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ORIGINAL PAPER

Behavioral and electrophysiological responses of *Helicoverpa assulta*, *H. armigera* (Lepidoptera: Noctuidae), their F_1 hybrids and backcross progenies to sex pheromone component blends

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Abstract Two sibling species, *Helicoverpa assulta* and *Helicoverpa armigera* both use (*Z*)-9-hexadecenal and (*Z*)-11-hexadecenal as their sex pheromone components but in almost reversed ratios, 93:7 and 3:97, respectively. *H. assulta* and *H. armigera* males performed upwind flight in response to the *H. assulta* sex pheromone blend (93:7). *H. armigera* responded strongly to the *H. armigera* blend (3:97), whereas *H. assulta* males remained inactive upon exposure to this blend. Both species gave clear dose-dependent electrophysiological responses to (*Z*)-11-hexadecenal. However, (*Z*)-9-hexadecenal evoked strong dose-dependent electrophysiological responses in *H. assulta* males but not in *H. armigera*. The two male F_1 hybrids exhibited similar behavioral responses to two sex pheromone blends and electrophysiological responses to two pheromone components as *H. armigera* males. This indicated that *H. armigera* genes appear dominant in determining the behavioral response and electrophysiological responses. Behavioral and electrophysiological responses of backcrosses of male F_1 hybrids (*H. armigera* female × *H. assulta* male) with female *H. assulta* and *H. armigera* were close to that of *H. assulta* and *H. armigera*, respectively. However, backcrosses of female F_1 hybrids (*H. assulta* female × *H. armigera* male) with male *H. assulta* and *H. armigera* showed reduced behavioral responses but normal electrophysiological responses compared to males of the respective parental line.

Keywords *Helicoverpa assulta* · *Helicoverpa armigera* · Sex pheromone · Electroantennogram · Wind tunnel

Abbreviations

AS	*Helicoverpa assulta*
AR	*Helicoverpa armigera*
EAG	Electroantennogram
F_1SR	*H. assulta* female × *H. armigera* male
F_1RS	*H. armigera* female × *H. assulta* male
*Z*5-12:Ac	(*Z*)-5-dodecenyl acetate
*Z*5-10:Ac	(*Z*)-5-decenyl acetate
*Z*7-12:Ac	(*Z*)-7-dodecenyl acetate
*Z*9-14:Ald	(*Z*)-9-tetradecenal
*Z*9-16:Ald	(*Z*)-9-hexadecenal
*Z*11-16:Ald	(*Z*)-11-hexadecenal
16:Ald	Hexadecanal
*Z*9-16:Ac	(*Z*)-9-hexadecenyl acetate
*Z*11-16:Ac	(*Z*)-11-hexadecenyl acetate
16:Ac	Hexadecenyl acetate
*Z*9-16:OH	(*Z*)-9-hexadecen-1-ol
*Z*11-16:OH	(*Z*)-11-hexadecen-1-ol
16:OH	Hexadecenol

Introduction

Two closely related species, the oriental tobacco budworm *Helicoverpa assulta* (Guenée) and the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) are key pests of crops in China and

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many other countries (Fitt 1989; Chen 1999). They occur sympatrically in China. Their phenology overlaps from middle May to middle October during which period five generations occur. However, *H. assulta* is an oligophagous species using several species in the family Solanaceae as hosts, such as tobacco and pepper, *H. armigera* is a widespread polyphagous pest using more than 60 crops such as cotton, corn, wheat, soybean, tomato and other solanaceous species as host plants (Fitt 1989; Chen 1999). To enable the use of the female sex pheromone to monitor and control the two pests, their sex pheromones have been isolated and identified (Piccardi et al. 1977; Nesbitt et al. 1979; Nesbitt et al. 1980; Kehat et al. 1980; ; Sugie et al. 1991; Cork et al. 1992; Liu et al. 1994; Boo et al. 1995; Wu et al. 1997). The composition of the sex pheromone differs between the two species. Chemical components in the sex pheromone gland extract of female *H. assulta* were identified as hexadecanal (16:Ald), (*Z*)-9-hexadecenal (*Z*9-16:Ald), (*Z*)-11-hexadecenal (*Z*11-16:Ald), hexadecyl acetate (16:Ac), (*Z*)-9-hexadecenyl acetate (*Z*9-16:Ac), (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac), hexadecane-1-ol (16:OH), (*Z*)-9-hexadecen-1-ol (*Z*9-16:OH), (*Z*)-11-hexadecen-1-ol (*Z*11-16:OH) (Cork et al. 1992; Liu et al. 1994). The sex pheromone gland components of female *H. armigera* are 16:Ald, *Z*9-16:Ald, *Z*11-16:Ald, (Z)-9-tetradecenal (*Z*9-14:Ald), 16:OH, and *Z*11-16:OH (Kehat and Dunkelblum 1990; Wu et al. 1997). Results from field studies showed that the major sex pheromone components of *H. assulta* and *H. armigera* are *Z*9-16:Ald and *Z*11-16:Ald, but the ratio of the two components is reversed between the two species and a slight variation among geographical populations of each species has been documented (Table 1). The ratio of *Z*9-16:Ald and *Z*11-16:Ald in the sex pheromone gland extract from Korean *H. assulta* is 93:7, and a 95:5 blend of the two components was the most attractive to *H. assulta* in the field (Cork et al. 1992). The ratio of *Z*9-16:Ald and *Z*11-16:Ald in the pheromone gland extract of *H. armigera* was 2:98 in Israel and 4:96 in Shandong, China, respectively (Kehat and Dunkelblum 1990; Wu et al. 1997), and a 3:97 blend showed the strongest attraction for males of *H. armigera* in the field (Kehat and Dunkelblum 1990; Wu et al. 1997). Peak activity of calling by *H. assulta* females was found to occur from the third hour to the fifth hour in the scotophase, but that of *H. armigera* was from the fifth hour to the seventh hour (C.-Z. Wang, Y.-H. Yan, H.-L. Wang, unpublished data) The species-specific sex pheromone component ratio might play a key role in maintaining reproductive isolation between these two related species. The successful interspecific hybridization between *H. assulta* and *H. armigera* in our laboratory provided an opportunity to investigate the genetic basis pheromone communication of the two species (Wang and Dong 2001). In the present study, we compared the responses of male moths to sex pheromones of *H. assulta* and *H. armigera*, and investigated male behavioral and electrophysiological responses of F_1 hybrids and backcross progenies to sex pheromone components.

Materials and methods

Insects

Larvae of *H. assulta* and *H. armigera* were collected from a tobacco field of Xuchang and a cotton field of Anyang, Henan province of China, respectively. They were reared in a climate chamber for many generations at a temperature of 27±1°C and relative humidity 60–80% with 16L:8D photoperiod. Larvae were fed with artificial diet described by Wu and Gong (1997).

