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Unusual pheromone receptor neuron responses in heliothine moth antennae derived from inter-species imaginal disc transplantation

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Abstract Single-cell electrophysiological recordings were obtained from olfactory receptor neurons housed in sensilla trichodea along the adult antennae arising from transplantation of the antennal imaginal discs between larval male *Helicoverpa zea* and *Heliothis virescens*. The olfactory receptor neurons from the majority of type C sensilla sampled on transplanted antennae displayed response characteristics consistent with those of the species that donated the antennae. However, some of the sensilla type C sampled in either transplant type contained olfactory receptor neurons that responded in a manner typical of the recipient species or other neurons that have not previously been found in the type C sensilla of either species. The single-cell data help to explain behavioral results showing that some transplant males do fly upwind to both species' pheromone blends, an outcome not expected based on known antennal sensory phenotypes. Our results suggest that host tissue can influence antennal olfactory receptor neuron development, and further that because of a common phylogenetic ancestry the donor tissue has the genetic capability to produce a variety of sensillar and receptor types.

Keywords Flight tunnel · *Helicoverpa zea* · *Heliothis virescens* · Imaginal disc transplants · Sex pheromone · Single cell recordings

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Abbreviations *AL* antennal lobe · *MGC* macroglomerular complex · *ORN* olfactory receptor neuron · *Z11–16:Ald* (*Z*)-11-hexadecenal · *Z9–16:Ald* (*Z*)-9-hexadecenal · *Z9–14:Ald* (*Z*)-9-tetradecenal · *Z11–16:Ac* (*Z*)-11-hexadecenyl acetate · *Z11–16:OH* (*Z*)-11-hexadecen-1-ol

Introduction

In all moth species investigated thus far that utilize sex pheromones for mate location, males possess olfactory receptor neurons (ORNs) on their antennae that differentially respond to behaviorally agonistic (attractive) pheromone components as well as to behaviorally antagonistic pheromone-related compounds. Each type of ORN associated with attraction is usually tuned to only one component of the agonistic conspecific pheromone blend and is housed in a long hair-like sensillum trichodeum (Hansson 1995). The ORNs involved in antagonistic behavioral responses such as cessation of upwind flight by males are tuned to respond only to the pheromone components of other species, thereby minimizing mating mistakes with females of the wrong species (Todd and Baker 1999).

The axons of the ORNs travel the length of the antenna before they synapse with central neurons in the portion of the brain called the antennal lobe (AL) (Christensen 1997; Hansson 1997). It is now known that the ORNs tuned to a specific pheromone component project to, and arborize in, a glomerulus in the AL that is dedicated to receiving information only about that specific component (Hansson 1997; Hansson and Anton 2000). Likewise, the ORNs tuned to antagonistic, pheromone-related compounds project to their own glomerulus, which together with the pheromone-component-specific glomeruli, comprise a set of AL glomeruli at the entrance of the antennal nerve called the macroglomerular complex (MGC) (Berg et al. 1998; Christensen 1997; Hansson 1997; Hansson et al. 1992). The MGC and other AL glomeruli are involved in

integration of incoming peripheral information about odor composition; the AL then sends out processed information to higher centers of the brain through a relatively few output projection interneurons (Christensen 1997; Christensen et al. 1995; Hansson 1997; Hansson and Anton 2000; Vickers et al. 1998).

Experiments by Schneiderman et al. (1982, 1986) using antennal imaginal discs that were transplanted from male to female *Manduca sexta* and vice versa showed that the ORNs growing into the AL influenced the glomerular structure of the AL, thus making it appear either 'male' (possessing an MGC) or 'female' (lacking an MGC). Subsequent studies using inter-sexually transplanted antennae in *M. sexta* have confirmed and extended these findings and defined further how glomerular organization is governed by antennal ORN input during development (Oland et al. 1990; Tolbert and Sirianni 1990; Rössler et al. 1999). The behaviors of the transplanted individuals were likewise influenced by the type of antennae they possessed. Female moths with grafted male antennae responded to sex pheromones with behaviors resembling both normal female tobacco-seeking and oviposition behaviors, and male mate-seeking and copulatory behaviors (Schneiderman et al. 1986). However, no studies have been performed on *M. sexta* to determine if transplanted antennae have a normal compliment of donor sensillar types.

The same imaginal disc transplantation technique has been successfully used to transfer antennae from the males of the 'Z' race of European cornborer, *Ostrinia nubilalis,* to males of the 'E' race (Linn et al. 1998, 1999). Although antennal sensory recordings were not conducted, flight tunnel behavioral tests showed that males exhibited upwind flight only to the pheromone blend of the recipient race, and never to that of the donor. The technique has also recently been used to transplant the antennae of males of the corn earworm, *Helicoverpa zea*, onto males of the closely related heliothine species, *Heliothis virescens*, and vice versa, to study possible changes in behavior and in MGC architecture (Linn et al. 2000). Females of both species produce (*Z*)-11-hexadecenal (Z11–16:Ald) as the principal component of their sex pheromone blends, but they emit different secondary components that help to give the blends their species-specificity (Roelofs et al. 1974; Tumlinson et al. 1975; Klun et al. 1980; Pope et al. 1984; Teal et al. 1981, 1986).

During the course of behavioral testing of the heliothine moth transplants, Linn et al. (2000) discovered that 24% of *H. zea* males possessing antennae derived from imaginal discs transplanted from *H. virescens* readily flew upwind in response to the two-component *H. zea* pheromone blend. Such attraction was unexpected because *H. virescens* males have not been observed to fly upwind to the *H. zea* pheromone blend and normal *H. virescens* antennae (now carried by the *H. zea* recipients) do not have an ORN type that is tuned to the *H. zea* secondary pheromone component, (*Z*)-9-hexadecenal (Z9–16:Ald) (Berg et al.1995; Cossé et al. 1998; T.C. Baker et al., unpublished observations). We therefore know of no pathway by which these transplanted antennae can receive information about the presence of Z9–16:Ald in the plume from the *H. zea* pheromone source.

