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# Unusual response characteristics of pheromone-specific olfactory receptor neurons in the Asian corn borer moth, *Ostrinia furnacalis*

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## Summary

**Male moth pheromone-detecting receptor neurons are known to be highly specific and very sensitive. We investigated physiological and behavioral responses to female sex pheromone components in male *Ostrinia furnacalis* moths (Lepidoptera: Crambidae). Using recordings from a cut-sensillum technique, trichoid sensilla could be grouped into four physiological types (1–4), according to the response of receptor neurons to the two major pheromone components, (*E*)-12- and (*Z*)-12-tetradecenyl acetate (E12- and Z12-14:OAc). These types could subsequently be characterized as four subtypes (A–D) depending on neural responses to pheromone components from various sister species of *O. furnacalis*, (*Z*)-9-, (*E*)-11- and (*Z*)-11-tetradecenyl acetate.**

**The peripheral pheromone detection system of *O. furnacalis* is different to that of other moths. A large majority of the neurons investigated responded to both of the two principal pheromone components. Dose–response and cross-adaptation studies showed that olfactory receptor neurons with large amplitude action potentials responded equally well to E12- and Z12-14:OAc in sensillum types 1–3. Field experiments showed that *O. furnacalis* males are sensitive to ratios of E12- and Z12-14:OAc and that (*Z*)-9-tetradecenyl acetate acts as a behavioral antagonist. *O. furnacalis* males thus display an unusual coding system for odors involved in sexual communication, mainly built on less specific neurons, but still have the ability to detect and respond to the correct female blend. We hypothesize that the pheromone detection system of *O. furnacalis* consists of two parts, where one is devoted to high sensitivity to Δ12 isomers of tetradecenyl acetate, E12- and Z12-14:OAc and the other to highly specific responses to the E12- or Z12-14:OAc. The unusual feature is thus that a large part of the system is devoted to sensitivity and only a minor part to selectivity. This could be explained by the fact that no other moth species are known to use E12- and/or Z12-14:OAc and that no strong selective pressure to increase selectivity between the isomers has been determined.**

Key words: olfaction, electrophysiology, single sensillum recording, *Ostrinia nubilalis*, sex pheromone communication, behavioral antagonist, field trapping, electron microscopy.

## Introduction

Female moths emit a volatile pheromone that is detected by males and used at a distance to locate the sexually receptive female. In most cases, moth sex pheromones consist of a mixture of several components. Species specificity of the pheromone is achieved by using unique combinations or ratios of components. The male antennae are equipped with a large number of sensilla containing olfactory receptor neurons tuned to components in the female sex pheromone. Male moth pheromone-detecting receptor neurons are known to be highly specific and extremely sensitive (Hansson, 1995; Todd and Baker, 1999).

Moth species in the genus *Ostrinia* (Lepidoptera: Crambidae) mainly use isomers of tetradecenyl acetates for sex pheromone communication (for a review, see Ishikawa et al., 1999b). *O. furnacalis* and sympatric congeners *O. scapulalis*,

*O. zaguliaevi* and *O. zealis* occur in Asia, whereas the European corn borer, *O. nubilalis*, occurs in Europe and North America. Field trapping and wind-tunnel experiments have shown that *O. furnacalis* is unique in utilizing (*E*)-12- and (*Z*)-12-tetradecenyl acetates (E12- and Z12-14:OAc, hereafter abbreviated accordingly), whereas the other species have (*E*)-11- and (*Z*)-11-tetradecenyl acetates (E11- and Z11-14:OAc) as pheromone components (Klun et al., 1975; Klun et al., 1980; Huang et al., 1997; Zhou and Du, 1999). In addition, (*Z*)-9-tetradecenyl acetate (Z9-14:OAc) is a pheromone component of *O. zaguliaevi* and *O. zealis* (Huang et al., 1998b; Ishikawa et al., 1999a). This compound also functions as a behavioral antagonist in *O. nubilalis* and *O. scapulalis* (Glover et al., 1989; Ishikawa et al., 1999a).

Sex pheromone communication of the Asian corn borer, *O. furnacalis*, has been studied intensely because of its status as a serious pest of maize in Asian countries. Ando et al. characterized the sex pheromone in Japan as E12- and Z12-14:OAc at a blend ratio of ~40% E and 60% Z isomer (Ando et al., 1980). Female pheromone blends differ slightly in four Japanese populations (35–43% E isomer) (Huang et al., 1998a). Concordant with variations in the female pheromone blend composition, field-trapping experiments have shown an optimal male behavioral response to a blend containing 36% E12-14:OAc in a Japanese population (Huang et al., 1998a). There are other reports of similar variations in female pheromone blend and male behavioral response in populations in other Asian countries (Kou et al., 1992; Boo and Park, 1998).

In *O. nubilalis*, two strains are attracted by pheromone blends dominated by E11- and Z11-14:OAc, respectively (Glover et al., 1987; Glover et al., 1989; Cossé et al., 1995; Linn et al., 2003). In both strains, two different receptor neurons respond specifically to one of the two pheromone components, and a third neuron responds to Z9-14:OAc (Hansson et al., 1987; Hansson et al., 1994; Cossé et al., 1995). The pheromone-specific receptor neurons reside in trichoid sensilla on the antenna and can always be distinguished by their amplitude, so that the large-amplitude neuron responds to the dominating pheromone component. Males could thus be typed according to these physiological characteristics (Hansson et al., 1987; Hansson et al., 1994; Cossé et al., 1995). In addition, interneurons responding to each pheromone component and the pheromone blend specifically occur in the male antennal lobe (Anton et al., 1997). In *O. furnacalis*, electroantennogram (EAG) responses to the pheromone components E12- and Z12-14:OAc and pheromone components of other *Ostrinia* species (Z9-, E11- and Z11-14:OAc) have been recorded (Ando et al., 1980). However, the EAG responses do not provide any bases to suggest how pheromone components are distinguished by the peripheral olfactory system, even though behavioral experiments have revealed the importance of the ratio between the components (Huang et al., 1998a; Boo and Park, 1998; Zhou and Du, 1999). Löfstedt and Hansson reported that pheromone receptor neurons of hybrid males between *O. nubilalis* and *O. furnacalis* responded to pheromone components of both species (Löfstedt and Hansson, 1989). However, detailed information regarding receptor neuron responses in *O. furnacalis* is absent. The external and internal morphology of sensilla on the antenna of *O. nubilalis* has been described, whereas only the external morphology has been described in *O. furnacalis* (Ren et al., 1987; Hallberg et al., 1994).

