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Tarsi of Male Heliothine Moths Contain Aldehydes and Butyrate Esters as Potential Pheromone Components

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Abstract The Noctuidae are one of the most speciose moth families and include the genera *Helicoverpa* and *Heliothis*. Females use (*Z*)-11-hexadecenal as the major component of their sex pheromones except for *Helicoverpa assulta* and *Helicoverpa gelotopoeon*, both of which utilize (*Z*)-9-hexadecenal. The minor compounds found in heliothine sex pheromone glands vary with species, but hexadecanal has been found in the pheromone gland of almost all heliothine females so far investigated. In this study, we found a large amount (0.5–1.5 μg) of hexadecanal and octadecanal on the legs of males of four heliothine species, *Helicoverpa zea*, *Helicoverpa armigera*, *H. assulta*, and *Heliothis virescens*. The hexadecanal was found on and released from the tarsi, and was in much lower levels or not detected on the remaining parts of the leg (tibia, femur, trochanter, and coxa). Lower amounts (0.05–0.5 μg) of hexadecanal were found on female tarsi. This is the first known sex pheromone compound to be identified from the legs of nocturnal moths. Large amounts of butyrate esters (about 16 μg) also were found on tarsi of males with lower amounts on female tarsi. Males deposited the butyrate esters while walking on a glass surface. Decapitation did not reduce the levels of hexadecanal on the tarsi of *H. zea* males, indicating that hexadecanal production is not under the same neuroendocrine regulation system as the production of female sex pheromone. Based on electroantennogram studies, female antennae had a relatively high response to hexadecanal compared to male antennae. We consider the possible role of aldehydes and butyrate esters as courtship signals in heliothine moths.

Keywords Hexadecanal · Octadecanal · Butyrate esters · Heliothine moths · Male pheromones

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Introduction

Sex attractant pheromones produced by female moths generally are either linear fatty acid-derived compounds, 12–18 carbons in chain length, with an oxygenated functional group and one to three double bonds, or based on hydrocarbons with a polyene and/or epoxide functional group. Pheromone blends comprised of multiple components are formed in species-specific ratios through pheromone biosynthetic pathways that involve fatty acid synthesis, desaturation, and limited chain-shortening along with reductases, oxidases, and acetyltransferases (Jurenka 2003; Tillman et al. 1999).

The Noctuidae are one of the most speciose moth families and includes the heliothine genera *Helicoverpa* and *Heliothis*. Because these two genera contain agriculturally important pests, their mating behaviors, sex pheromone components and blend ratios, and application of their pheromones in pest

management have been well studied. To date all *Helicoverpa* and *Heliothis* females investigated utilize (*Z*)-11-hexadecenal (Z11–16:Ald) as a major component of the female sex pheromone, except for *Helicoverpa assulta* and *Helicoverpa gelotopoeon*, which use (*Z*)-9-hexadecenal (Z9–16:Ald) as the major pheromone component (Table S1) (Cork and Lobos 2003; El-Sayed 2014; Sugie et al. 1991). Each species produces a specific pheromone blend. In particular, the ratio of two components, Z11–16:Ald and Z9–16:Ald, is crucial to prevent inter-specific attraction between species of *Helicoverpa* and *Heliothis* (Cork et al. 1992; El-Sayed 2014). The identified minor components in pheromone glands of heliothines are hexadecanal (16:Ald), (*Z*)-7-hexadecenal, tetradecanal, (*Z*)-9-tetradecenal, (*Z*)-11-hexadecenyl acetate, (*Z*)-9-hexadecenyl acetate, (*Z*)-11-hexadecenyl alcohol, and (*Z*)-9-hexadecenyl alcohol. These minor components in heliothines are found in varying amounts, and their possible roles in mate attraction generally have not been clearly determined. To date, the minor gland component 16:Ald has been found in all heliothine species.