The pheromone-specific ORNs in *H. virescens* and *H. zea* are housed in long antennal sensilla trichodea depicted diagrammatically in Fig. 1. In *H. zea* males, approximately 71% of the antennal hairs belong to a class known as *sensilla trichodea* type A. Each sensillum contains a single ORN tuned to the major pheromone component, Z11–16:Ald. A second sensillar type (called type B), comprising about 7% of the sensilla trichodea, house an ORN specific to (*Z*)-9-tetradecenal (Z9–14:Ald), a secondary pheromone component of *H. virescens*. A third sensillar type (called type C), comprising about 20% of the antennal hairs, houses two ORNs, one being a large-spiking neuron tuned to both the *H. zea* secondary pheromone component Z9–16:Ald, and also to Z9–14:Ald. A smaller-spiking neuron in this sensillar type is tuned to two compounds known to act as behavioral antagonists of *H. zea*, (*Z*)-11-hexadecen-1-ol (Z11–16:OH) and (*Z*)-11-hexadecenyl acetate (Z11–16:Ac). This ORN also responds, but with less sensitivity, to another behavioral antagonist, Z9–14:Ald (Cossé et al. 1998; Teal et al. 1984). In comparison, about 90% of the *H. virescens* sensilla trichodea type A contain a single ORN tuned to the *H. virescens* major pheromone component, Z11–16:Ald, and ca. 3% of the sensilla trichodea are of type B, containing an ORN tuned to the *H. virescens* secondary pheromone component, Z9–14:Ald (T.C. Baker et al., unpublished observations). Type C sensilla (comprising 7%) contain two ORNs, one with large amplitude spike tuned to Z11–16:Ac and the other with small amplitude spike to

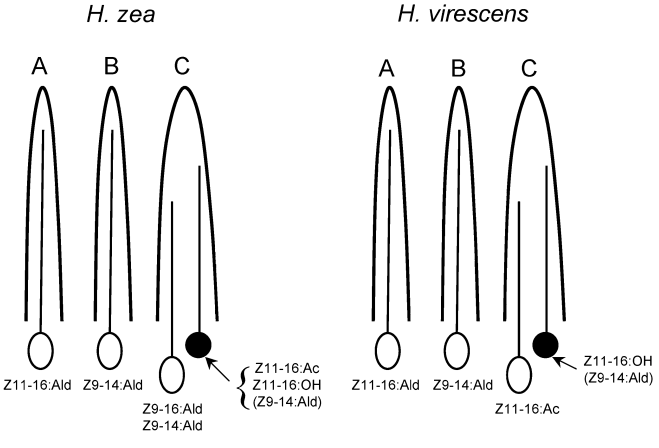


Fig. 1 A depiction of the relationship of pheromone specific sensilla types on the antennae of male *Helicoverpa zea* and *Heliothis virescens*. Olfactory receptor neurons (ORNs) housed in sensilla types A are specific to the principal pheromone component in both species. The main difference between *H. zea* and *H. virescens* is that no ORN in *H. virescens* has been found that is specific to the *H. zea* secondary pheromone component (*Z*)-9-hexadecenal (Z9–16:Ald). ORNs with large action potentials are drawn with *large open ellipses* and those with smaller action potentials are shown with *small closed circles*

Z11–16:OH; this second ORN also responds to Z9–14:Ald, but with 1,000 times lower sensitivity than to Z11–16:OH (T.C. Baker et al., unpublished observations).

The main difference between *H. zea* and *H. virescens* (Fig. 1), is that no ORN in *H. virescens* has been found that responds to the *H. zea* secondary pheromone component Z9–16:Ald, despite hundreds of sensilla having been sampled on this species. In the current study, all our recordings were conducted from ORNs housed within sensillum type C in order to characterize the transplanted antennae and determine whether they belonged to the species that donated the imaginal discs or to the recipient species. ORN types A and B were not considered in this study since their response profiles are similar in both species (Fig. 1).

Materials and methods

Insects

Colonies of the two-heliothine moth species were maintained at the Geneva, New York lab as described in Jurenka et al. (1991), on a 16:8 L:D photoperiod, 25°C, 40–50% RH. Normal pupae of each species were sexed and adults separated daily to obtain individuals of known age. Transplanted adults were tested behaviorally in the flight tunnel 1–3 days after emergence, and then shipped overnight to Ames, Iowa, for electrophysiological investigations.

Transplant procedure

The transplant procedure (Linn et al. 1999) is modified from Schneiderman et al. (1982). The sexes were separated in the larval stage (Lavenseau 1982), and imaginal disks were transplanted in the middle of the last instar. Larvae were restrained in aluminum tubes and anesthetized by chilling in ice water. The antennae with intact disks were excised and held in chilled drops of saline (calcium Ringer's). The donor disks were placed in the recipient and sealed with VetBond (3M). The recipient was removed and placed in a 50-ml plastic cup containing fresh media. Larvae were then checked daily for activity, feeding, and subsequent eclosion.