In the present study, we observed the structure of the antenna and its sensilla in male *O. furnacalis* by scanning and transmission electron microscopy. We then recorded male olfactory receptor neuron responses to conspecific sex pheromone components (E12- and Z12-14:OAc) and to pheromone components of other *Ostrinia* species (Z9-, E11- and Z11-14:OAc) by using a cut-sensillum technique. Pheromone-specific olfactory receptor neurons were found in sensilla trichodea and were characterized by the size of their action potentials. An unusual coding system for pheromone information characterized by a large number of less specific neurons was revealed. In addition, field-trapping experiments were performed to investigate the effect of Z9-14:OAc as a behavioral antagonist.

## Materials and methods

### *Animals*

Male and female *Ostrinia furnacalis* Guenée were obtained from a laboratory culture reared for several generations on a semi-synthetic diet (Mani et al., 1978). The culture originated from progenies of three female moths collected at Iruma (35.5°N, 139.2°E), Saitama, Japan in 2001 by J. Tabata. All stages of insects were kept at 25°C and 70% relative humidity with a 16 h:8 h L:D cycle. Pupae were collected from the diet and the sex was determined. The sexes were allowed to eclose separately. Male adults were provided with sugar water and were used for electrophysiological recordings two to five days after emergence.

### *Chemicals*

All compounds tested [(*E*)-12-tetradecenyl acetate (E12-14:OAc), (*Z*)-12-tetradecenyl acetate (Z12-14:OAc), (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), (*E*)-11-tetradecenyl acetate (E11-14:OAc), (*Z*)-11-tetradecenyl acetate (Z11-14:OAc)] were purchased from Pherobank (Wageningen, The Netherlands). The isomeric purity of each compound was >99.5%. All compounds were diluted in redistilled hexane.

### *Electron microscopy*

For scanning electron microscopy, excised antennae from male *O. furnacalis* were fixed in 70% ethanol, dehydrated and air-dried. The antennae were mounted on holders with double-sided tape and coated with gold:palladium (3:1) in an ion sputter (Polaron SC-7640; Quorum, Newhaven, UK). The specimens were studied in a scanning electron microscope (LEO 435VP; Cambridge, UK) with a secondary electron detector at a high voltage of 10 kV. For transmission electron microscopy, excised antennae were cut into pieces and immersed in a mixture of 3% paraformaldehyde and 2%

glutaraldehyde in 0.1 mol $l^{-1}$ phosphate buffer, pH 7.2. The specimens were vacuum-pumped six times for 5 min and left for 8 h at room temperature with gentle shaking and were washed with buffer and postfixed with 1% osmium tetroxide for 2 h. After dehydration with dimethoxypropane and acetone, the specimens were embedded in Spurr's resin and polymerized. Ultrathin sections were cut with a diamond knife and counterstained with uranyl acetate and lead citrate (2168 Ultrostainer; LKB, Bromma, Sweden). The sections were observed in a JEM-1010 (JEOL, Tokyo, Japan) transmission electron microscope at 60 kV.

### *Electrophysiology*

Single-unit recordings were performed using a tip-cutting technique (Kaissling, 1974; Van der Pers and Den Otter, 1978). The moth was restrained in holders cut from plastic pipette tips, and the head and antennae were fixed with dental wax. A sensillum on the antenna was cut by microscopic glass knives, and a recording glass electrode filled with Beadle-Ephrussi Ringer (128 mmol $l^{-1}$ NaCl, 4.69 mmol $l^{-1}$ KCl and 1.97 mmol $l^{-1}$ $CaCl_2$) was placed in contact with the cut surface of the sensillum. A grounded reference silver electrode was inserted into the abdomen. A binocular microscope with up to 300× magnification and two Leica micromanipulators (Wetzlar, Germany) were used to position the moths, the recording electrode and the glass knife. The antenna was continuously flushed with a charcoal-filtered and moisturized air stream through a glass tube (8 mm i.d.) at a speed of 0.5 m $s^{-1}$. The outlet of the tube was 10 mm from the antenna. The stimulus was injected into the air stream in the glass tube 150 mm upstream of the antenna. The stimulus was delivered in a 2.5 ml air puff for 0.5 s by a stimulus controller (SFC-1/b; Syntech, Hilversum, The Netherlands). Stimulus sources consisted of Pasteur pipettes containing 8×15-mm pieces of filter paper onto which each synthetic compound diluted in 10 μl hexane was applied. The stimulus pipettes were stored at –20°C when they were not in use and were renewed every third day.

In screening tests, a blank cartridge plus pipettes loaded with 10 μg of E12-, Z12- and Z9-14:OAc were tested. Stimuli were tested in random order and were applied with a 30 s interstimulus interval. If the quality of the contact was still acceptable, dose–response tests and/or cross-adaptation tests were performed. In the dose–response tests, $10^2$–$10^5$ ng of each compound were tested in decadic steps, starting with lowest doses. Cross-adaptation experiments were performed to determine whether neural activity could be attributed to the same neuron or to different neurons that produced similar-sized spikes (Kaissling et al., 1989; Kalinová et al., 2001). Using a Syntech SFC-2 stimulus controller with dual channels and single-unit recording as described above, a sensillum was stimulated twice for 0.5 s at 10 μg dosages with an interval of 0.2 s. For sensilla containing both large- and small-spiking neurons responding to E12- and Z12-14:OAc, the following stimulus pairs were tested: (1) E12-14:OAc/E12-14:OAc, (2) Z12-14:OAc/Z12-14:OAc, (3) E12-14:OAc/Z12-14:OAc and (4) Z12-14:OAc/E12-14:OAc. At the end of screening, dose–response and cross-adaptation tests, the antenna was stimulated with 10 μg of either E12-14:OAc or Z12-14:OAc to verify the initial neural activity. Data from sensilla containing neurons that failed to respond in a repeatable way at this stage were excluded from analysis.

The signal was amplified using a custom-built high-impedance amplifier with a low-pass/high-pass filter. During experiments, neural responses were visualized on an oscilloscope. The signal from the amplifiers was fed into a Syntech IDAC A/D converter and transferred to a Compaq ProLinea 4/66 computer (Houston, TX, USA) for analysis with the software program Syntech Auto Spike v. 3.0. The separation of individual neurons was based on differences in the amplitude of their action potentials. The response to a test compound was calculated as the number of spikes during the 0.5 s after stimulation minus the number of spikes during the 0.5 s before stimulation.