Table 1 Ratios of *Z*9-16:Ald and *Z*11-16:Ald in *H. assulta* and *H. armigera* from different localities

Species	Ratios for extract	Ratios for attractant	Origin	References
H. assulta	97:3	97:3	Japan	Sugie et al. (1991)
H. assulta	93:7	95:5	Korea	Cork et al. (1992)
H. assulta	83:17	88:12	Thailand	Cork et al. (1992)
H. assulta	–	98:2 and 83:17	Hunan (China)	Cork et al. (1992)
H. assulta	94:6	93:7	Beijing (China)	Liu et al. (1994)
H. assulta	93:7	–	Henan (China)	Yan et al. (unpublished)
H. armigera	2:98	2.5:97.5	Israel	Kehat and Dunkelblum (1990)
H. armigera	4:96	3:97	Shandong (China)	Wu et al. (1997)
H. armigera	3:97	–	Henan (China)	Yan et al. (unpublished)

Ratios for extract: ratios of *Z*9-16:Ald to *Z*11-16:Ald in sex pheromone gland extract. *Ratios for attractant*: ratios of blends of *Z*9-16:Ald and *Z*11-16:Ald that were most attractive for males. The ratios shown here were converted from original data in references with the total amount of *Z*9-16:Ald and *Z*11-16:Ald set at 100

Male and female pupae were separated. Adults always had access to a 10% honey solution in water.

Reciprocal crosses, *H. assulta* (AS) female × *H. armigera* (AR) male and *H. armigera* female × *H. assulta* male were made by placing females and males in a cylindrical cage (diameter 15 cm, height 15 cm) to produce two lines of hybrids, F_1SR and F_1RS, respectively. Backcross offspring were obtained by mating F_1 hybrids with both parental species.

Chemicals

The pheromone compounds used were *Z*9-16:Ald and *Z*11-16:Ald and were purchased from Shin-estu Company (Tokyo, Japan). The purity upon purchase was 95% and further purification to 99% was done using a silica gel column. Verification was made by capillary column gas chromatograph, BP-20 (i.d. 0.22 mm, length 25 m). *N*-hexane and paraffin oil (Sigma) were used as solvents for behavioral and electroantennogram (EAG) tests, respectively. Blends of *Z*9-16:Ald and *Z*11-16:Ald with ratios of 93:7 and 3:97 were used as the sex pheromones of *H. assulta* and *H. armigera*, respectively.

Wind tunnel behavioral assays

The 2–4-day-old virgin males were tested during the fourth–sixth hour of their scotophase in a clear plexiglass wind tunnel (2.5 m long, 1 m wide, 1 m high). The conditions in the wind tunnel were 22–25°C, 40–60% relative humidity and 0.3 lux of red light. The air speed was 30 cm/s. Prior to behavioral observation, the males were moved to the flight-tunnel room from the beginning of scotophase to acclimatise to these conditions. Rubber septa were loaded with 10 µl of a 100 µg/µl solution of the blends of *Z*9-16:Ald and *Z*11-16:Ald with ratios of 93:7 and 3:97. The impregnated septum was hung on a hook as pheromone source at the upwind end of the tunnel, 40 cm above its floor. Each male was released downwind 2 m from the pheromone source and 30 cm above the tunnel floor from a mesh cage (10 cm long and 5 cm in diameter). The intensity of male response to the pheromone source was estimated by (1) the proportion of males that initiated flight; (2) the proportion that performed upwind flight, zigzagging in the pheromone plume; (3) the proportion that flew to within 10 cm of pheromone source; (4) the proportion that contacted the pheromone source, relative to the number of males released. Males that did not take off from the release cage within 5 min after introduction into the wind tunnel were not scored and were replaced by new moths.

Electroantennogram recording

The EAG technique was applied as described by Visser (1979). The antenna of 2–3-day-old virgin males was cut at the base of the flagellum. The tip of the terminal segment was removed. The antennal preparation was made under a stereomicroscope under cold light illumination. The antenna was fixed between two glass electrodes filled with Kaissling's haemolymph solution (Kaissling 1995). The indifferent electrode was inserted into the base of the antennal preparation and the tip connected with the recording electrode. Silver wires, coated with AgCl, were inserted in the glass electrodes and connected with the input probe which was mounted on a micromanipulator (Syntech MP-12, Hilversum, The Netherlands) and then connected with an AC/DC amplifier (Syntech UN-06). The signal from the DC amplifier was sent to a computer. Syntech EAG-software v2.6c was used to record the EAG response.

A continuous air flow of 30 ml/s was produced by a stimulus controller (Syntech CS-05) and led over the antennal preparation through a glass tube (i.d. 1.2 cm) the outlet of which was at 1 cm from the antennal preparation. The stimuli were delivered from a Pasteur pipette. A filter paper (0.5 cm×5 cm) loaded with 10 µl solution of the pheromone components was put into the Pasteur pipette. One end of the Pasteur pipette (i.d. 7 mm) was linked to a second outlet of the stimulus controller. The other end of the Pasteur pipette (i.d. 2 mm) was inserted into a hole of the glass tube delivering the air stream to the preparation. The two single components *Z*9-16:Ald and *Z*11-16:Ald and two blends, with ratios of 93:7 and 3:97 were dissolved into paraffin oil and diluted to 1, 10^1, 10^2, 10^3, 10^4 and 10^5 ng/µl. Paraffin oil was used as blank. The sequence of stimulus delivery was from low to high concentration with 1-min intervals. Each antennal preparation was stimulated by only one stimulus, either a pure chemical or a blend. The duration of stimulation was 0.1 s. The blank was applied at the start and at the end of a stimulation series, and the average of EAG amplitudes of the blank was subtracted from the EAG amplitude of the stimuli.

Statistical analyses

The proportions of males performing each behavior were subjected to χ^2 2×2 test of independence with Yates' continuity correction using software Poptools 2.6.2 (Hood 2004). EAG data were analyzed by the one-way ANOVA for analysis of variance, and the least significant difference (LSD) test was used for means multiple comparisons. Independent sample *t* test was used for determining the difference in means between *Z*9-16:Ald and

*Z*11-16:Ald at each dose in each species. Both ANOVA and *t* test were performed using SPSS 11.01 (SPSS 2001). Statistical significance was determined at the P=0.05 level.

