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Broadly and narrowly tuned odorant receptors are involved in female sex pheromone reception in *Ostrinia* moths☆

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ABSTRACT

Mate-finding communication in many moths is mediated by sex pheromones produced by females. Since the differentiation of sex pheromones is often associated with speciation, it is intriguing to elucidate how the changes in sex pheromones are tracked by the pheromone recognition system of the males. Moths of the genus *Ostrinia*, which show distinct differentiation in female sex pheromones, are good models to study this. The present study was initiated with the aim of identifying ORs from *Ostrinia scapulalis* that respond to its own pheromone components, (*E*)-11- and (*Z*)-11-tetradecenyl acetates. We isolated six OR gene candidates (*OscaOR3–8*) from *O. scapulalis*. The same set of genes homologous to *OscaOR3–8* were conserved in all (eight) *Ostrinia* species examined in addition to the previously reported OscaOR1 (tuned to (*E*)-11-tetradecenol) and the Or83b homologue OscaOR2. OscaOR3 not only responded to (*E*)-11- and (*Z*)-11-tetradecenyl acetates, but also to the pheromone components of the congeners, (*Z*)-9-, (*E*)-12-, and (*Z*)-12-tetradecenyl acetates. OscaOR4 responded with a relatively high specificity to (*E*)-11-tetradecenyl acetate. While OscaOR5 responded only marginally to a few pheromone components, OscaOR6–8 did not respond to any of the compounds tested. A few conserved ORs, including a unique one with very broad responsiveness, appear to be involved in the sex pheromone reception in *O. scapulalis*. The findings of the present study are discussed with reference to knowledge on electrophysiological response profiles of olfactory receptor neurons in *Ostrinia* moths.

1. Introduction

In many moths, mate-finding communication is mediated by the sex pheromones produced by females, which are usually a blend of a few compounds (Witzgall et al., 2004). The species specificity of pheromones is conferred by the combination and blend ratio of components, and the males' response is tuned to the specific blend to facilitate finding of conspecific females. Since the differentiation of sex pheromones is often associated with speciation, it is intriguing to know how the changes in female sex pheromones have been tracked by the pheromone recognition system of the males.

Moths of the genus *Ostrinia* (Lepidoptera: Crambidae), which show distinct differentiation in sex pheromones (Ishikawa et al., 1999b), are good materials for studying the evolution of the pheromone recognition system. Among the 21 species of the genus *Ostrinia* recorded worldwide (Mutuura and Munroe, 1970; Ohno, 2003a), the sex pheromones of nine species have been characterized to date (Fig. 1). The following six compounds have been found as the components of sex pheromones produced by females; Z9-14:OAc, E11-14:OAc, Z11-14:OAc, E12-14:OAc, Z12-14:OAc and E11-14:OH (Roelofs et al., 1985; Ishikawa et al., 1999b; Takanashi et al., 2000; Ohno, 2003b). Here it should be noted that Z9-14:OAc, a sex pheromone component of *Ostrinia zaguliaevi* and *Ostrinia zealis*, functions as a behavioral antagonist against *Ostrinia scapulalis*, *Ostrinia nubilalis* and *Ostrinia furnacalis* (Fig. 1) (Glover et al., 1989; Huang et al., 1998; Ishikawa et al., 1999a; Takanashi et al., 2006).

Abbrevations: 14:OAc, tetradecyl acetate; Z9-14:OAc, (*Z*)-9-tetradecenyl acetate; E11-14:OAc, (*E*)-11-tetradecenyl acetate; Z11-14:OAc, (*Z*)-11-tetradecenyl acetate; E12-14:OAc, (*E*)-12-tetradecenyl acetate; Z12-14:OAc, (*Z*)-12-tetradecenyl acetate; E11-14:OH, (*E*)-11-tetradecenol; Z11-16:OAc, (*Z*)-11-hexadecenyl acetate; Z11-16:Ald, (*Z*)-11-hexadecenal; DMSO, dimethyl sulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered saline; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-polymerase chain reaction.

☆ The nucleotide sequences reported in this paper have been submitted to the DDBJ/GenBank™/EBI Data Bank with accession numbers shown in supplementary material Table S1.

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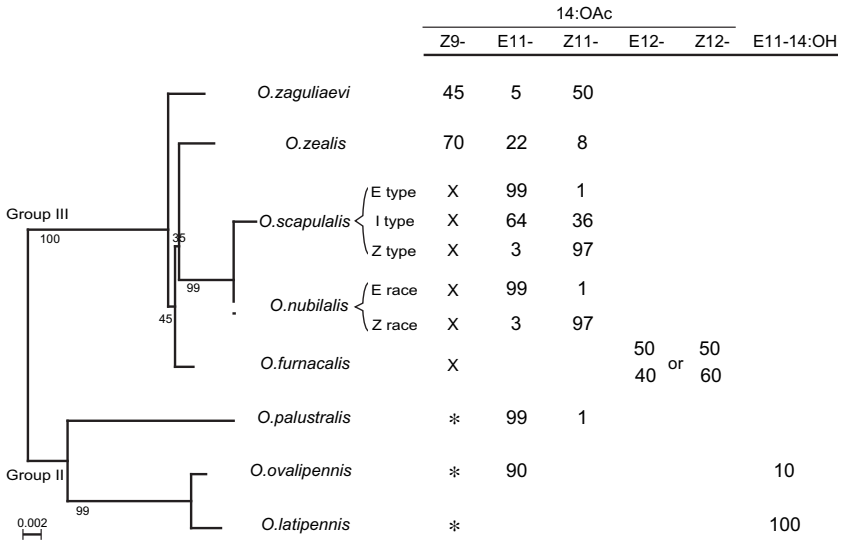

Fig. 1. Phylogenetic tree and sex pheromone components of the moths of genus *Ostrinia*. The left figure is a tentative neighbor-joining tree constructed based on mitochondrial COII gene sequences. The right table shows sex pheromone components and their ratios. The symbol X denotes that the component works as a behavioral antagonist. *Not tested for behavioral antagonism.