### *Field trapping*

Field-trapping experiments were performed at Tanashi (35.7°N, 139.5°E), Tokyo, Japan by methods described in Huang et al. (Huang et al., 1998a). Test chemicals were dissolved in hexane at a dosage of 100 μg and loaded on rubber septa (Aldrich Chemical Co., Milwaukee, WI, USA). Traps (Nitolure; Nitto Denko, Osaka, Japan) were hung 50 cm above the ground in an experimental block and were set 10 m apart from each other. The distance between blocks was more than 50 m. In the first series of experiments, the number of moths captured by traps baited with different blends of E12- and Z12-14:OAc during 19–29 May 1997 was compared at four experimental blocks. In the second series of experiments, a reference bait with the blend of 36% E12-14:OAc was compared with another bait with the same blend plus 1% Z9-14:OAc during 28 August–4 September 1997 in three experimental blocks.

## Results

### *Morphology*

The medial and lateral parts of the ventral surface of the male antenna were occupied by sensilla in *Ostrinia furnacalis*. The surface sculpturing was honeycomb-like on the ventral surface, whereas the dorsal surface was covered by scales. In the electrophysiological experiments, we discriminated medially situated sensilla from laterally situated ones based on a keel-like ventral surface of the antenna (Fig. 1A). According to morphological criteria, six types of sensilla occurred on the antenna: sensilla trichodea, s. basiconica, s. chaetica, s. auricillica, s. coeloconica and s. styloconica. The structure of the sensilla and their distribution patterns conform to patterns published in other crambids such as *Ostrinia* and *Chilo* species (Ren et al., 1987; Hallberg et al., 1994; Hansson et al., 1995).

The most common sensillum type was s. trichodeum (~80% of total sensilla). The trichoid sensilla were 30–60 μm long, with a basal diameter of ~3.0 μm. The wall thickness was

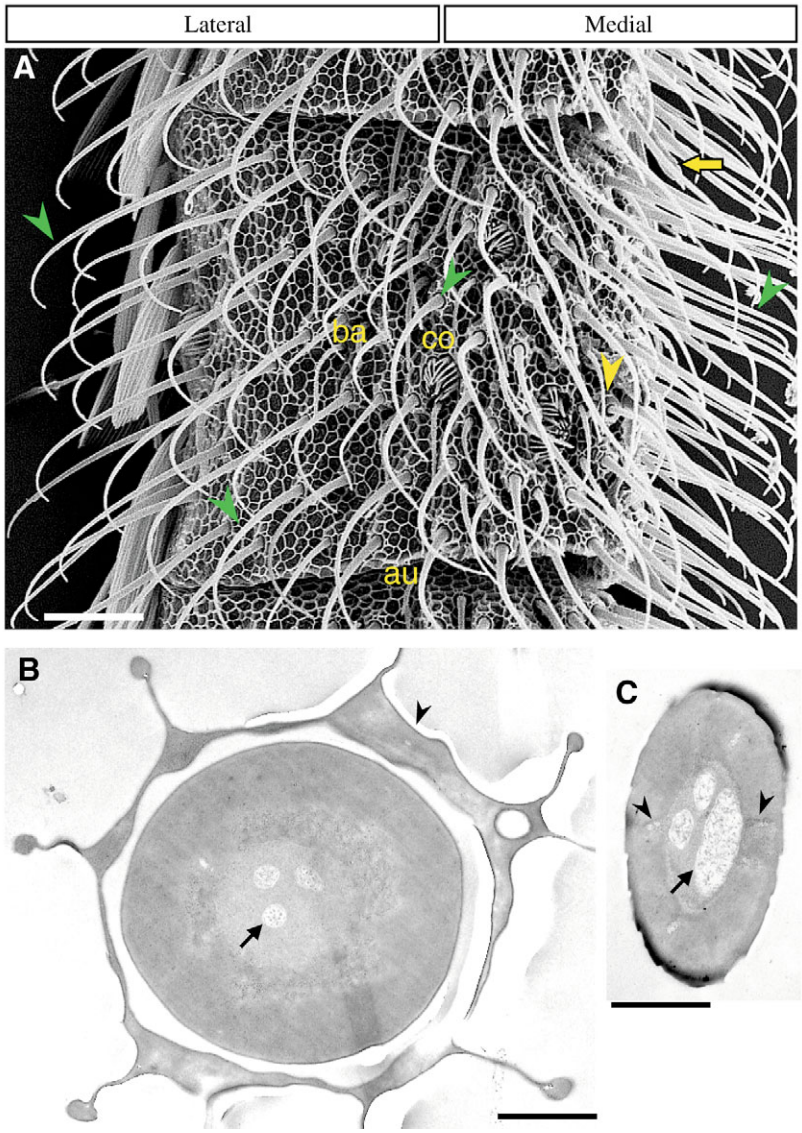


Fig. 1. Electron micrographs of the male antenna in *Ostrinia furnacalis*. (A) Scanning micrograph of the basal flagellomer of the antenna. Large numbers of sensilla trichodea (green arrowheads) occur at the medial and lateral part of the ventral surface of the antenna. Basiconic sensillum (ba), coeloconic sensillum (co), auricillic sensillum (au), chaetic sensillum (yellow arrowhead) and styloconic sensillum (yellow arrow, behind s. trichodeum) are also visible. For morphological details of the sensilla, see text. Note the honeycomb-like surface sculpturing of the antennal cuticle. Scales occur on the dorsal surface. Bar, 20 μm. (B) Transmission micrograph of a basal transverse section through a sensillum trichodeum containing three unbranched outer dendritic segments (arrow) in the sensillum cavity. Honeycomb-like surface cuticle (arrowhead) surrounds the sensillum. This region lacks wall pores. Bar, 1 μm. (C) Middle section of an s. trichodeum containing three unbranched outer dendritic segments (arrow). Wall pores (arrowheads) were seen as less electron-dense areas in the wall cuticle. Note that the sensillum was cut obliquely and that inner structures of the outer dendritic segments were unclear because of incomplete fixation. Bar, 1 μm.

~0.5 μm. No obvious variation in length was found between 12 sensilla situated medially (46.4–58.7 μm) and 12 situated laterally (41.9–58.7 μm) (Fig. 1A). At the base, s. trichodea were surrounded by a honeycomb-like surface and lacked wall pores. Unbranched dendritic outer segments were observed in this region (Fig. 1B). In the middle of the sensillum, pores penetrated the sensillum wall into the sensillum lymph. Branched and unbranched dendritic outer segments were observed at this height (Fig. 1C). Out of 59 trichoid sensilla observed on two male antennae, 57 were innervated by three sensory cells and two were innervated by two sensory cells.