Results

Interspecific hybridization between *H. assulta* and *H. armigera*

The reciprocal crossings between AS and AR produced different results. The crossing of females *H. assulta* with males *H. armigera* produced viable and fertile F_1SR hybrids. The F_1RS hybrid derived from the reverse cross of females *H. armigera* with males *H. assulta* included fertile males and abnormal individuals, but no fertile females. The abnormal F_1 hybrid individuals have no penis and valve, but have *H. armigera*-male-like color pattern on their forewings. Backcrosses from F_1SR female × AS male, F_1SR female × AR male, AS female × F_1RS male, and AR female × F_1RS male were obtained. In the backcross of AR female × F_1RS male, there were some sterile and abnormal individuals besides normal male and female offspring. Due to low fertility of the F_1SR hybrid, F_2 progeny could not be generated.

Behavioral response to sex pheromone blends

The four sequential behavioral responses of males of AS, AR, the hybrids, and backcross lines to the sex pheromone blend of *H. assulta*, a 93:7 blend of *Z*9-16:Ald and *Z*11-16:Ald are shown in Fig. 1a. More than 70% of *H. assulta* males initiated flight from the mesh cage, 59% showed upwind flight, 41% flew up to within 10 cm of the pheromone source, and 15% reached the pheromone source. For males of *H. armigera*, 64% initiated flight, 36% flew upwind, 32% approached to the source, and 14% reached the source. No significant difference in full-flight behavioral responses to *H. assulta* sex pheromone blend occurred between males of *H. assulta* and *H. armigera*. The percentages for males of F_1SR that flew close to the pheromone source and reached it were 60 and 32%, respectively, which proportions were significant higher than that of *H. assulta* or *H. armigera* (Fig. 1a). Of the males of hybrid F_1RS 35% flew closely to and 10% reached the *H. assulta* sex pheromone blend, which percentages were similar to that of both parental species (Fig. 1a). A significantly lower percentage of various male backcrosses flew up to the *H. assulta* pheromone source than that of both parental species and both F_1 hybrids (Fig. 1a). Male backcrosses from AS female × F_1RS male and AR female × F_1RS male exhibited proportions that reached the source that were similar to that of *H. assulta* and *H. armigera*. A low percentage of male backcrosses of F_1SR female × AS male and no male backcrosses of F_1SR female × R male reached the *H. assulta* pheromone source (Fig. 1a).

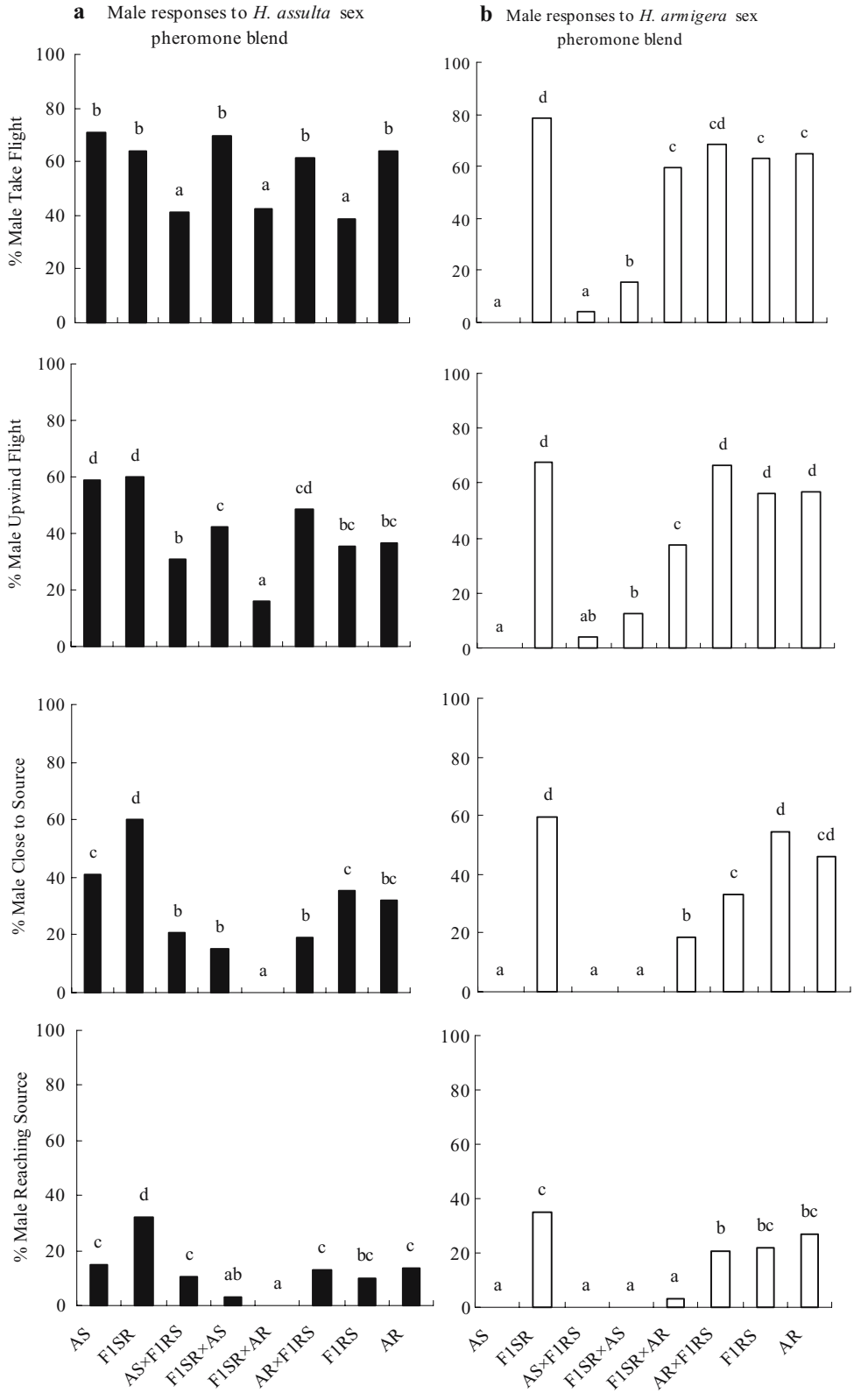
When the sex pheromone blend of *H. armigera*, 3:97 blend of *Z*9-16:Ald and *Z*11-16:Ald, was used as pheromone source in the wind tunnel, males of *H. assulta* remained inactive upon exposure and showed none of the sequential behavioral responses (Fig. 1b). For males of *H. armigera*, 65% of the males initiated flight from the mesh cage, 57% flew upwind, 46% came up to within 10 cm of the source, and 27% reached the source (Fig. 1b). Males of both F_1 crosses showed complete flights to this pheromone source in proportions similar to those displayed by males of *H. armigera* (Fig. 1b). Few male backcross offspring of AS female × F_1RS male and F_1SR female × AS male took off from the mesh cage in the plume of *H. armigera* sex pheromone blend. No males of offspring of both these backcrosses showed flight behavior up to the *H. armigera* sex pheromone source until reaching the source, which was similar to the behavior recorded for *H. assulta* males. However, males from backcrosses of AR female × F_1RS male and F_1SR female × AR male exhibited complete flights to the *H. armigera* sex pheromone source. The levels of behavioral responsiveness of males of AR female × F_1RS male were close to that of *H. armigera*, while the percentages of males of F_1SR female × AR male that flew up to and reached this pheromone source were significantly lower than that of *H. armigera* (Fig. 1b).