Flight tunnel

Adult transplant animals were tested in the sustained-flight tunnel during the 2nd to 4th hours of scotophase (16:8 L:D photoperiod), 22°C, 60% RH, a wind speed of 0.5 m s^{-1}, and illumination of 11 lx of red light at the tunnel floor (Linn et al. 1999). Moths were scored for activation, with no locking on to the pheromone plume, and upwind flight in the pheromone plume over a distance of 1.5 m to the pheromone source. Adults of each transplant type and control males from each colony, were tested for their upwind flight response to standard 300-μg dose lures of both pheromone blends (Z11–16:Ald with either 10% Z9–14:Ald for *H. virescens*, or 5% Z9–16:Ald for *H. zea*). Lures were tested one at a time, with the first lure randomly selected on each test day. During each test, five to ten normal males from each colony were also tested to each pheromone blend. Chemical solutions were prepared in HPLC grade hexane, and applied to 5 mm×9 mm red rubber septa (Arthur H. Thomas).

Test compounds

For electrophysiological recordings, synthetic pheromone compounds, Z11–16:Ald, Z9–16:Ald, Z9–14:Ald, Z11–16:Ac and Z11–16:OH (purities >95%; verified by gas chromatography) were obtained from Bedoukian Research, Danbury, Conn., USA. Serial dilutions of the compounds were made in HPLC-grade hexane. Each odorant was applied as 10-μl aliquots on a piece of Whatman No.1 filter paper that was then inserted into a Pasteur pipette. Filter papers containing 10 μl of the solvent were used as controls. The solvent was allowed to evaporate in a fume hood before sealing the wide end of the Pasteur pipette with aluminum foil. Stimulus cartridges were stored at –20°C when not in use, and brought to room temperature prior to a recording session. For dose-response profiles, stimulus compounds were selected in random order, beginning with the 1-μg odor cartridges and working upward to the 100-μg odor cartridges. Interstimulus intervals ranged from 30 s to 1 min depending on the magnitude of the previous response.

Single-cell recordings and stimulations

We used the cut-sensillum technique (Kaissling 1974), to record from the ORNs within individual antennal sensilla. The insect 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 silver wire and mounted on a Syntech Portable Recording Unit INR-2 (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 its tip hanging over the edge. The sensillum tip was cut off using a horizontally oriented mobile tungsten knife placed in a second micromanipulator. The cut end was immediately contacted with a saline-filled glass micropipette containing an Ag/AgCl recording electrode. The a.c. signal from the recording electrode was connected to the built-in amplifier of the portable recording unit and the a.c. output fed into a computer. The neural activity was monitored by a loudspeaker and displayed on the computer.

A stream of purified and humidified air continuously blew over the antenna (10 ml s^{-1}), and was directed through a 14-cm-long stainless steel tube (8 mm i.d.) whose outlet was positioned 2 cm from the antenna. With a stimulus flow-controller device (SFC-2, Syntech), a 20-ms air pulse at 40 ml s^{-1}-flow rates was injected through the odor cartridge and into the air stream flushing the antenna. Recordings were made from randomly selected sensilla close to the dorsal (scale-covered) surface on the proximal end of the antenna, known to house long sensilla trichodea (Grant et al. 1989; Cossé et al. 1998). We processed the data with a PC-based signal processing software, Syntech AutoSpike4.0 (Syntech). A response was determined as the difference in number of spikes 200 ms before and after stimulus onset. Classification of sensilla types were made according to the following criteria: (1) "*H. zea* C-type" sensillum: two ORNs one exhibiting larger amplitude spikes in response to both Z9–16:Ald and Z9–14:Ald and a second ORN exhibiting smaller amplitude spikes in response to Z11–16:Ac, Z11–16:OH, and Z9–14:Ald; (2) "*H. virescens* C-type": two ORNs one exhibiting larger amplitude spikes in response to Z11–16:Ac and a second ORN exhibiting smaller amplitude spikes in response to Z11–16:OH, occasionally to Z9–14:Ald at the highest concentrations; and (3) "atypical C-type": responses from one or more ORNs to more than one of the following: Z9–14:Ald, Z9–16:Ald, Z11–16:OH and Z11–16:Ac in a combination not recorded previously from either *H. virescens* or *H. zea*.

Results

H. virescens disc-*H. zea* recipient (V-Z transplants)

From a total of 185 last instar *H. zea* male larvae having *H. virescens* antennal imaginal discs transplanted onto

Table 1 Electrophysiological expression characteristics of transplanted antennae of *Heliothis virescens* on the body of *Helicoverpa zea* (V-Z transplants)

Expression type	Character of expressed receptor responses			
	No. of moths[a]	No. of sensilla[b]		
		virescens C type	*zea* C type	Atypical
virescens	14	30	–	–
zea	1	–	2	–
Atypical	3	–	–	5
virescens + atypical	2	2	–	3
zea + atypical	1	–	5	1
Total	21	32	7	9

[a]Number of moths having corresponding ORN response characteristics
[b]Number of sensilla identified in each type of moth antennae

them (V-Z transplants), 69 (38%) emerged to adult. Of these, 55 were tested in the flight tunnel with the following results for the 10 moths that made upwind progress in the plume: 5 flew upwind only to the *H. virescens* blend; 2 flew upwind only to the *H. zea* blend; and 3 flew upwind to both blends. A total of 430 normal *H. zea* males were tested to both pheromone blends, with 163 of the males reaching the *H. zea* pheromone source. None of these *H. zea* males flew upwind to the *H. virescens* blend.