Sensilla basiconica (Fig. 1A), the second most common sensillum type (~15%), had a shorter length (~25 μm), thinner wall with more numerous wall pores and profusely branched dendritic outer segments compared with s. trichodea. Chaetic, auricillic, styloconic and coeloconic sensilla were much less numerous compared with trichoid and basiconic sensilla (Fig. 1A). S. chaetica were hair-like and short with a distinct basal socket. Styloconic sensilla were located at the distal edge of the flagellomeres. Auricillic sensilla were similar in length to basiconic sensilla but were flattened. Coeloconic sensilla were of different external morphology with cuticular spines surrounding a sensory hair. These sensillum types all adhere to morphologies reported in other moth species (for a review, see Hansson, 1995).

### *Electrophysiology*

The quality of electrophysiological contacts was tested by stimulation of long trichoid sensilla on the ventral surface of the middle and basal antenna with the two pheromone components. In 105 contacted sensilla from 39 males, we obtained 71 recordings that lasted long enough for physiological characterization of the response to E12- and Z12-14:OAc (Figs 2, 3). In 47 recordings, a full characterization of the response to Z9-, E11- and Z11-14:OAc, in addition to E12- and Z12-14:OAc, was performed (Fig. 4; Table 1). Recordings typically revealed activity of two or three sensory neurons based on differences in action potentials with small, medium and large spike amplitudes. In 71 sensilla recorded, 67 had no background firing, whereas four sensilla had relatively low background firing prior to stimulation (mean number of spikes per 0.5 s: 1.75). Blank stimuli (hexane control) elicited no or very few spikes above background firing.

### *Responses to pheromone components (Types 1–4)*

In sensillum type 1 (28% of the tested sensillum population), a neuron characterized by a large spike amplitude always responded to both pheromone components in an identical (Mann–Whitney *U*-test, $P>0.05$), dose-dependent fashion (Fig. 3). A small spike amplitude

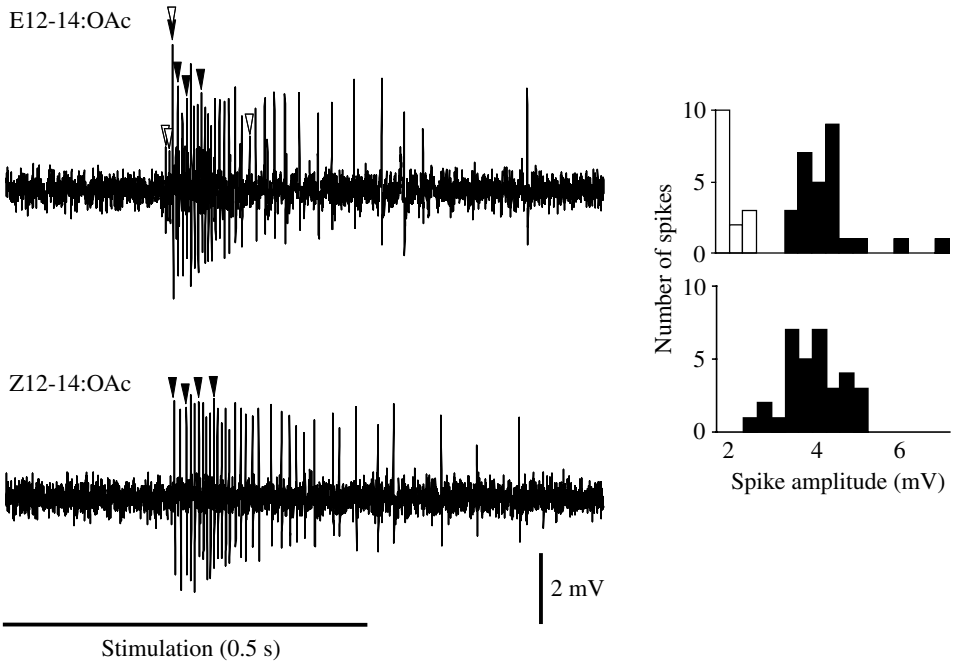


Fig. 2. Single sensillum recordings from sensillum type 2 (see Fig. 3) containing two olfactory receptor neurons. In spike-amplitude histograms on the right-hand side of recordings, the neurons are distinguished by having different spike amplitudes for large spike amplitude (filled columns) and small spike amplitude (white columns). The figure shows responses to the two major pheromone components (*E*)-12- and (*Z*)-12-tetradecenyl acetates (E12- and Z12-14:OAc) at a 10 μg loading. The two neurons, characterized by a large (filled arrowhead) and small (open arrowhead) spike amplitude, respond to E12-14:OAc. The large spiking neuron is also stimulated by Z12-14:OAc. The correctness of the characterizations was corroborated by differential adaptation experiments (see Fig. 5).

neuron also responded to both pheromone compounds but with a significantly higher affinity to E12-14:OAc. The response to E12-14:OAc was dose-dependent, whereas responses elicited by Z12-14:OAc were not.

In sensillum type 2 (38% of the tested sensillum population) the large spike neuron responded identically to type 1, i.e. to both pheromone components. The small spiking neuron responded exclusively to E12-14:OAc in a dose-dependent manner (Figs 2, 3).

In sensillum type 3 (31% of the tested sensillum population) a large spiking neuron again responded identically to type 1 and 2. No other responses to pheromone stimulation were registered, and no activity of a small spiking neuron was registered (Fig. 3).

In sensillum type 4 (3% of the tested sensillum population) the large spiking neuron responded exclusively to Z12-14:OAc at the 10 μg dosage. When the dosage was elevated to 100 μg, a weak response was also elicited by the E isomer. The small spiking neuron did not respond to any of the pheromone components at the 10 μg dosage but did respond to the E isomer at the 100 μg level (Fig. 3).

*Responses to interspecific signals (subtypes A–D)*

In three of the four pheromone-responding sensillum types (1–3), four subtypes (A–D) could be recognized depending on the response of associated neurons to the interspecific signals tested; Z9-, E11- and Z11-14:OAc (Fig. 4; Table 1). Type 4 sensilla occurred only as one subtype.

In subtype A (26%) the large spiking neuron excited by the pheromone components was also stimulated by E11- and Z11-14:OAc. The responses to Z9-, E11- and Z11-14:OAc were significantly different at the 100 μg level (Freidman test; $P<0.05$) but were all dose dependent. This sensillum type also housed a medium spiking neuron responding to stimulation by all the interspecific stimuli tested. No significant differences were found in responses to Z9-, E11- and Z11-14:OAc in the medium spiking neuron ($P>0.1$).