Electrophysiological responses to sex pheromone blends

Dose–response profiles of males of *H. assulta*, *H. armigera* and both F_1 crosses to blend ratios of 93:7 and 3:97 are compared in Fig. 2a and b, respectively. No significant differences in responses to the 93:7 blend at high concentrations occurred among males of *H. assulta*, *H. armigera* and the F_1SR hybrid (Fig. 2a). The responses of F_1RS males were significantly lower than those of *H. assulta* and *H. armigera* at 10^4 and 10^5 ng/μl (Fig. 2a). Males of *H. assulta*, *H. armigera* and both F_1 crosses displayed similar dose–response profiles to the 3:97 blend (Fig. 2b).

Electrophysiological responses to single sex pheromone components

In males of *H. assulta*, both *Z*9-16:Ald and *Z*11-16:Ald evoked dose-dependent EAG responses, but EAG response to *Z*9-16:Ald was higher in the dose range



10^3–10^5 ng/μl (Fig. 3a). In males of *H. armigera*, dose-dependent EAG responses were observed to *Z*11-16:Ald whereas *Z*9-16:Ald evoked weak responses even at 10^5 ng/μl (Fig. 3b). EAG-responses of both F_1 progenies to the two single components were similar to each other (Fig. 3c, d). The abnormal F_1RS hybrid

Fig. 1 Percentage of males performing four sequential behaviors in response to the sex pheromone blends of (*Z*)-9-hexadecenal (*Z*9-16:Ald) and (*Z*)-11-hexadecenal (*Z*11-16:Ald) with ratios of 93:7 and 3:97. *Take Flight* males initiated flight leaving the mesh release cage. *Upwind Flight:* males flew zigzagging in the pheromone plume to the pheromone source. *Close to Source:* males approached within 10 cm from the pheromone source. *Reaching Source:* males contacted the pheromone source. Different letters indicated with the bars in the same behavioral category indicate values that are significantly different according to χ^2 2×2 test of independence with Yates' correction (P=0.05). **a** Behavioral responses to the sex pheromone of *H. assulta*, 93:7 blend (*black bar*). AS, males of *H. assulta*, n=34. AR, males of *H. armigera*, n=22. F_1SR, males of F_1 hybrid *H. assulta* female × *H. armigera* male, n=25. F_1RS, males of F_1 hybrid *H. armigera* female × *H. assulta* male, n=31. AS × F_1RS, males from backcross AS female × F_1RS male, n=29. F_1SR × AS, males from backcross F_1SR female × AS male, n=33. F_1SR × AR, males from backcross F_1SR female × AR male, n=19. AR × F_1RS, males from backcross AR female × F_1RS male, n=31. **b** Behavioral responses to the sex pheromone of *H. armigera*, 3:97 blend (*white bars*). AS, males of *H. assulta*, n=25. AR, males of *H. armigera*, n=37. F_1SR, males of F_1 hybrid *H. assulta* female × *H. armigera* male, n=37. F_1RS, males of F_1 hybrid *H. armigera* female × *H. assulta* male, n=46. AS × F_1RS, males from backcross AS female × F_1RS male, n=25. F_1SR × AS, males from backcross F_1SR female × AS male, n=32. F_1SR × AR, males from backcross F_1SR female × AR male, n=32. AR × F_1RS, males from backcross AR female × F_1RS male, n=48

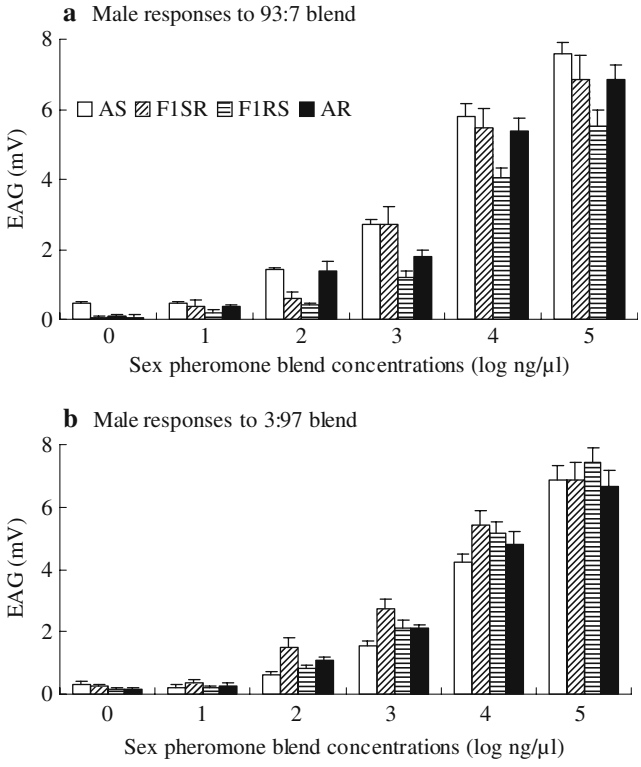


Fig. 2 Electrophysiological responses of males to different sex pheromone blend concentration of *Z*9-16:Ald and *Z*11-16:Ald with ratios of 93:7 and 3:97. **a** Responses to 93:7 blend. **b** Responses to 3:97 blend. The *bars* indicate standard error of means (SEM). AS (*white bars*), n=12. AR (*black bars*), n=12. F_1SR (*diagonal bars*), n=6. F_1RS (*horizontal bars*), n=12. Abbreviations of insect lines refer to Fig. 1

individuals derived from crossing females *H. armigera* and males *H. assulta* showed no EAG responses to *Z*9-16:Ald and *Z*11-16:Ald (Fig. 3e).

EAG response amplitudes of males of *H. assulta, H. armigera*, their F_1 hybrids and backcross progenies to *Z*9-16:Ald and *Z*11-16:Ald at a dose rate of 10^5 ng/µl were compared in Fig. 4a and b. EAG response amplitude of *H. assulta* males to 10^5 ng/µl *Z*9-16:Ald was significantly higher than that of *H. armigera* (Fig. 14a); the response of males of both F_1 hybrids from the reciprocal crossing between *H. assulta* and *H. armigera* was the same as that of *H. armigera* males (Fig. 4a). The response of males of backcrosses between AS female × F_1RS male and that between F_1SR female × AS male were significantly higher than that of *H. armigera* (Fig. 4a). Males of backcross of F_1SR female × AS male produced a response similar to that of *H. assulta*, while males of AS female × F_1RS male produced a lower response. In males of F_1SR female × AR male and AR female × F_1RS male, the responses to *Z*9-16:Ald were similar to that of *H. armigera* (Fig. 4a).