Forty-five of the 69 transplants were shipped to the Iowa State University lab, and 37 of these were part of the group tested in the flight tunnel. We recorded from a total of 48 sensilla from 21 V-Z transplants. Overall, although only a few sensilla on each male could be

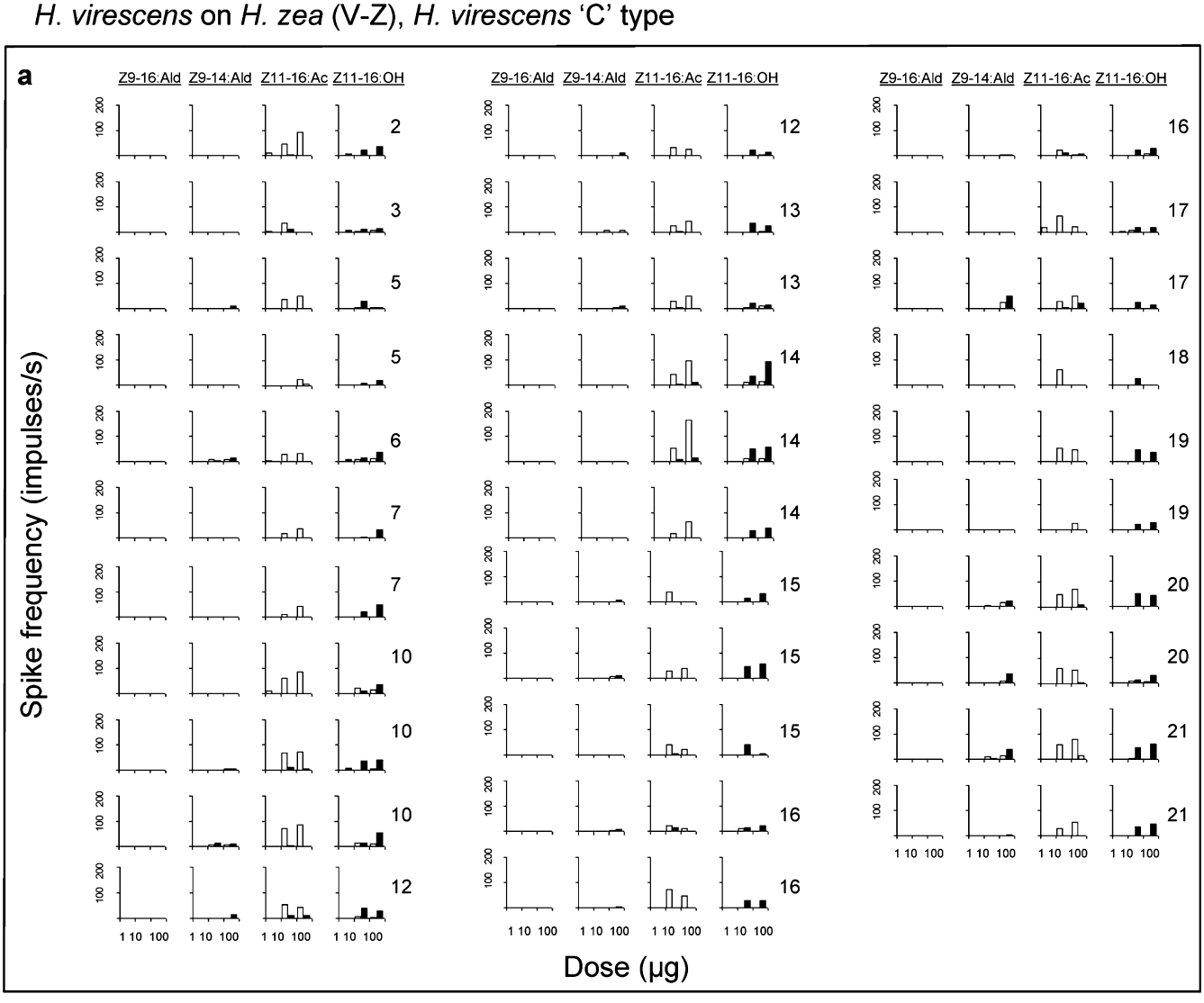
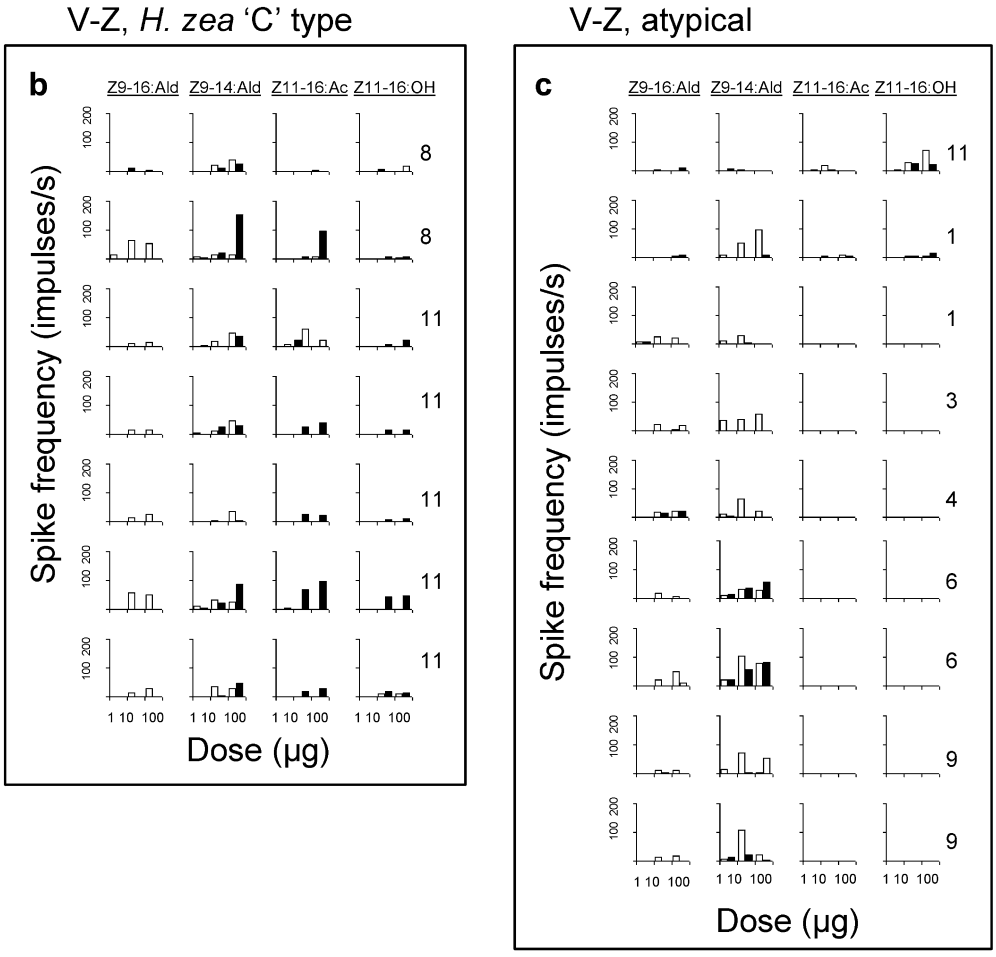