In subtype B (23%) the large spiking neuron did not respond to any of the interspecific signals. The medium spiking neuron response to all the interspecific signals was identical to that in subtype A.

In subtype C (30%) the large spiking neurons were again non-responsive to interspecific signals. The medium spiking neuron responded to Z9-14:OAc exclusively in a dose-dependent manner.

In subtype D (21%) no response was recorded after stimulation with interspecific signals.

*Cross-adaptation tests*

As some neurons were found to respond to E12- and Z12-14:OAc (Figs 2, 3), cross-adaptation tests of large and small spiking neurons housed in sensillum type 1 ($N=5$) were performed to clarify whether one or two neurons were present within each spike class (Fig. 5). Among the large spiking neurons, pre-exposure and subsequent stimulation with the same compound resulted in a significantly reduced response. In addition, crosswise adaptation resulted in the same phenomenon. This strongly indicates that the same neuron was excited by both pheromone components. Cross-adaptation tests of large spiking neurons in sensillum type 3 ($N=3$) produced the same results (data not shown).

Similarly, small spiking neurons of sensillum type 1 were adapted when stimulated by homologous pairs, although their responses to stimulation by Z12-14:OAc were weak (Fig. 5). In crosswise adaptation using E12- and Z12-14:OAc, the neurons showed a similar pattern as elicited by the same compound combination. However, these neurons did respond to E12-14:OAc after exposure to Z12-14:OAc, a response similar to the

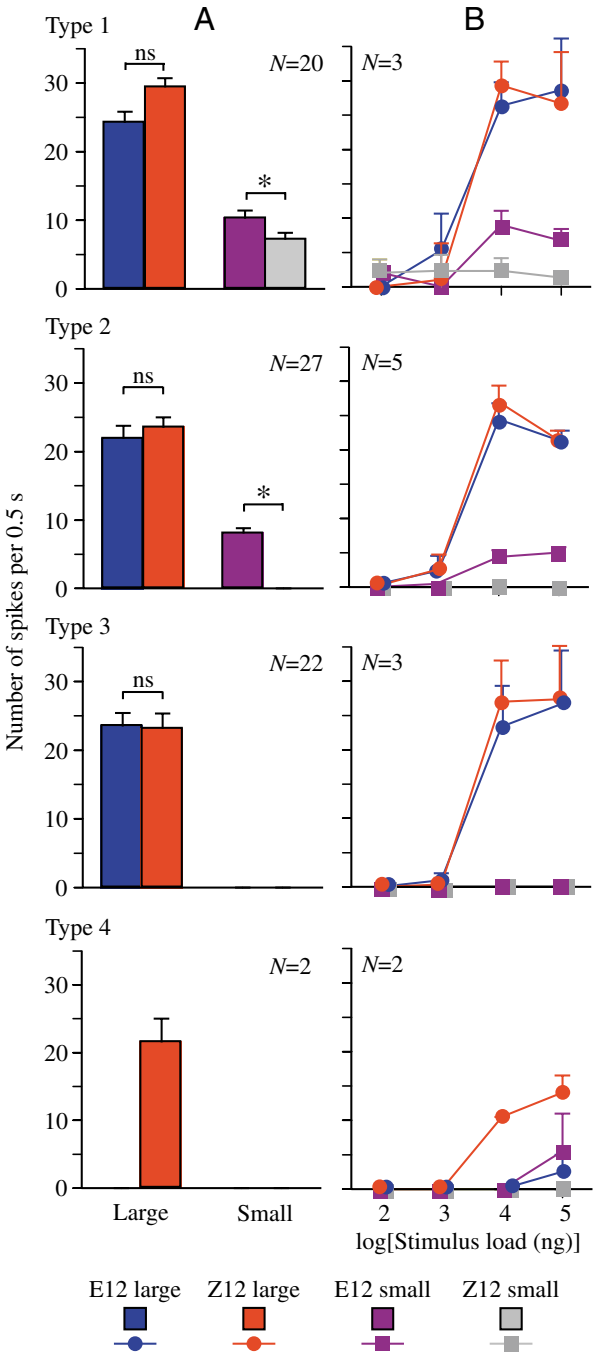


Fig. 3. Four different types of trichoid sensilla (1–4) characterized by their responses to E12- and Z12-14:OAc. Values showing the number of spikes per 0.5 s (means + s.e.m.) of receptor neurons with large and small spike amplitudes. (A) Responses of large and small spiking neurons housed within sensillum types 1–4 to E12-14:OAc (E12 large and E12 small) and to Z12-14:OAc (Z12 large and Z12 small) at dosages of 10 μg ($N$=71). *, significant difference between responses to E12- and Z12-14:OAc as tested by Mann–Whitney $U$-test ($P$<0.05); ns, not significant. (B) Dose–response curves were obtained from sensillum types 1–4 housing large and small spiking neurons responding to E12-14:OAc (E12 large and E12 small) and to Z12-14:OAc (Z12 large and Z12 small). The stimulus was loaded at 100 ng, 1 μg, 10 μg and 100 μg.

