論文の内容の要旨

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論文題目：Studies on the sex pheromone biosynthesis in *Ostrinia scapulalis* with special reference to the sex pheromone gland specific desaturases

（アズキノメイガにおける性フェロモン生合成に関する研究）

In many moths, females produce a sex pheromone to attract conspecific males. Sex pheromones thus play a very important role in pre-mating reproductive isolation among moth species. To date, sex pheromones of more than 1,500 moths have been chemically characterized. Moth pheromones are usually a blend of a few chemical components, and the species specificity is conferred by the combination of components and their blend ratios. Therefore, regulation of sex pheromone blend in females is crucial for the existence of a species. Despite the importance, the regulation of sex pheromone synthesis in female moths, the mechanism of blend ratio control in particular, is not well understood.

The genus *Ostrinia* Hübner (Lepidoptera: Crambidae) worldwide includes 21 species, among which eight species inhabit Japan. The adzuki bean borer moth *O. scapulalis*, one of the eight species, uses a blend of (*E*)-11- and (*Z*)-11-tetradecenyl acetates (E11- and Z11-14:OAc) as the sex pheromone, whereas the Asian corn borer moth *O. furnacalis*, a congener found in Japan, uses a blend of the positional isomers, (*E*)-12- and (*Z*)-12-tetradecenyl acetates (E12- and Z12-14:OAc). In this thesis, I studied sex pheromone biosynthesis in *O. scapulalis* with special reference to the pheromone-gland-specific desaturase,

one of the key enzymes that determine the carbon chain configuration of the pheromone components. In parallel, I also investigated the genetic regulation of pheromone biosynthesis by crossing *O. scapulalis* with *O. furnacalis*, which uses pheromone components distinctly different from those of *O. scapulalis*.

1. Fatty acid sex pheromone analogs (FAPA) in *Ostrinia scapulalis*

Most of the lepidopteran sex pheromone components are derived from common fatty acids through chain-shortening, desaturation, reduction, and then acetylation (in case of acetates) or oxidation (in case of aldehydes). Unsaturated fatty acids that have the same carbon chain configuration as the sex pheromone components are often called as fatty acid pheromone analogs (FAPAs). Since FAPAs are found specifically in the pheromone gland, these compounds have been assumed to be the immediate precursors of the sex pheromone components. Although control of FAPA production is likely to underlie the control of pheromone production, the dynamics of FAPA biosynthesis remained to be studied.

(*E*)-11- and (*Z*)-11-tetradecenoic acids are FAPAs of *O. scapulalis*, which are produced from myristic acid (C14) by the function of Δ11-desaturase. I measured the titers of FAPAs in the pheromone gland for three days after the emergence. The titers started to increase at the beginning of the scotophase, reaching a peak at the end of the same phase. FAPAs decreased continuously during the photophase, and started to increase again in the next scotophase. This diel pattern was repeated for three days. These findings suggest that biosynthesis of FAPAs is under photoperiodic control, as in the case of sex pheromone biosynthesis. Strong suppression of the increase of FAPA titers by decapitation suggested that signals from cephalic organs play an important role in the control of FAPA biosynthesis.

O. scapulalis is known for the intraspecific pheromone blend polymorphism; E-type females produce a pheromone with a 99:1 blend of E11- and Z11-14:OAc, whereas Z-type females produce a pheromone with an opposite blend (E:Z = 3:97). I found that, regardless of the pheromone blend phenotypes, E/Z ratio in the FAPA geometric isomers was stably maintained at about 7:3. This finding suggests that E- and Z-type *O. scapulalis* use the same pathway for the production of FAPAs, and the blend ratio is regulated at a step in the downstream of FAPA biosynthesis, i.e., either reduction or acetylation.

2. cDNA cloning and *in situ* hybridization of Δ11-desaturase in *O. scapulalis*

Sex pheromones are considered to be produced in a specialized tissue, the "pheromone gland" located in the terminal abdominal segments (8th–10th, TAS) of a moth; however, in many moth species the cells that produce pheromones have not actually been specified. I investigated cells in the TAS that synthesize pheromones in the adzuki bean borer *Ostrinia scapulalis*, by locating pheromones and their precursors, and mRNA for ∆11-desaturase, a key enzyme in pheromone biosynthesis. I demonstrated that the pheromone components, E11- and Z11-14:OAc, and their fatty acyl precursors were specifically contained in the dorsal part of the TAS. From the TAS of E-type *O. scapulalis* females, I then cloned a 1217-base cDNA of ∆11-desaturase gene (*OscZ/E11*, AB232855) that included a complete open reading frame encoding 329 amino acid residues. The deduced amino acid sequence has three histidine boxes which are highly conserved within acyl-CoA desaturases in animals and yeast. RT-PCR and *in situ* hybridization unequivocally showed that *OscaZ/E11* is specifically expressed in the modified epidermal cells located at the dorsal end of the 8th–9th intersegmental membrane.