Fig. 2a–c Response profiles of the antennal ORNs within sensilla type C resulting from transplantation of *H. virescens* antennal imaginal discs onto *H. zea* male recipients (V-Z transplants). ORN recordings indicated type C sensilla typical of *H. virescens* (**a**), *H. zea* (**b**), as well as atypical sensilla (**c**). Recordings from the same animal are indicated by the same number next to each profile. Animal no. 9 flew upwind to pheromone blends of both species, whereas animal no. 17 flew upwind to the *H. virescens* pheromone blend only. *Open and closed bars* represent ORN responses with large and small amplitude spikes, respectively. Dose is the amount, in micrograms, applied to the filter paper in each odorant stimulus pipette

Fig. 2a–c (Contd.)



sampled, the majority of males exhibited high fidelity to possessing C type sensilla characteristic of either *H. zea* or *H. virescens* (Table 1). For example, 14 of these 21 males had only *H. virescens*-type sensilla and one had only *H. zea*-type sensilla. The remaining 6 males had one or more atypical sensilla sometimes in addition to normal *H. zea* or *H. virescens* C-type sensilla. Of these 21 V-Z transplant males sampled, none had sensilla of both the *H. virescens* and *H. zea* types.

Of the 48 sensilla sampled, 32 showed response profiles that were typical of *H. virescens* C type (Fig. 2a; cf. Fig. 3) and 7 sensilla were of the *H. zea* C type (Fig. 2b; cf. Fig. 5). Nine sensilla were atypical and all but one had a similar response profile with a large amplitude ORN responding to Z9–16:Ald and Z9–14:Ald (Fig. 2c). One of the latter individuals (no. 9) was one of the three that had flown upwind to both pheromone blends. The atypical sensillar response profiles have never been found on normal *H. virescens* or *H. zea* antennae (Figs. 3, 5).

H. zea disc-*H. virescens* recipient (Z-V transplants)

From a total of 94 last instar *H. virescens* larvae having *H. zea* discs transplanted onto them (Z-V transplants), 28 (30%) eclosed successfully. Of these, 17 were tested in the flight tunnel, with the following results for the 5 males that made upwind progress in the plume: 4 flew upwind only to the *H. virescens* blend and one male flew upwind to both blends. This individual died before recordings could be made. None of these males flew upwind to the *H. zea* blend alone. A total of 411 normal *H. virescens* males were tested to both pheromone blends, with 218 of these males reaching the *H. virescens* pheromone source. None of these *H. virescens* males flew upwind in response to the *H. zea* pheromone blend.

We recorded successfully from 31 sensilla from ten Z-V transplant males. Of these ten males, five had sensilla response profiles typical of *H. zea* (donor) antennae (Table 2). The five remaining males that were investigated possessed sensilla that had at least one ORN response profile categorized as atypical (Table 2). A total of 18 sensilla had ORN responses typical of *H. zea* C type (Fig. 4a; cf. Fig. 5). One of the animals (no. 10) is one of the four that had flown upwind to the *H. virescens* pheromone blend. The remaining 13 sensilla were classified as atypical (Fig. 4b) but this group was much more heterogeneous than the atypical sensilla described for the V-Z transplants (Fig. 2c). Five sensilla had only a small amplitude ORN responding to Z9–14:Ald, Z11–16:OH and Z11–16:Ac. Four sensilla had a large-spiking ORN that responded to both Z9–16:Ald and Z9–14:Ald.