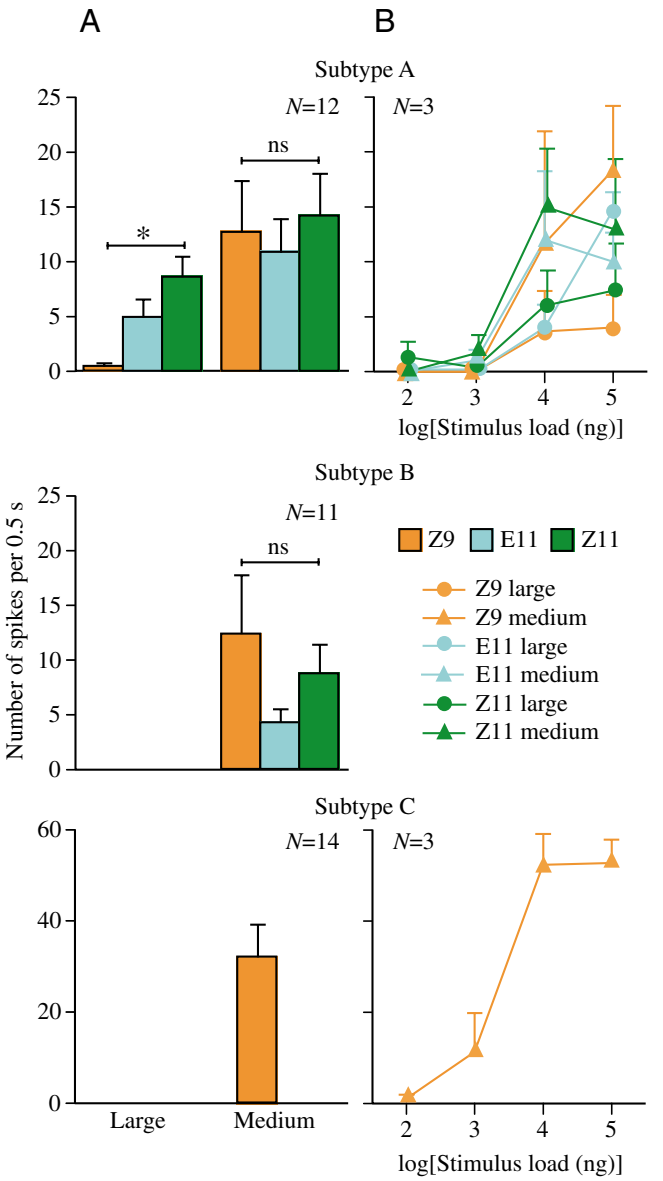


Fig. 4. Three subtypes of trichoid sensilla (A–C) characterized by their responses to Z9-, E11- and Z11-14:OAc. Values showing number of spikes per 0.5 s (means + s.e.m.) of receptor neurons with large and medium spike amplitudes. (A) Responses of large and medium spiking neurons housed within sensillum subtype A, B and C to Z9-, E11- and Z11-14:OAc (Z9, E11 and Z11) at a stimulus loading of 10 μg ($N$=47). *, significant difference among responses to E12-, Z12- and Z9-14:OAc as tested by Friedman test ($P$<0.05); ns, not significant. (B) Dose–response curves of large and medium spiking neurons housed in medially situated sensilla (subtype A) and of medium spiking neurons in laterally situated sensilla (subtype C) stimulated by E11-14:OAc, Z11-14:OAc and Z9-14:OAc, respectively (Z9 large, Z9 medium, E11 large, E11 medium, Z11 large and Z11 medium). The stimulus was loaded at 100 ng, 1 μg, 10 μg and 100 μg. Values showing spike frequencies per 0.5 s (means + s.e.m.). No dose–response curve data were obtained for subtype B.

first response to E12-14:OAc in the E12-14:OAc/Z12-14:OAc adaptation sequence. This response pattern could be expected, as the response to the E isomer was significantly higher in this neuron, as shown in the dose–response curve in Fig. 3B. Large and small spiking neurons had no background firing prior to stimulation and no response to blank stimuli.

*Topographical location of physiological types*

No topographical difference in distribution was found among the sensillum types 1–4. However, the different subtypes described above were found more or less frequently, depending on the topographical location of the sensillum on the antenna. Among medial sensilla, subtypes A and B were frequently found, whereas subtype C was common among lateral sensilla (Table 1). The frequencies of subtypes A (1 and 11 for lateral and medial sensilla, respectively), B (2 and 9), C (15 and 1) and D (2 and 6) differed significantly among lateral and medial parts [extended Fisher's exact test (Mehta and Patel, 1983); $P<0.001$].

*Field-trapping test*

In the first series of field-trapping tests, trap catches of *O. furnacalis* males with different blends of E12- and Z12-14:OAc were compared (Fig. 6A). Unbaited control traps caught no males. In traps baited with a single component, E12-14:OAc (100% E12-14:OAc) or Z12-14:OAc (0% E12-14:OAc), almost no males were captured either. In traps baited with 36% E12-14:OAc, the pheromone blend of *O. furnacalis* in Japan, the catch was significantly the highest, whereas traps with 10% and 90% E12-14:OAc caught less but these caught significantly more males than control traps.

In the second test, the effect of Z9-14:OAc on trap catches with the optimal blend of E12- and Z12-14:OAc was investigated (Fig. 6B). Z9-14:OAc significantly suppressed trap catches of males compared with the reference trap of 36% E12-14:OAc. This indicates that the electrophysiologically active Z9-14:OAc works as an antagonist for behavioral response to the pheromone blend.

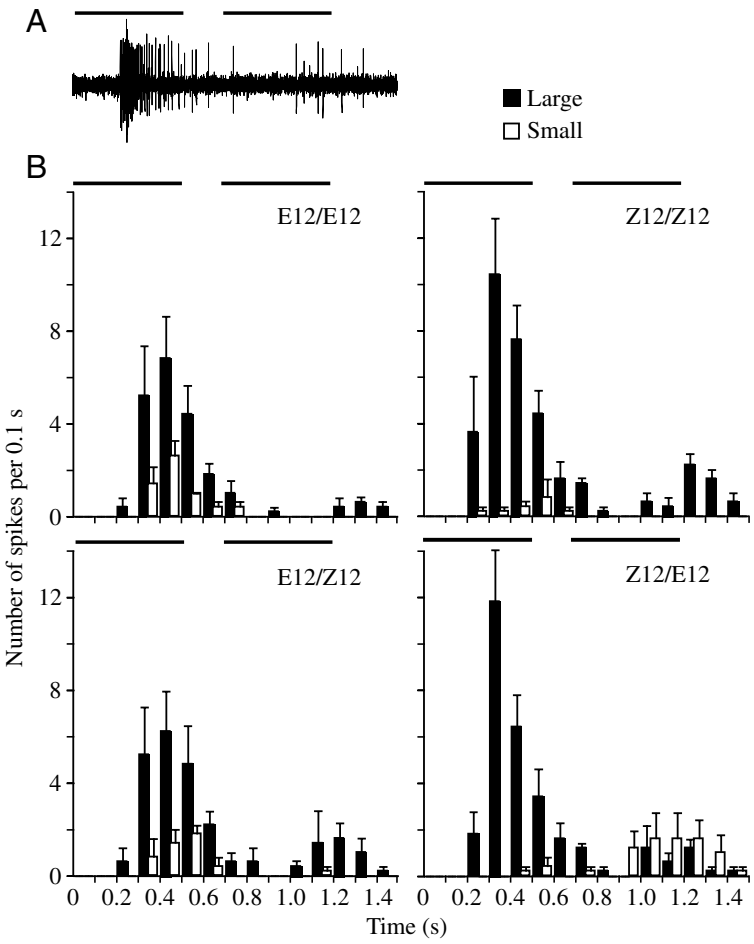


Fig. 5. Responses of large and small spiking neurons during cross-adaptation experiments. (A) Typical adapted response to two 0.5 s stimuli of E12-14:OAc (10 μg) with an interval of 0.2 s, indicated as horizontal bars. (B) Histograms showing number of spikes per 0.1 s (means + s.e.m.) of large and small spiking neurons (large and small) in sensillum type 1 ($N$=5) before and after adaptation. Two stimuli [E12-14:OAc (E12) and Z12-14:OAc (Z12) in different stimulus combinations] (10 μg) were presented successively. Note how the activity of the second stimulation is equally affected by prestimulation of both the same and the opposite isomer.