EAG response amplitude of *H. assulta* males to 10^5 ng/µl *Z*11-16:Ald was significantly lower than that of *H. armigera* (Fig. 4b). Males of F_1 hybrids F_1SR and F_1RS exhibited the same response to *Z*11-16:Ald, which was intermediate between that of *H. assulta* and *H. armigera* (Fig. 4b). EAG responses to *Z*11-16:Ald of males of the backcrosses between AS female × F_1RS male and F_1SR female × AS male were similar to *H. assulta* males and responses of males of the backcrosses between F_1SR female × AR male and AR female × F_1RS male were close to that of males of *H. armigera* (Fig. 4b).

Discussion

Behavioral response to sex pheromone blends

Males of *H. assulta* and *H. armigera* showed similar levels of behavioral response in terms of flying close to and reaching the *H. assulta* sex pheromone blend in the wind tunnel. Our data show that *H. armigera* responds equally to the two blends which are characterized by a reversed ratio of the two major sex pheromone components. The behavioral responses of male hybrid F_1RS to *H. assulta* sex pheromone blend were similar to their both parental species, but male hybrid

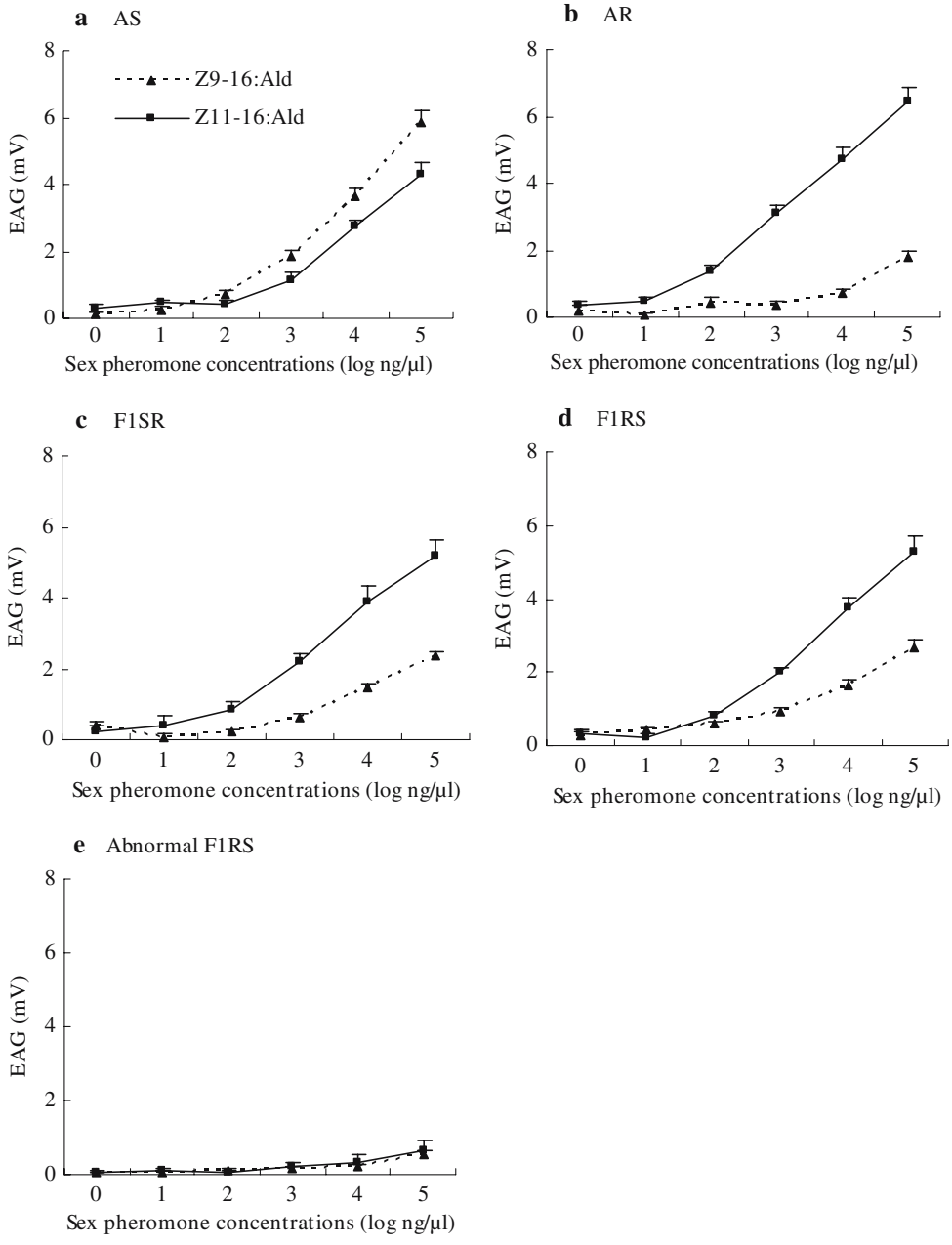


Fig. 3 Dose–response curves of males to *Z*9-16:Ald (*dashed lines*) and *Z*11-16:Ald (*solid lines*). The *bars* indicate SEM. Abbreviations of insect lines refer to Fig. 1

F_1SR showed a response that was stronger than that of the parental species. The hybrid F_1SR males might show heterosis in their responses to the *H. assulta* sex pheromone blend. Males from the backcrosses AS female × F_1RS male and AR female × F_1RS male showed levels of behavioral responses similar to that of *H. assulta* and *H. armigera*. However, a low percentage of males from the backcrosses F_1SR female × AS male and no males from the backcrosses F_1SR female × AR male reached the source releasing *H. assulta* pheromone. These findings indicate that the behavioral response to *H. assulta* sex pheromone was reduced in the latter backcrosses.