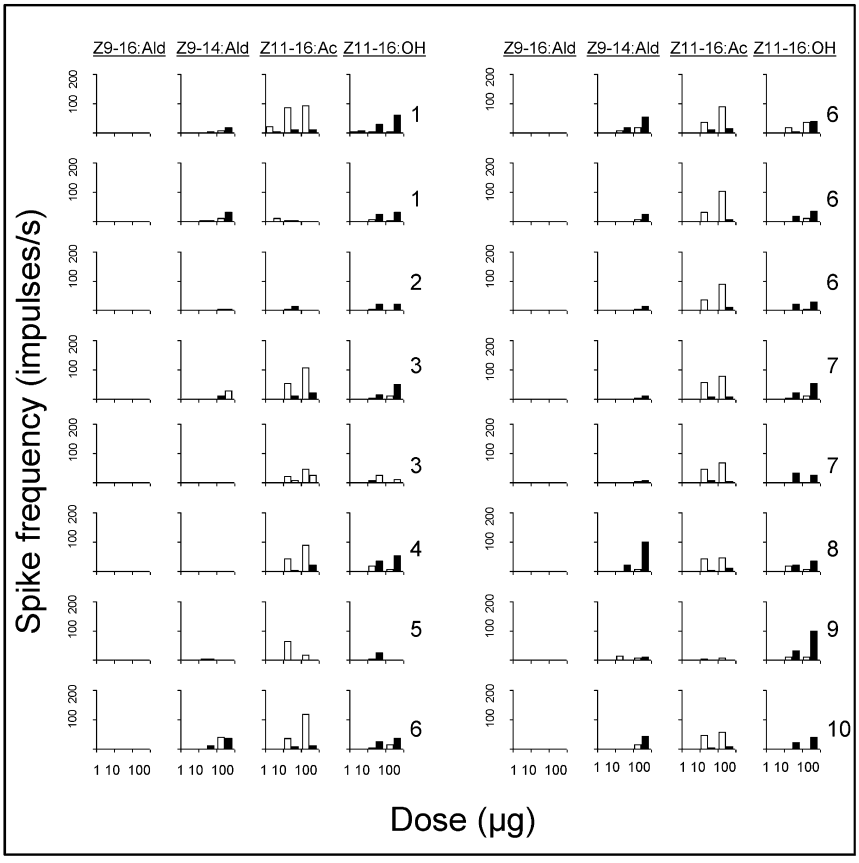


Fig. 3 Response profiles of antennal ORNs within sensilla type C on the antennae of normal *H. virescens*. Note that none responded to stimulation with Z9–16:Ald, the *H. zea* secondary pheromone component. Recordings from the same animal are indicated by the same number next to each profile. *Open and closed bars* represent ORN responses with large and small amplitude spikes, respectively. Dose is the amount, in micrograms, applied to the filter paper in each odorant stimulus pipette

Table 2 Electrophysiological expression characteristics of transplanted antennae of *H. zea* on the body of *H. virescens* (Z-V transplants)

Expression type	Character of expressed receptor responses		
	No. of moths[a]	No. of sensilla[b]	
		zea C type	Atypical
zea	5	11	–
Atypical	3	–	11
zea + atypical	2	7	2
Total	10	18	13

[a]Number of moths having corresponding ORN response characteristics
[b]Number of sensilla identified in each type of moth antennae

The other four sensilla housed ORNs exhibiting a variety of responses. The response profiles of the ORNs in the atypical category (Fig. 4b) differ markedly from ORNs in the C type sensilla of males taken from the *H. zea* colony in Geneva, NY (Fig. 5), and from those previously recorded from males in the Ames, IA colony (Cossé et al. 1998).

Discussion

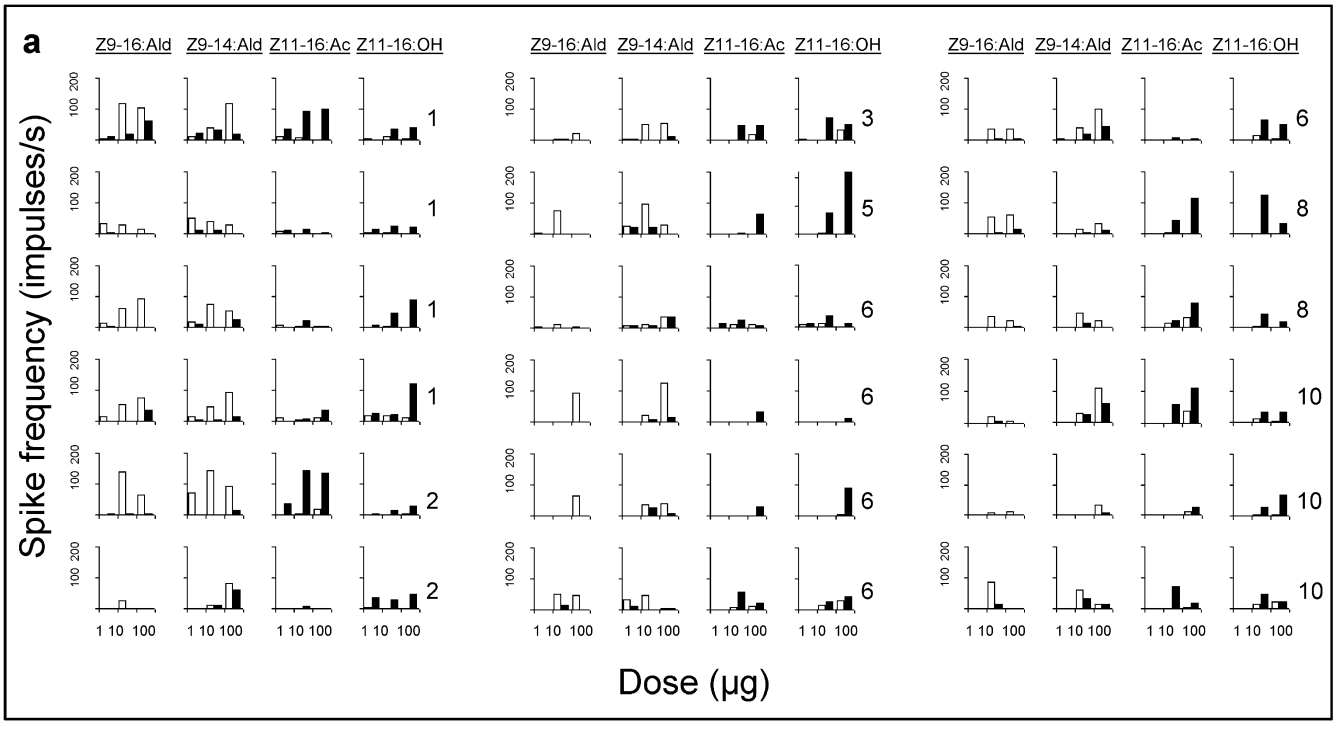
Our results show that in a high percentage of the sensilla sampled, ORNs developed on the transplant animals that displayed response profiles of ORNs housed in C-type sensilla not differing from those that would be expected from undisturbed donor species' antennae (Figs. 2a and 4a). However, a variety of other additional sensilla containing ORNs with responses typical of either normal host C-type sensilla (Fig. 2b) or atypical profiles (Figs. 2c and 4b) were also discovered. The ability of V-Z transplant males to fly upwind in response to the *H. zea* blend may be explained by their antennae possessing ORNs that are responsive to Z9–16:Ald as well as their MGC architecture being changed to appropriately receive and integrate these new inputs (Linn et al. 2000; Vickers et al. 1998). Some of our V-Z transplants did possess Z9–16:Ald-sensitive ORNs in their sensilla, and such ORNs have not been found on normal *H. virescens* antennae (Fig. 3; Almaas et al. 1991; Berg et al. 1995; Hansson et al. 1995; T.C. Baker et al., unpublished observations). The occurrence of these ORNs provides a