## Discussion

The male Asian corn borer, *Ostrinia furnacalis*, displays a highly sensitive peripheral detection system for female-produced sexual pheromone components. From the results presented above, it is clear that this system is unusual, compared with other moth species investigated. A major part of the peripheral coding system for the two principal pheromone components resides in neurons responding to both of these components. This condition contrasts with previous studies stating that insect pheromone receptor neurons are highly specific in their response spectrum (Van der Pers and Den Otter, 1978; Liljefors et al., 1987; Akers and O'Connell, 1991; Hansson et al., 1995; Mustaparta, 1997; Wojtasek et al., 1998; Kalinová et al., 2001; Larsson et al., 2002). This has been demonstrated in the closely related European corn borer, *O. nubilalis* (Hansson et al., 1987; Hansson et al., 1994; Cossé et al., 1995).

Adaptation studies further strengthened the result that one and the same large spiking neuron in sensillum types 1–3 responds to the two principal pheromone components E12- and Z12-14:OAc equally well (Figs 3, 5). The large spiking neurons were devoted to co-detection of the two pheromone components in 97% of the investigated sensilla, whereas the small spiking neurons were in 28% of those. Still, it is clear from the present and previous studies of field-trapping experiments that *O. furnacalis* males respond to the sex pheromone blend selectively and not at all to the single

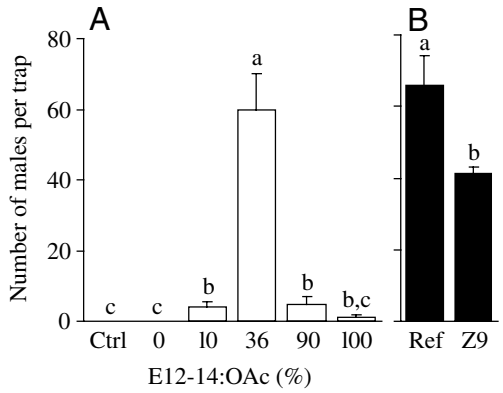


Fig. 6. Number of males (means + s.e.m.) captured by traps baited with different blends of E12-14:OAc and Z12-14:OAc (shown as % E12-14:OAc) during 19–29 May 1997 ($N$=4) (A). Number of males captured in traps baited with the optimal blend [36% E12-14:OAc (Ref)] as compared with those baited with the optimal blend plus 1% Z9-14:OAc (Z9) during 28 August–4 September 1997 ($N$=3) (B). The dosage used was 100 μg in each experiment. Bars with the same letters are not significantly different according to Tukey-Kramer's multiple-range test at the level of 5%. Data were transformed to log ($x$+1) prior to the statistical analysis. Ctrl, control traps.

components E12- and Z12-14:OAc (Huang et al., 1998a; Boo and Park, 1998). In addition, in wind-tunnel studies male Asian corn borers respond selectively to the correct pheromone blend (Zhou and Du, 1999). This means that the male Asian corn borer is indeed able to determine both the presence of each of the components and their specific ratio in relation to each other.

What then is the rationale behind this unusual system? There is a high premium for sensitivity in the moth pheromone detection system, i.e. to localize the female over long distances (Hansson et al., 1995). By using receptor neurons tuned to both pheromone components the male doubles the number of detecting units, as compared with when each neuron is specifically tuned to a single component. In this way, the sensitivity of the system should be higher than in the European corn borer, for example. However, the Asian corn borer male loses specificity. To solve the specificity problem, the system contains two crucial neuron types: small spike neuron in type 2 (2S) and large spike neuron in type 4 (4L). The 2S neuron responds specifically to the E isomer and the 4L to the Z isomer. These two neurons must thus be responsible for isomeric discrimination and ratiometric coding. The 2S neuron was present in 38% of the sensilla investigated, whereas the 4L was found in only 3%. To further strengthen E isomer identification, the small spike neuron in Type 1 shows a differential response to the components, with a significantly stronger response to the E isomer. The detection of E isomer thus seems to be robust, but is 3% of the sensillum population enough to allow reliable detection of the Z isomer and ratiometric coding? From earlier investigations we know that it is indeed sufficient, with a very low percentage of neurons detecting behaviorally crucial odors (Kalinová et al., 2001; Baker et al., 2004). In the turnip moth, *Agrotis segetum*, approximately 1–2% of the male olfactory receptor neurons are tuned to an important pheromone component, and in this case no other neurons respond to this specific component (Löfstedt et al., 1982; Hansson et al., 1990).

The pheromone detection system of *O. furnacalis* can thus be divided into two parts: one 'sensitivity' part formed by the non-specific pheromone-detecting neurons and one 'specificity' part formed by the two neuron types responding differentially to the two isomers. It thus seems as though evolution has 'allowed' the development of a specific part of the system towards less sensitivity. This could be the result of the unusual pheromone composition of *O. furnacalis*. No other species of insects are known to use E12- and/or Z12-14:OAc (Witzgall et al., 2004). Could it thus be the case that the specificity demands are less restrictive in a detection system when the signals are highly specific and not used by any other species in the communication channel? A similar case of less restrictive specificity of pheromone receptor neurons has been reported in *Yponomeuta rorellus*, which use another unusual pheromone component, saturated tetradecyl acetate (Löfstedt et al., 1990).

Z9-, E11- and Z11-14:OAc are not included in sex pheromone gland extracts of *O. furnacalis* (Ando et al., 1980) but elicited clear responses in male olfactory receptor neurons. Similar to the pheromone-detecting neurons, some neurons displayed a very broad response profile to Z9-, E11- and Z11-14:OAc. Medium spiking neurons present in sensillum subtypes A and B responded equally well to Z9-, E11- and Z11-14:OAc. In sensillum subtype C the medium spiking neurons responded specifically to Z9-14:OAc and had a considerably higher sensitivity than other neurons responding to this compound.

