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Comparative study of sex pheromone composition and biosynthesis in *Helicoverpa armigera*, *H. assulta* and their hybrid

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Abstract

Two *Helicoverpa* species, *H. armigera* and *H. assulta* use (*Z*)-11-hexadecenal and (*Z*)-9-hexadecenal as their sex attractant pheromone components but in opposite ratios. Since both female and male interspecific hybrids produced by female *H. assulta* and male *H. armigera* have been obtained in our laboratory, we can make a comparative study of sex pheromone composition and biosynthesis in the two species and their hybrid. With GC and GC–MS analyses using single gland extracts, the ratio of (*Z*)-9-hexadecenal to (*Z*)-11-hexadecenal was determined as 2.1:100 in *H. armigera*, and 1739:100 in *H. assulta*. The hybrid has a ratio of 4.0: 100, which is closer to that of *H. armigera*, but significantly different from *H. armigera*. We investigated pheromone biosynthesis with labeling experiments, using various fatty acid precursors in *H. armigera*, *H. assulta* and the hybrid. In *H. armigera*, (*Z*)-11-hexadecenal is produced by Δ11 desaturation of palmitic acid, followed by reduction and terminal oxidation; (*Z*)-9-hexadecenal results from Δ11 desaturation of stearic acid, followed by one cycle of chain shortening, reduction and terminal oxidation. Δ11 desaturase is the unique desaturase for the production of the two pheromone components. In our Chinese strain of *H. assulta*, palmitic acid is used as the substrate to form both the major pheromone component, (*Z*)-9-hexadecenal and the minor one, (*Z*)-11-hexadecenal. Our data suggest that Δ9 desaturase is the major desaturase, and Δ11 desaturase is responsible for the minor component in *H. assulta*, which is consistent with previous work. However, the weak chain shortening acting on (*Z*)-9 and (*Z*)-11-octadecenoic acid, which is present in the pheromone glands, does occur in this species to produce (*Z*)-7 and (*Z*)-9-hexadecenoic acid. In the hybrid, the major pheromone component, (*Z*)-11-hexadecenal is produced by Δ11 desaturation of palmitic acid, followed by reduction and terminal oxidation. The direct fatty acid precursor of the minor component, (*Z*)-9-hexadecenoic acid is mainly produced by Δ9 desaturation of palmitic acid, but also by Δ11 desaturation of stearic acid and one cycle of chain shortening. The greater relative amounts of (*Z*)-9-hexadecenal in the hybrid are due to the fact that both palmitic and stearic acids are used as substrates, whereas only stearic acid is used as substrate in *H. armigera*. The evolutionary relationships between the desaturases in several *Helicoverpa* species are also discussed in this paper.

Keywords: Noctuidae; *Helicoverpa armigera*; *Helicoverpa assulta*; Interspecific hybrid; Sex pheromone biosynthesis; Desaturases

1. Introduction

Sex pheromones produced by female moths are generally 12- to 18-carbon straight chain compounds, usually containing an oxygenated functional group (acetate ester, alcohols, or aldehydes) and zero to three double bonds (Tamaki et al., 1985). Moths rely on variation of sex pheromones in the chain length, the type of oxygenated functional group, and the number, location, and isomeric nature of the double bonds, as well as the precise ratios of components in multi-component pheromones to promote reproductive isolation. Closely related moth species or strains usually share common sex pheromone components but have

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different component ratios. Comparisons of pheromone communication systems of closely related species or strains have given insight into how diversification of pheromone blends may have occurred (e.g. Roelofs and Comeau, 1969; Tumlinson et al., 1974; Klun et al., 1979; Roelofs and Brown, 1982; Löfstedt et al., 1991). Further, pheromone studies involving moth hybridization as well as their pheromone biosynthesis have been extremely informative to elucidate the nature of genetic changes that have occurred (Roelofs et al., 1987; Löfstedt et al., 1989). However, information on pheromone genetics from pheromone biosynthesis in parent species and their hybrids is limited to a study of regulation of the specific ratio of the sex pheromone components in two pheromone strains of the European corn borer, *Ostrinia nubilalis*, and the intraspecific hybrid (Zhu et al., 1996). The sex pheromone of E strain of *O. nubilalis* consists of E11-14:OAc[1] with 1–3% Z11-14:OAc, whereas the sex pheromone of the Z strain of *O. nubilalis* consists of the approximately opposite blend. The female hybrids produce a 70:30 ratio between the two acetates (Klun and Maini, 1979; Roelofs et al., 1987). The *in vivo* experiments with labeled FAs as pheromone precursors suggest that the reductase system converts the E11 and Z11-14:Acid to the corresponding alcohols rather than other biosynthetic enzymes regulating the specific ratios of the two pheromone components in the two strains and their hybrid (Zhu et al., 1996).