The adzuki bean borer *O. scapulalis*, the species mainly focused on in the present study, uses a mixture of E11-14:OAc and Z11-14:OAc as a sex pheromone (Huang et al., 2002). In this species, three sex pheromone blend phenotypes have been identified, type E (E isomer: Z isomer ≈ 99:1), type Z (E:Z ≈ 3: 97), and their intermediate, type I (E:Z ≈ 64: 36) (Huang et al., 1997; Huang et al., 2002). These three types are mainly determined by an autosomal locus with two alleles, E^{sca} and Z^{sca} (Takanashi et al., 2005).

To detect and track the sex pheromones emitted by the conspecific females, males should have a system that can discriminate pheromone components and their ratios. In this system, pheromone receptors in the olfactory sensilla of male moths should play an important role in the specific recognition of pheromone molecules. Recently, information on the molecular characteristics of pheromone receptors in moths has amassed rapidly (Krieger et al., 2004; Sakurai et al., 2004; Krieger et al., 2005; Nakagawa et al., 2005; Gohl & Krieger, 2006; Syed et al., 2006; Kurtovic et al., 2007; Mitsuno et al., 2008). Along with the characterization of pheromone receptors, the essential role of Or83b family proteins, co-expressed with odorant receptors (ORs) in most olfactory receptor neurons (ORNs), has been demonstrated (Vosshall et al., 2000; Krieger et al., 2003; Larsson et al., 2004; Benton et al., 2006; Sato et al., 2008; Wicher et al., 2008).

In a previous study, we isolated a gene encoding a male-specific odorant receptor (*OscaOR1*) and an *Or83b* homologue (*OscaOR2*) from E-type *O. scapulalis*. Unexpectedly, *Xenopus* oocytes co-expressing OscaOR1 and OscaOR2 specifically responded to E11-14:OH, a sex pheromone component of the congener *Ostrinia latipennis*, not of *O. scapulalis* (Miura et al., 2009). The present study was initiated with the aim of identifying ORs from male *O. scapulalis* that respond to E11- and Z11-14:OAc, the sex pheromone components produced by conspecific females. Six new candidate odorant receptor genes (*OscaOR3–8*) isolated from the antennae of E-type male *O. scapulalis* were subjected to functional assays. In parallel, homologues of *OscaOR3–8* were cloned from seven other *Ostrinia* species to examine if these ORs are conserved in this genus. The findings of the present study are discussed with reference to vast knowledge on electrophysiological response profiles of ORNs in the pheromone-sensitive sensilla on the male antennae of *O. nubilalis* and *O. furnacalis*.

2. Materials and methods

2.1. Insects

In the present study, E-type *O. scapulalis* maintained as a culture in our laboratory was mainly used for the investigation of ORs. Males of *O. scapulalis* were considered to have ORs of E11-14:OAc and Z11-14:OAc, the two pheromone components used in this species, irrespective of their pheromone type. The *O. nubilalis* culture used was of the Z race. Seven species of *Ostrinia* were reared as reported previously (Huang et al., 1997; Huang et al., 1998; Ishikawa et al., 1999a; Takanashi et al., 2000; Fukuzawa et al., 2004). One-day-old adults and the 5th instar larvae were used in the present study.

2.2. RNA extraction and cDNA synthesis

Total RNA was isolated from the antennae, legs, proboscis, testes, head, midgut and fat body of an individual of *Ostrinia* using RNeasy Micro Kit (Qiagen, Hilden, Germany). The first-strand cDNA was synthesized with Takara RNA PCR kit (AMV) Ver.3.0 or a PrimeScript 1st strand cDNA Synthesis kit (Takara Bio, Shiga, Japan). The quality of the cDNA was verified by testing the PCR amplification of the beta-actin gene using as primers, sBACT, AACTGGGATGACATG GAGAAGATCTGGC, and aBACT, GAGATCCACATCTGCTGGAAGGTGGA CA (Miura et al., 2005).

2.3. RT-PCR and cloning of OscaORs

A fragment of cDNA encoding odorant receptors of *O. scapulalis* (*OscaORs*) was amplified from the first-strand cDNA, which was prepared from the antennae of an E-type male moth of *O. scapulalis*, using Ex Taq DNA polymerase (Takara bio) and the following degenerate primers designed based on the consensus sequences of ORs of *Bombyx mori* (BmORs) and *Heliothis virescens* (HRs): sORA (TGGGSNCAYYTNAARAT), sORB (TNAYNCCNATGTWYMAYAA), sORC (TGGGSNCAYYTNMGNAT), sORD (ACNYTNTGGGGNCAYYT), aORA (TCCCANGGNARNNNRTANAC), aORB (TCCCANGGNACNNNRT ANAC), aORC (RAARTANWSNATNWSNGTYTT), aORD (GTNAGNGG NCCRTANC), and aORE (GTYAANGGNCCRTANC). The amplification was performed according to the following program: 2 min at 94 °C;