Our field-trapping experiments showed that Z9-14:OAc works as an antagonist for behavioral responses toward a conspecific pheromone with E12- and Z12-14:OAc in *O. furnacalis* (Fig. 6). This compound also works as a behavioral antagonist in *O. nubilalis* and *O. scapulalis* (Glover et al., 1989; Ishikawa et al., 1999a). In addition, Z9-14:OAc is a sex pheromone component in *O. zaguliaevi* and *O. zealis* (Huang et al., 1998b; Ishikawa et al., 1999a). Z9-14:OAc may have played an important role in pre-mating isolation between *Ostrinia* species. E11- and Z11-14:OAc, which are also pheromone components of closely related *Ostrinia* species (Huang et al., 1997; Ishikawa et al., 1999b), may have played a similar role for *O. furnacalis*. *O. furnacalis* and *O. nubilalis* have been shown to detect the antagonist Z9-14:OAc by specifically tuned receptor neurons (present study) (Hansson et al., 1987; Cossé et al., 1995). Several other moth species are known to have neurons responding to pheromone components of their closely related species as behavioral antagonists, presumably for pre-mating isolation (Löfstedt et al., 1991; Mustaparta, 1997; Larsson et al., 2002).

When contemplating detection systems for behavioral antagonists the demand for sensitivity remains the same as for pheromone detection. The reasoning regarding specificity can, however, be more or less the opposite. One unspecific neuron,

Table 1. *Response spectrum, frequency and location of sensillum types 1–4 stimulated with E12- and Z12-14:OAc (E12 and Z12) and sensillum subtypes A–D stimulated with Z9-, E11- and Z11-14:OAc (Z9, E11 and Z11) including large (L), medium (M) and small (S) spiking neurons*

| Sensillum | | | Stimuli | | | | | *N* | |
|---|---|---|---|---|---|---|---|---|---|
| Type | Subtype | Neuron | E12 | Z12 | Z9 | E11 | Z11 | Lateral | Medial |
| 1 | A | L | ++ | ++ | + | + | + | 0 | 7 |
| | | M | 0 | 0 | + | + | ++ | | |
| | | S | + | + | 0 | 0 | 0 | | |
| 1 | B | L | ++ | ++ | 0 | 0 | 0 | 2 | 1 |
| | | M | 0 | 0 | ++ | + | + | | |
| | | S | + | + | 0 | 0 | 0 | | |
| 1 | C | L | ++ | ++ | 0 | 0 | 0 | 2 | 1 |
| | | M | 0 | 0 | ++ | 0 | 0 | | |
| | | S | + | + | 0 | 0 | 0 | | |
| 1 | D | L | + | + | 0 | 0 | 0 | 0 | 1 |
| | | S | + | + | 0 | 0 | 0 | | |
| 2 | A | L | ++ | ++ | 0 | + | + | 1 | 3 |
| | | M | 0 | 0 | ++ | + | + | | |
| | | S | + | 0 | 0 | 0 | 0 | | |
| 2 | B | L | ++ | ++ | 0 | 0 | 0 | | |
| | | M | 0 | 0 | + | + | + | 0 | 4 |
| | | S | + | 0 | 0 | 0 | 0 | | |
| 2 | C | L | ++ | ++ | 0 | 0 | 0 | 5 | 0 |
| | | M | 0 | 0 | ++ | 0 | 0 | | |
| | | S | + | 0 | 0 | 0 | 0 | | |
| 2 | D | L | ++ | ++ | 0 | 0 | 0 | 2 | 1 |
| | | S | + | 0 | 0 | 0 | 0 | | |
| 3 | A | L | ++ | ++ | 0 | ++ | + | 0 | 1 |
| | | M | 0 | 0 | + | + | + | | |
| 3 | B | L | ++ | ++ | 0 | 0 | 0 | 0 | 4 |
| | | M | 0 | 0 | + | + | + | | |
| 3 | C | L | ++ | ++ | 0 | 0 | 0 | 6 | 0 |
| | | M | 0 | 0 | ++ | 0 | 0 | | |
| 3 | D | L | ++ | ++ | 0 | 0 | 0 | 0 | 4 |
| 4 | C | L | 0 | ++ | 0 | 0 | 0 | 2 | 0 |
| | | M | 0 | 0 | ++ | 0 | 0 | | |

Location is given as lateral or medial placement of the sensillum on the antenna. For detailed data for types 1–4, see Fig. 3. For subtypes A–C, see Fig. 4. Subtype D did not contain any neurons responding to Z9-, E11- or Z11-14:OAc. ++, strong response by receptor neuron after stimulation with a 10 μg stimulus (mean number of spikes per 0.5 s is more than 17); +, mean number of spikes per 0.5 s is less than 16; 0, no response; *N*, number of observed sensillum types with respect to lateral and medial parts of the antenna.

tuned to all antagonists, can serve as a general antagonist detector. The only message that needs to be conveyed is to abort flight towards the source, irrespective of which antagonist is present. The antagonist neurons in subtypes A and B (Fig. 4) thus make perfect sense in being non-specific. The neuron in subtype C specific to Z9-14:OAc shows higher sensitivity than the neuron to other potential antagonists, but can this antagonist be more important than the others? In one neuron type, the large spiking neuron in type 1, subtype A (Fig. 3; Table 1), conditions were further complicated. In the neuron types, both the sex pheromone components, E12- and Z12-14:OAc, and the potential antagonists, E11- and Z11-14:OAc, were stimulating. This neuron thus seems to transcend the absolute border in specificity; between attractant and their antagonists. A similar function, with a neuron responding to both pheromone components and antagonists,

has been indicated in *Yponomeuta rorellus* (Löfstedt et al., 1990).

*O. furnacalis* has a peculiar coding system for its two major pheromone components, whereas the close relative *O. nubilalis* has specialized neurons tuned to each pheromone component, as reported in many other moth species (Hansson et al., 1987; Akers and O'Connell, 1991; Mustaparta, 1997; Kalinová et al., 2001; Larsson et al., 2002). In rare cases, males of *O. nubilalis* show behavioral response to E12- and Z12-14:OAc (Roelofs et al., 2002; Linn et al., 2003), suggesting that their neurons can sometimes respond to the compounds of *O. furnacalis*. In addition, *O. furnacalis* has neurons responding to E11- and Z11-14:OAc. It thus seems that pheromone-detecting neurons of *Ostrinia* species can inherently respond to isomers of 11- and 12-14:OAc, but the response specificity to the compounds differs dramatically among the species. The *Ostrinia* species complex thus offers an excellent system for future studies of the evolution of pheromone communication systems, both at the sender and receiver levels.

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