In the genera *Helicoverpa* and *Heliothis*, almost all species investigated so far, use Z9-16:Ald and Z11-16:Ald as their sex attractant pheromone, which is necessary for eliciting male attraction to the conspecific female, with Z11-16:Ald being the major component in the ratio, except for *Helicoverpa assulta* in which Z9-16:Ald dominates (Arn et al., 2001; Cork et al., 1992). *H. armigera* and *H. assulta* are sympatric, closely related species in and around China. In *H. armigera*, the ratio of the major pheromone component, Z11-16:Ald and the minor component, Z9-16:Ald varied from 100:6.5 to 100:2.5 (Nesbitt et al., 1980; Wu et al., 1997; Dunkelblum et al., 1980; Kehat and Dunkelblum, 1990). The variation could have been caused by collection of experimental insects from different areas. An approximately opposite ratio (7:100) of the two components was produced by females of *H. assulta* in China as their sex attractant pheromone (Liu et al., 1994) and this ratio was shown to vary with insect distribution and the pheromone release times in a light–dark cycle, but Z9-16:Ald was always a dominant component (Cork et al., 1992; Park et al., 1996). With species-specific ratios of the same sex pheromone components, these closely related species achieve reproduction isolation. A major question is how the species-specific ratio of the pheromone components is regulated. It has been shown that the desaturation of saturated FAs, one of the key steps in pheromone biosynthesis of *H. zea* and *H. assulta*, plays a major part in such regulation. In *H. assulta*, Δ9 desaturase is the major desaturase, which acts on palmitic acid to form Z9-16:Acid, the precursor of the main pheromone component Z9-16:Ald. Also Δ11 desaturase catalyses the same substrate to produce Z11-16:Acid which is then converted to the minor component, Z11-16:Ald. In *H. assulta* pheromone glands, no β-oxidation (limited chain shortening) activity was detected (Choi et al., 2002). However, the occurrence of *Z*7-16:Ald and *Z*7-16:OAc as minor components of pheromone blends from a Japanese strain of *H. assulta* suggests that residual β-oxidation of the *Z*9-18:Acid precursor probably occurs in this species in some geographic regions (Jeong et al., 2003). In *H. zea*, Δ11 desaturase acts on palmitic and stearic acid, the Z9-16:Acid is mainly produced by chain shortening of Z11-18:Acid, and finally the opposite ratio of the two pheromone components is produced (Choi et al., 2002).

Wang and Dong (2001) discovered that *H. armigera* could be hybridized with *H. assulta*. Only male but not female offspring was produced by the cross of female *H. armigera* and male *H. assulta*. However, the reverse cross (female *H. assulta* × male *H. armigera*) produced both fertile male and female offspring with a sex ratio of nearly 1:1. This discovery gives us a chance to make a comparative study of pheromone composition and pheromone biosynthetic pathways in both sibling species and their hybrid. In this study, in addition to pheromone composition, we report on the pheromone biosynthetic pathway in *H. armigera*, *H. assulta* and the interspecific hybrid by labeling experiments using various FA precursors. We were especially interested in determining how Δ9 and Δ11 desaturases are involved in the pheromone biosynthesis in the hybrid.

2. Materials and methods

2.1. Insect sources and pheromone extraction

Laboratory cultures of *H. armigera* and *H. assulta* were collected in Zhengzhou, Henan province of China. The larvae were reared on an artificial diet (Wang and Dong, 2001) at 26 ± 1 °C under a reversed 16:8 light:-

[1]Abbreviations used: Pheromone compounds and fatty acyl moieties are abbreviated in a standard way including (in order) geometry of the double bond, position of unsaturation, chain length followed by a colon and functionality. For example, Z5-12:Ald is (*Z*)-5-dodecenal, Z9-16:OH is (*Z*)-9-hexadecenol, Z9-16:OAc is (*Z*)-9-hexadecenyl acetate, Z9-16:Me is methyl (*Z*)-9-hexadecenoate. FA = fatty acid; ME = methyl ester; FAME = fatty acid methyl ester; DMSO = dimethylsulfoxide; D_3-16:Acid = [16,16,16 $^2H_3$]-hexadecanoic acid; D_3-Z11-16:Ald = [16,16,16 $^2H_3$]-(*Z*)-11-hexadecenal; D_3-18:Acid-[18,18,18-$^2H_3$]-octadecanoic acid; D_4-Z11-18:Acid = [15,15,16,16-$^2H_4$]-(*Z*)-11-octadecenoic acid. GC = gas chromatography; GC-MS = gas chromatography- mass spectrometry; PBAN = pheromone biosynthesis activating neuropeptide.