acad Sci USA 99:13621–13626.

Roelofs WL, Glover TJ, Tang X-H, Sreng I, Robbins P, Eckenrode CJ, Löfstedt C, Hansson BS, Bengtsson BO (1987) Sex pheromone production and perception in European corn borer moths requires both autosomal and sex-linked genes. Proc Natl Acad Sci USA 84:7585–7589.

Sparks AN, Raulston JR, Lingren PD, Carpenter JE, Klun JA, Mullinix BG (1979) Field response of male *Heliothis virescens* to pheromone stimuli and traps. ESA Bull 2:268–274.

Steinbrecht RA (1999) V. Olfactory Receptors. In: Atlas of Arthropod Sensory Receptors: Dynamic Morphology in Relation to Function (Eguchi E, Tominaga Y, eds), pp 155–176. Tokyo: Springer-Verlag.

Stopfer M, Jayaraman V, Laurent G (2003) Intensity versus identity coding in an olfactory system. Neuron 39:991–1004.

Teal PEA, Tumlinson JH (1997) Effects of interspecific hybridization between *Heliothis virescens* and *Heliothis subflexa* (Lepidoptera: Noctuidae) on the sex pheromone communication system. In: Insect Pheromone Research: New Directions (Cardé RT, Minks AK, eds), pp 535–547. New York: Chapman and Hall.

Teal PEA, Heath RR, Tumlinson JH, McLaughlin JR (1981) Identification of a sex pheromone of *Heliothis subflexa* (GN.) (Lepidoptera: Noctuidae) and field trapping studies using different blends. J Chem Ecol 7:1011–1022.

Teal PEA, Tumlinson JH, Heath RR (1986) Chemical and behavioral analyses of volatile sex pheromone components released by calling *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae). J Chem Ecol 12:107–125.

Tumlinson, JH, Hendricks PE, Mitchell ER, Doolittle RE, Brennan MM (1975) Isolation, identification and synthesis of the sex pheromone of the tobacco budworm. J Chem Ecol 1:203–214.

Van der Pers JNC, Den Otter CJ (1978) Single cell responses from olfactory receptors of small ermine moths to sex-attractants. J Insect Physiol 24:337–343.

Vetter RS, Baker TC (1983) Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female glands. J Chem Ecol 9: 747–759.

Vickers NJ (2002) Defining a synthetic pheromone blend attractive to male *Heliothis subflexa* under wind tunnel conditions. J Chem Ecol 28: 1255–1267.

Vickers NJ (2006a) Inheritance of olfactory preferences. I. Pheromone-mediated behavioral responses of *Heliothis subflexa* x *Heliothis virescens* hybrid male moths. Brain Behav Evol 68:63–74.

Vickers NJ (2006b) Inheritance of olfactory preferences. III. Processing of pheromonal signals in the antennal lobe of *Heliothis subflexa* x *Heliothis virescens* hybrid male moths. Brain Behav Evol 68:90–108.

Vickers NJ, Christensen TA (2003) Functional divergence of spatially conserved olfactory glomeruli in two related moth species. Chem Senses 28:325–338.

Vickers NJ, Christensen TA, Mustaparta H, Baker TC (1991) Chemical communication in heliothine moths. III. Flight behavior of male *Helicoverpa zea* and *Heliothis virescens* in response to varying ratios of intra- and interspecific sex pheromone components. J Comp Physiol A 169:275–280

Vickers NJ, Christensen TA, Hildebrand JG (1998) Combinatorial odor discrimination in the brain: Attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. J Comp Neurol 400:35–56.