►

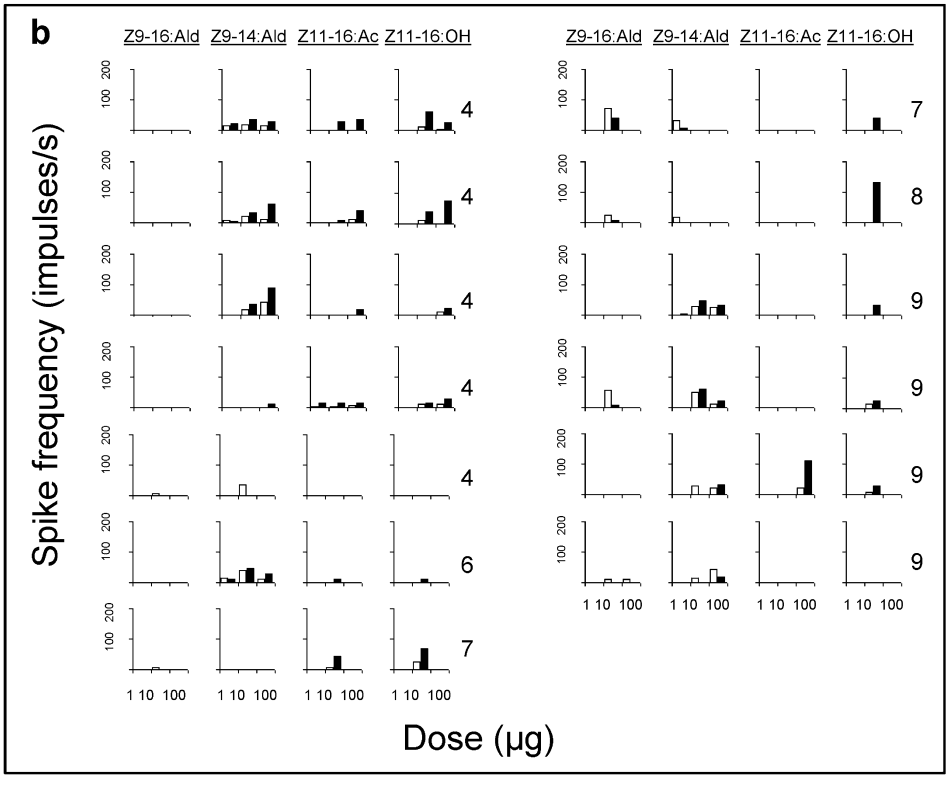
Fig. 4a,b Response profiles of the antennal ORNs within sensilla type C resulting from transplantation of *H. zea* antennal imaginal discs onto *H. virescens* recipients (Z-V transplants). ORN recordings indicated type C sensilla typical of *H. zea* (**a**), as well as atypical sensilla (**b**). Recordings from the same animal are indicated by the same number next to each profile. Animal no. 10 flew upwind to the pheromone blend of *H. virescens*. *Open and closed bars* represent ORN responses with large and small amplitude spikes, respectively. Dose is the amount, in micrograms, applied to the filter paper in each odorant stimulus pipette

possible neurophysiological explanation for the ability of transplant males to respond with upwind flight to both species pheromonal blends as documented by Linn et al. (2000). Such behavior would not be expected if only the predicted donor antennal sensory phenotypes were found on transplanted antennae.