Males of *H. assulta* and *H. armigera* responded differently to the sex pheromone blend of *H. armigera*. The majority of *H. armigera* males flew upwind and many reached the sex pheromone source, but all males of *H. assulta* failed to respond when exposed to the plume of *H. armigera* sex pheromone, and none initiated flight. This suggests that the sex pheromone blend produced by *H. armigera* does not attract *H. assulta* males. Both male F_1 hybrids exhibited a similarly high level of complete flight response to the *H. armigera* sex pheromone as males of *H. armigera*. This indicates dominance of *H. armigera* genes in the inheritance of the behavioral responses to *H. armigera* sex pheromone. We found no

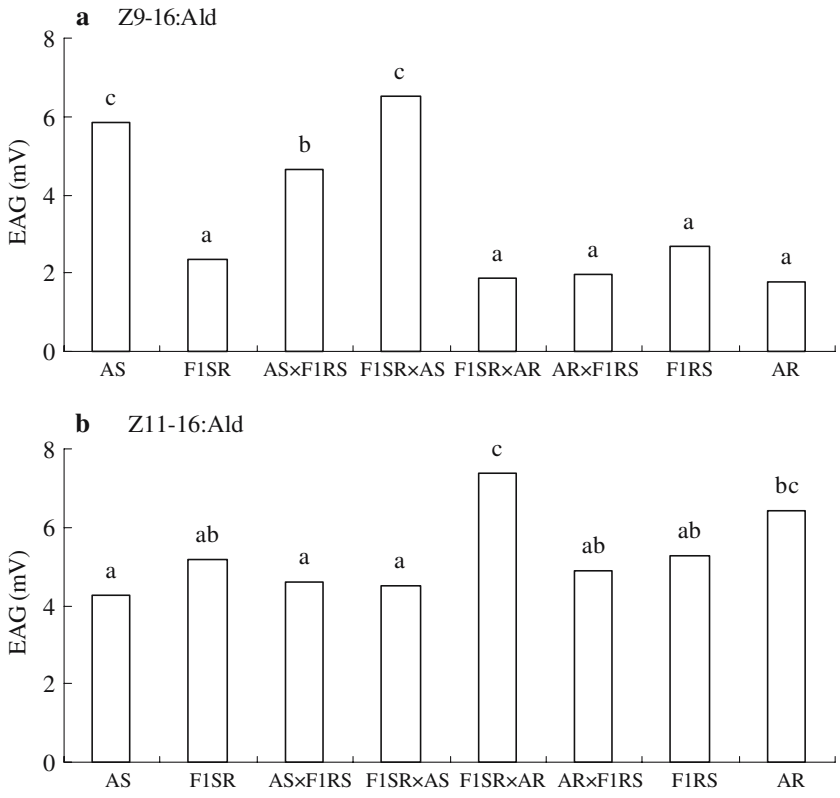


Fig. 4 Electroantennogram responses of males to single sex pheromone components at a dose rate of 10^5 ng/µl. **a** Responses to *Z*9-16:Ald. **b** Responses to *Z*11-16:Ald. AS, *n*=12. AR, *n*=12. F_1SR, males of F_1 hybrid *H. assulta* female × *H. armigera* male, *n*=6. F_1RS, males of F_1 hybrid *H. armigera* female × *H. assulta* male, *n*=12. AS × F_1RS, males from backcross AS female × F_1RS male, *n*=12. F_1SR × AS, males from backcross F_1SR female × AS male, *n*=12. F_1SR × AR, males from backcross F_1SR female × AR male, *n*=6. AR × F_1RS, *n*=13. *Different letters* indicate values that are significantly different (*P*=0.05). The *bars* denote SEM. Abbreviations of insect lines refer to Fig. 1

indication for the involvement of a cytoplasmic factor that might affect such behavioral responses. The responses of males from the backcrosses AS female × F_1RS male and F_1SR female × AS male were close to that of *H. assulta*. Responses of males from the backcrosses AR female × F_1RS male and F_1SR female × AR male resembled those of *H. armigera*. The low percentage of males from the backcross F_1SR female × AR male that reached the pheromone source indicated that their response ability was reduced.

The species-specific pheromone responses of *H. assulta* promotes reproductive isolation from other species. Populations of *H. assulta* from different geographic origin differ in the composition of the sex pheromone blend that is extracted and in the ratio that produces highest attraction (Table 1). Higher specificity of behavioral responses of *H. assulta* to sex pheromone blends at different localities may facilitate its diversification. Conversely, lower specificity of behavioral response of *H. armigera* may lead to large panmictic populations which might prevent diversification of their sex pheromone communication system.

Electrophysiological responses to sex pheromone components

We found no significant differences in EAG responses of males of *H. assulta* and *H. armigera* to the two blends. This might be due to the fact that the EAG technique records only the summed changes in potential of numerous antennal receptor neurons (Howse et al. 1998).

*Z*9-16:Ald elicited a strong dose-dependent EAG response in males of *H. assulta*, and a weak response lacking a relationship with the dose applied in males of *H. armigera* (Fig. 3b). Single sensillum recordings revealed that *H. assulta* receptor neurons are tuned to *Z*9-16:Ald (Berg and Mustaparta 1995). The receptor neuron tuned to *Z*9-16:Ald occurs in a sensillum of males of *H. assulta* together with another neuron tuned to *Z*9-14:Ald, which is a secondary pheromone component of females of *H. virescens* (Berg and Mustaparta 1995). No receptor neurons specifically tuned to *Z*9-16:Ald have been found in the antenna of male *H. armigera* (Mustaparta 1997). Our results confirmed that there are specific *Z*9-16:Ald sensitive receptor neurons in antenna of *H. assulta*, but few or no *Z*9-16:Ald sensitive receptor neurons in the antenna of *H. armigera*. The discrepancies in specificity and sensitivity of receptor neurons tuned to *Z*9-16:Ald in *H. assulta* and *H. armigera* might be ascribed to differences in their membrane receptors (Berg and Mustaparta 1995; Wu 1993). The EAG response of males of both F_1 hybrids to *Z*9-16:Ald were similar to *H. armigera* indicating that *H. armigera* genes were dominant in the genetic control of EAG responses to *Z*9-16:Ald.

*Z*11-16:Ald elicited a significant dose-dependent EAG response in males of both *H. assulta* and *H. armigera*. Receptor neurons tuned to *Z*11-16:Ald in both *H. assulta* and *H. armigera* antennae displayed a large spike amplitude (Berg and Mustaparta 1995; Wu 1993). The similarity in specificity and sensitivity of receptor neurons tuned to *Z*11-16:Ald in heliothine moths suggest that the membrane receptors are functionally similar (Mustaparta 1997). The difference in EAG response to *Z*11-16:Ald between *H. assulta* and *H. armigera* males may imply that they possess different numbers of the receptor neurons. Males of both F_1 hybrids showed an intermediate responses to *Z*11-16:Ald compared with their parents indicating that EAG responsiveness to *Z*11-16:Ald inherits through incomplete dominance.