3. The same set of desaturases are expressed in the pheromone glands of *O. scapulalis* and *O. furnacalis*

While Δ11-desaturase is involved in the pheromone biosynthesis in many moth species including *O. scapulalis*, involvement of Δ14-desaturase is only known in *O. furnacalis*. A cDNA of Δ14-desaturase gene (*OfuZ/E14*) has been cloned from *O. furnacalis*. Interestingly, although Δ11-desaturase is not utilized in *O. furnacalis,* transcript of Δ11-desaturase gene has been found in the pheromone gland of this species. Analysis of the cDNA (*OfuZ/E11*) has shown a very high homology to *OscZ/E11*. In this chapter, I first examined whether or not Δ14-desaturase gene is expressed in *O. scapulalis*, which do not use this enzyme for the pheromone production. I found that Δ14-desaturase gene is expressed in *O. scapulalis*, and I was able to clone a cDNA of this gene from this species (*OscZ/E14*), which showed a very high homology to *OfuZ/E14*. Analyses of sex pheromone gland extracts by gas chromatography – mass spectrometry showed that (*E*)-11- and (*Z*)-11-tetradecenoic acids, the expected products of OfuZ/E11, were not detected in the pheromone gland of *O. furnacalis.* Likewise, any traces of E14-16:Acid, Z14-16:Acid, E12-14:Acid and Z12-14:Acid, the expected products of OscZ/E14 and their derivatives, were not detected in *O. scapulalis*. These findings suggest that functional OfuZ/E11 protein is not present in the pheromone gland of *O. furnacalis*, and functional OscZ/E14

protein is not present in *O. scapulalis*. Non existence of OfuZ/E11 in *O. furnacalis* was finally demonstrated by immunocytochemical analysis of the pheromone gland.

4. Alternative use of two pheromone-gland-specific desaturases in *O. scapulalis* and *O. furnacalis*

To gain an insight into the regulation of sex pheromone biosynthesis in *O. scapulalis*, which uses E11- and Z11-14:OAc as the pheromone components, and *O. furnacalis*, which uses E12- and Z12-14:OAc, I crossed these two species and investigated the female sex pheromone composition in F1, F2 and backcross progenies. Surprisingly, all F1 hybrid females predominantly produced saturated tetradecyl acetate, with a small amount of the parental components. Tetradecyl acetate has not been regarded as the sex pheromone component of any *Ostrinia* species examined to date. Analyses of the pheromone gland components in the backcross and F2 progenies have shown that the pheromone phenotypes were divided into *O. scapulalis*-like, *O. furnacalis*-like and F1-like. Based on the frequencies of the phenotypes in the backcross and F2 progenies, the variation in the phenotype was found to be explainable by the existence of a major autosomal locus with two alleles.

In the pheromone gland of F1 females, the expression level of Δ11-desaturase gene was comparable to that in *O. scapulalis*, a parent that uses Δ11-desaturase for pheromone biosynthesis, and the level of Δ14-desaturase gene transcript was comparable to that of *O. furnacalis*, the other parent that uses Δ14-desaturas. Nevertheless, the titters of FAPAs, the products of these enzymes, were remarkably low. Taking all findings in the previous and present chapters together, it is inferred that *O. scapulalis* has a factor that inhibits production of Δ14-desaturase protein, and that *O. furnacalis* reciprocally has a factor that inhibits production of Δ11-desaturase protein.

In this thesis, I studied sex pheromone biosynthesis in *O. scapulalis* in comparison with that in a close relative *O. furnacalis*, with special reference to the pheromone-gland-specific desaturases. I clarified that two species have the same set of desaturase genes (Δ11- and Δ14-), but only one of them is functionally expressed in each species, resulting in a production of completely different set of pheromone components.