H. zea on *H. virescens* (Z-V), *H. zea* 'C' type



Z-V, atypical



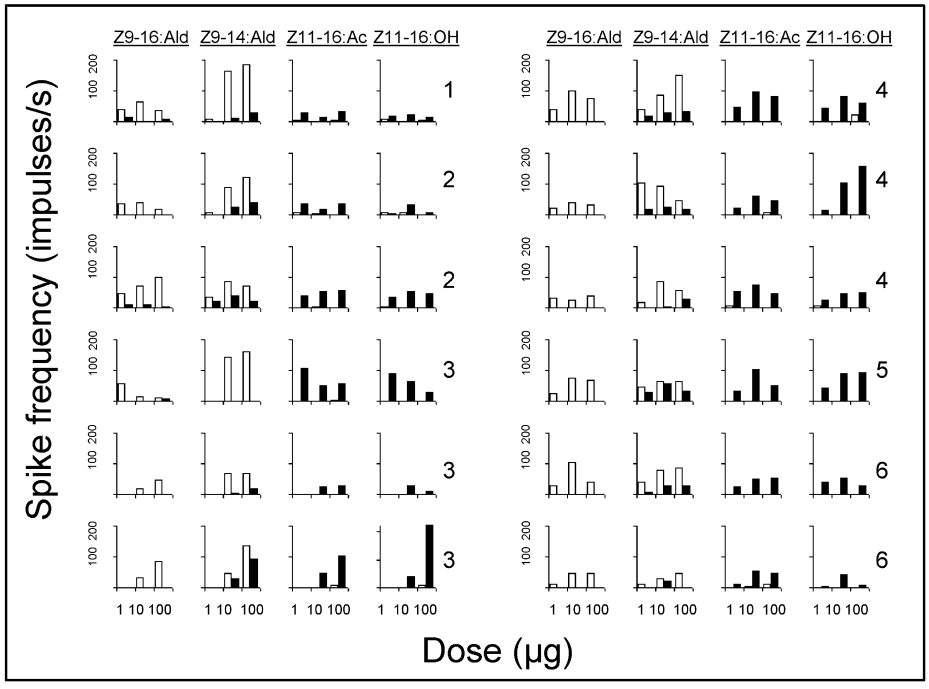


Fig. 5 Response profiles of antennal ORNs within sensilla type C on the antennae of normal *H. zea*. In all cases two ORNs were present, one with large spike amplitude responded to both Z9–16:Ald and (*Z*)-9-tetradecenal (Z9–14:Ald), whereas the other with small spike amplitude responded to both (*Z*)-11-hexadecenyl acetate (Z11–16:Ac) and (*Z*)-11-hexadecen-1-ol (Z11–16:OH). Recordings that came from the same animal are indicated by the same number next to each profile. *Open and closed bars* represent ORN responses with large and small amplitude spikes, respectively. Dose is the amount, in micrograms, applied to the filter paper in each odorant stimulus pipette

The observation that response properties of the donor antenna may be affected in some way by the host environment was not anticipated based on previous antennal imaginal disc transplants in *M. sexta* (Schneiderman et al. 1982, 1986; Rössler et al. 1999). In those experiments male or female antennal discs were transplanted into the opposite sex. In every case, whether or not complete antennae were formed, the antennae were reported to be that of the donor disc sex. Since there is considerable sexual dimorphism in the antennal morphology between the sexes, differences would presumably be easy to detect, and yet this phenomenon has not been reported. The AL that develops in the *M. sexta* inter-sexual transplant is always that of the donor antennal type (Schneiderman et al. 1982, 1986; Oland et al. 1990; Rössler et al. 1998, 1999). Our working hypothesis for the heliothine inter-species transplant studies was that the host tissue would not affect the antennal sensillar array, and if the antenna developed, it would have an array characteristic of the normal donor type.

Our data suggest otherwise, that, in fact, there are interactions between host and donor tissue that can affect antennal ORN development. Further, the results imply that the donor tissue has the genetic capacity to construct a variety of sensillar and ORN types that would not normally develop in the undisturbed donor environment, but could develop because of a common phylogenetic ancestry. Current models suggest that regulatory factors that control the expression of genes play a key role in the temporal and spatial expression of genes and that many genes may be silent but still exist as part of the genome (Carroll 2000; Truman 1996; Bayer et al. 1996; Force et al. 1999).

Studies on the development of the antenna and receptor cells in *M. sexta* indicate that differentiation of cellular types and growth of axons to the AL occur several days after evagination of the imaginal disc and accompanying changes in cell shape and number (Sanes and Hildebrand 1975; Oland et al. 1990; Rössler et al. 1998, 1999). Presumably during this period regulatory pathways initiate the expression of genetic elements that will give rise to the specific antennal cell types, and affect their developmental fate. We do not at present have experimental evidence to explain how, or when, these developmental pathways are altered on the transplant males, but we offer several points for consideration. First, there is the possibility that the expression of alternative ORNs is due to trauma associated with the transplant procedure itself. Loosli (1959) reported that in some cases involving transplantation of dorsal metathoracic discs between individual *Drosophila melanogaster*, a pattern of adventitious bristles developed that were like those found in sepsid flies. The appearance of the altered pattern was attributed to mechanical disturbance during transplantation as well as properties of the saline used to hold the disc during the procedure. While this may be the case in our transplant studies, it does not explain the presence of atypical sensilla types.

A second possibility, again dealing with the transplant procedure, is that if the disc is not completely removed during the surgical procedure, the remaining small part of the host disc could fuse with the donor disc, creating a mosaic of epidermal tissue that can give rise to different sensillar types (see Schneiderman 1984). We did find antennae in many of the transplants that exhibited the unusual morphological appearance of being a mosaic of *H. zea* and *H. virescens* antennal tissue. The transplant surgery is performed during the midpoint of the 5th instar, at a time when the disc is stable enough for removal. The imaginal disc in both

heliothine moths is very similar in appearance to the one described and illustrated for *M. sexta* in Sanes and Hildebrand (1975). The presence of an intact peripodial membrane and severed segments of the larval antennal nerve and trachea serve as key morphological features indicating that a complete disc has been removed. When taken earlier in the instar the disc, upon removal, does not remain intact and can easily fragment; later in the instar the disc can elongate into a tube-like structure, bearing a clear segmentation pattern. In both cases the disc cannot be re-implanted into a different larva. The fact that we do not always see a mosaic pattern on the transplant antenna suggests that timing of the transplant procedure may again be important; that there may be a critical window during development when donor tissues can be influenced by host signals. Critical experiments need to be run involving carefully staged individuals in order to ascertain the effect of timing on the presence of the unusual ORNs. Experiments involving transplantation of discs between males of the same species could also help determine the extent to which the surgical procedure affects antennal morphology.

A final consideration is that there are in fact a very small number of pheromone-specific sensillar types on the antennae of normal male *H. zea* and *H. virescens* that differ from those depicted in Fig. 1. These may be at such a low frequency that the probability of recording from them would be remote in normal animals. However, their frequency might increase as a result of the different conditions in the transplant host.

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