Electrophysiological response patterns to two single compounds could predict the behavioral responses to the two pheromone blends in both parental species and their hybrids well. In insect chemical communication, the antennal receptors detect chemicals and send signals into the central nervous system, where integration determines behavior. Males of *H. armigera* not only showed a significant level of behavioral response to the sex pheromone blend of *H. armigera*, but also to the sex pheromone blend of *H. assulta*, while males of *H.assulta* showed a significant level of behavioral response to the sex pheromone blend of *H. assulta* and exhibited no behavioral response to the sex pheromone of *H. armigera.* This can be explained by assuming that males of *H. armigera* have receptor neurons sensitive only to Z11-16:Ald, but males of *H. assulta* have receptor neurons to both components. This would provide the central nervous system of *H. assulta* with information allowing a better distinction between *Z*9-16:Ald, *Z*11-16:Ald and their blend ratio, but not so in males of *H. armigera.* Cobalt–lysine staining showed that the cumulus of the subunit of the macroglomerular complex in antennal lobe of *H. assulta* contained neurons responding to *Z*9-16:Ald and that the ventral glomerulus contained neurons responding to *Z*11-16:Ald (Berg et al. 2005). This distinctly differed from that of other heliothine moths including *H. armigera, Helicoverpa zea*, *Heliothis virescens* and *Heliothis subflexa*, in which species the largest glomerulus, the cumulus, contain neurons responding to their primary pheromone component, *Z*11-16:Ald (Christensen et al. 1991; Vickers and Christensen 2003). *Z*9-16:Ald elicited weak electrophysiological responses of *H. armigera* but significantly enhanced its behavioral responses. Similar results had also been found in *Helicoverpa zea* and *Heliothis subflexa* which both use *Z*9-16:Ald as secondary pheromone components. Single sensillum recording showed that no neurons specifically tuned to *Z*9-16:Ald, but that neurons tuned to *Z*9-14:Ald could also respond to *Z*9-16:Ald in *H. zea* and *H. subflexa* (Cossé et al. 1998; Baker et al. 2004), and this may be also true in *H. armigera*. The behavioral response to sex pheromone blends of the two F_1 hybrids was similar to that of *H. armigera.* This was consistent with their EAG response patterns which were close to *H. armigera* as well (Table 2). However, males from the backcross F_1SR female × AR male showed reduced responsiveness to sex pheromone, but their electrophysiological responses were not affected (Table 2), which indicates that changes occurred in the central nervous system of these males. It would be rewarding to investigate the functioning of the macroglomerular complex in *H. assulta*, *H. armigera*, their hybrids, and backcrosses.

Hybridization has revealed the genetic differences underlying sex pheromone communication of some insects (Roelofs et al. 1987; Hendrikes 1988; Foster et al. 1997; Gadenne et al. 1997; Haynes 1997; Laforest et al. 1997; Monti et al. 1997; Teal and Tumlinson 1997). Differences in sex pheromone perception in closely related species or subspecies were controlled by a small number of Mendelian genes (Löfstedt 1990, 1993). A single autosomal gene controlled the difference in male electrophysiological responses between the two races of the European corn borer, *O. nubilalis* and a single sex-linked gene controlled *O. nubilalis* behavioral responses (Hansson et al. 1987; Roelofs et al. 1987). Electrophysiological responses of males of the hybrids between two brownheaded leafrollers species *Ctenopseustis obliquana* and *C. herana* suggested that the differences in responses can be ascribed largely to a single sex-linked locus. The considerable variability in responses of the hybrids

Table 2 Summary for behavioral and electrophysiological responses of males from different insect lines indicated

Insect lines	Behavioral responses		Electrophysiological responses	
	H. assulta sex pheromone blend	*H. armigera* sex pheromone blend	*Z*9-16: Ald	*Z*11-16: Ald
AS	+++	−	+++	+++
F_1SR	++++	+++	+	+++
AS × F_1RS	+++	+	++	+++
F_1SR × AS	++	+	+++	+++
F_1SR × AR	+	++	+	++++
AR × F_1RS	+++	+++	+	+++
F_1RS	+++	+++	+	+++
AR	+++	+++	+	++++

Degree of responses was represented as "+" according to Figs. 1 and 4. "−" Indicated no responses. Abbreviations of insect lines refer to Fig. 1

suggested that another gene may be involved in bringing about electrophysiological differences (Hansson et al. 1989; Foster et al. 1997). The mode of inheritance for behavioral responses of the two *Ctenopseustis* species is similar to that found for antennal reception (Foster et al. 1997). In our case, the results in behavioral and EAG responses of F_1 hybrids revealed no involvement of cytoplasmic factors controlling such responses. Genes on autosomes or sex chromosomes might control these responses. Behavioral and EAG responses of males from backcross F_1SR female × AS were more similar to that of *H. assulta* than *H. armigera*, suggesting that autosomal genes regulated the differences in behavioral and EAG responses to sex pheromone components between *H. assulta* and *H. armigera*, and that the genes from *H. armigera* are dominant in most cases. However, the low success rate of the hybrid cross *H. assulta* female × *H. armigera* male and the lack of female hybrids from the cross *H. armigera* female × *H. assulta* male are obstacles for a complete genetic analysis of the differences in pheromone responses between these two species. In the near future, results from single sensillum recordings should be designed to unravel the sensory encoding in *H. assulta*, *H. armigera*, and their hybrids and backcrosses by identifying receptor neurons tuned to the constituents of pheromone blends. The hybridization system also gives us an opportunity to develop a genetic linkage map for *Helicoverpa* species and locate the major genes conferring their sexual communication.

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References

Baker TC, Ochieng SA, Cossé AA, Lee SG, Todd JL, Quero C, Vickers NJ (2004) A comparison of responses from olfactory receptor neurons of *Heliothis subflexa* and *Heliothis virescens* to components of their sex pheromone. J Comp Physiol A 190:155–165

Berg BG, Mustaparta H (1995) The significance of major pheromone components and interspecific signals as expressed by receptor neurons in the oriental tobacco budworm moth, *Helicoverpa assulta*. J Comp Physiol A 177:683–694

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H (2005) Projections of male-specific receptor neurons in the antennal lobe of the oriental tobacco budworm moth, *Helicoverpa assulta*:a unique glomerular organization among related species. J Comp Neurol 486:209–220

Boo KS, Park KC, Hall DR, Cork A, Berg BG, Mustaparta H (1995) (*Z*)-9-tetradecenal: a potent inhibitor of pheromone-mediated communication in the oriental tobacco budworm moth; *Helicoverpa assulta*. J Comp Physiol A 177:695–699

Chen YX (1999) Fauna Sinica: Insecta, Lepidoptera, Noctuidae, vol 16. Science Press, Beijing, pp 145–147

Christensen TA, Mustaparta H, Hildebrand JG (1991) Chemical communication in heliothine moths II. Central processing of intra- and interspecific olfactory messages in the male corn earworm moth *Helicoverpa zea*. J Comp Physiol A 169:259–274

Cork A, Boo KS, Dunkelblum E, Hall DR, Jee-Rajunga K, Kehat M, Kong Jie E, Park KC, Tepgidagarm P, Liu X (1992) Female sex pheromone of oriental tobacco budworm, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae): identification and field testing. J Chem Ecol 18:403–418

Cossé AA, Todd JL, Baker TC (1998) Neurons discovered in male *Helicoverpa zea* antennae that correlate with pheromone-mediated attraction and interspecific antagonism. J Comp Physiol A 182:585–594

Fitt GP (1989) The ecology of *Heliothis* species in relation to agroecosystems. Ann Rev Entomol 34:17–52

Foster SP, Muggleston SJ, Löfstedt C, Hansson BS (1997) A genetic study on pheromonal communication in two *Ctenopseutis* moths. In: Cardé RT, Minks AK (eds) Insect pheromone research: new directions. Chapman and Hall, New York, pp 514–524

Gadenne C, Picimbon J-F, Becard J-M, Lalanne-Cassou B, Renou M (1997) Development and pheromone communication systems in hybrids of *Agrotis ipsilon* and *Agrotis segetum* (Lepidoptera: Noctuidae). J Chem Ecol 23:191–209

Hansson BS, Löfstedt C, Roelofs WL (1987) Inheritance of olfactory response to sex pheromone components in *Ostrinia nubilalis*. Naturwissenchaften 74:497–499

Hansson BS, Löfstedt C, Foster SP (1989) *Z*-linked inheritance of male olfactory response to sex pheromone components in two species of tortricid moths, *Ctenopseustis obliquana* and *Ctenopseustis* sp. Entomol Exp Appl 53:137–145

Haynes KF (1997) Genetics of pheromone communication in the cabbage looper moth, *Trichoplusia ni*. In: Cardé RT, Minks AK (eds) Insect pheromone research: new directions. Chapman and Hall, New York, pp 525–534

Hendrikes A (1988) Hybridization and sex-pheromone responses among members of the Yponomeuta padellus-compex. Entomol Exp Appl 48:213–223

Hood GM (2004) Poptools version 2.6.2. Available on the Internet. URL http://www.cse.csiro.au/poptools

Howse P, Stevens I, Jones O (1998) Insect pheromone and their use in pest management. Chapman and Hall, London, pp 105–132

Kaissling K-E (1995) Single unit and electroantennogram recordings in insect olfactory organ. In: Spielman AI, Brand JG (eds) Experimental cell biology of taste and olfaction. CRC press, Boca Raton, pp 361–377

Kehat M, Dunkelblum E (1990) Behavioral response of male *Heliothis armigera* (Lepidoptera: Noctuidae) Moths in a flight tunnel to combinations of components identified from female sex pheromone glands. J Insect Behav 3:75–83

Kehat M, Gothilf S, Dunkelblum E, Greenberg S (1980) Field evaluation of female sex pheromone components of the cotton bollworm, *Heliothis armigera*. Entomol Exp Appl 27:188–193

Laforest S, Wu WQ, Löfstedt C (1997) A genetic analysis of population differences in pheromone production and

response between two populations of the turnip moth, *Agrotis segetum*. J Chem Ecol 23:1487–1503
Liu MY, Cai JP, Tian Y (1994) Sex pheromone components of the oriental tobacco budworm, *Helicoverpa assulta* Guenée: identification and field trials. Entomol Sinica 1:77–85
Löfstedt C (1990) Population variation and genetic control of pheromone communication systems in moths. Entomol Exp Appl 54:199–218
Löfstedt C (1993) Moth pheromone genetics and evolution. Phil Trans R Soc Lond B 340:167–177
Monti L, Genermont J, Malosse C, Lalanne-Cassou B (1997) A genetic analysis of some components of reproductive isolation between two closely related species, *Spodoptera latifascia* (Walker) and *S. descoinsi* (Lalanne-Cassou and Silvain) (Lepidoptera: Noctuidae). J Evol Biol 10:121–134
Mustaparta H (1997) Olfactory coding mechanism for pheromone and interspecific signal information in related moth species. In: Cardé RT, Minks AK (eds) Insect pheromone research:new directions. Chapman and Hall, New York, pp 144–163
Nesbitt BF, Beevor PS, Hall DR, Lester R (1979) Female sex pheromone components of the cotton bollworm, *Heliothis armigera*. J Insect Physiol 25:535–541
Nesbitt BF, Beevor PS, Hall DR, Lester R (1980) (*Z*)-9-hexadecenal: a minor component of the female sex pheromone of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae). Entomol Exp Appl 27:306–308
Piccardi P, Capizzi A, Cassani G, Spinelli P, Arsura E, Massardo P (1977) A sex pheromone component of the old world bollworm *Heliothis armigera*. J Insect Physiol 23:1443–1445
Roelofs W, Glover T, Tang XH, Sreng I, Robbins P, Eckenrode C, Löfstedt C, Hansson BS, Bengtsson BO (1987) Sex pheromone production and perception in European corn borer mothe is determined by both autosomal and sex-linked genes. Proc Natl Acad Sci USA 84:7585–7589
SPSS (2001) SPSS 11.01 for Windows, SPSS Inc, Chicago, Illinois
Sugie H, Tatsuki S, Nakagaki S, Rao CBJ, Yamamoto A (1991) Identification of the sex pheromone of the oriental tobacco budworm, *Heliothis assulta* (Guenée) (Lepidoptera: Noctuidae). Appl Entomol Zool 26:151–153
Teal PEA, Tumlinson JH (1997) Effects of interspecific hybridization between *Heliothis virescens* and *Heliothis subflexa* on the sex phermone communication system. In: Cardé RT, Minks AK (eds) Insect pheromone research: new directions. Chapman and Hall, New York, pp 535–547
Vickers NJ, Christensen TA (2003) Functional divergence of spatially conserved olfactory glomeruli in two related moth species. Chem Senses 28:325–338
Visser JH, (1979) Electroantennogram response of the colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. Entomol Exp Appl 25:86–97
Wang CZ, Dong JF (2001) Interspecific hybridization of *Helicoverpa armigera* and *H.* assulta (Lepidoptera: Noctuidae). Chin Sci Bull 46:489–491
Wu CH (1993) Response from sensilla on the antennae of male *Heliothis armigera* to its sex pheromone components and analogs. Acta Entomol Sin 36:385–389
Wu KJ, Gong PY (1997) A new and practical artificial diet for the cotton bollworm. Entomol Sin 4:277–282
Wu DM, Yan YH, Cui JR (1997) Sex pheromone components of *Helicoverpa armigera*: chemical analysis and field tests. Entomol Sin 4:350–356