Female sex pheromone glands in heliothine moths typically are located on the intersegmental cuticle between the 8th and 9th abdominal segments just anterior to the ovipositor (Ma and Ramaswamy 2003). Male pheromone glands, on the other hand, have developed from abdominal tips, and form a brush or hair pencil that is concealed within a pocket and everted by sclerotized levers (Birch et al. 1990). The hair pencil is a male scent organ that produces volatiles that differ chemically from female sex pheromones, and that can play a role in close-range attraction of conspecific females during courtship (Baker et al. 1981). In butterflies, male sex pheromones usually are produced from androconial organs or tissues associated with wings or legs (Nieberding et al. 2008). A day-flying moth, the palm borer, *Paysandisia archon* (Lepidoptera: Castiniidae) utilizes visual cues to find conspecific partners (Delle-Vedove et al. 2012, 2014; Monteys et al. 2012). Recently, it has been discovered that *P. archon* males produce a short-range sex pheromone from androconia located on the tarsi of the mesothoracic legs (Frerot et al. 2013).

In this study, we investigated the presence of potential pheromone components on the legs of four species of heliothine moths and measured their electroantennographic activity on the antennae of both male and female moths.

Methods and Materials

Insects Pupae of the corn earworm, *H. zea*, and the tobacco budworm, *H. virescens*, were purchased from Bio-Serv (USA) and maintained at 25 °C ± 1 under a photoperiod of L:D 15:9 h until adult emergence. *Helicoverpa assulta* and *H. armigera* larvae were collected from fields near Suwon, South Korea, and were reared individually with corn-soy based artificial diet at 25 °C ± 1 under a photoperiod of L:D 16:8 h and adults were provided with 10 % sucrose solution-soaked cotton ball until tarsal extraction or bioassays. *Helicoverpa armigera* used for electroantennogram (EAG) studies was supplied from a stock culture kept at Plant & Food Research, Auckland, New Zealand.

Tarsal Extraction and Analysis Legs were detached from 2 to 3-d-old adults, and tarsi were excised from each leg by cutting the joint between tibia and tarsus. The six tarsi were immersed in 50 μl hexane containing (*Z*)-9-tetradecenal (10 ng/μl) as an internal standard. Some extracts contained *n*-tetratriacontane as an internal standard. The tarsi were removed from the extraction vial after 10 min to 1 h at room temperature, and the extract was kept at −70 °C prior to chemical analysis.

Tarsal extracts from *H. zea*, *H. virescens*, *H. armigera*, and *H. assulta* were analyzed by GC (6890 N; Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a mass-selective detector (5975C; Agilent) and a DB-5 column (30 m × 0.25 mm ID, 0.25 μm film thickness, J&W Scientific, USA). The oven temperature was programed at 80 °C for 1 min, increased to 170 °C at 5 °C/min, held for 3 min, and increased to 220 °C at 5 °C/min. Injector temperature was 250 °C.

Tarsi of *H. zea* also were analyzed using solid phase microextraction (SPME) with a black fiber (75um Carboxen™ - PDMS, Supelco, USA). Tarsi were removed from 2- to 3-day-old adult males and placed in a 4 ml vial with a hole in the cap that allowed the insertion point of the SPME fiber. Adsorption of air space volatiles occurred over 4 h and they then were analyzed by desorbing the volatiles collected on the SPME fiber in the injection port of a gas chromatograph connected to a mass-selective detector and fitted with a DB-5 column as above. The GC oven was programmed starting at 60 °C for 5 min, increased to 230 °C at 10 °C/min, and held for 15 min.

Tarsal extracts from *H. zea* were separated into butyrate esters and hydrocarbon fractions using a column with 300 mg of silica gel. Hydrocarbons were eluted with 4 ml of hexane, and butyrates were eluted with 4 ml of 80:20 hexane:diethyl ether. The two fractions were concentrated and analyzed by GC and mass-selective detector using a DB-5 column as described above.

Pheromone glands of *Helicoverpa zea* were dissected during the scotophase and extracted for 0.5–1 h with hexane containing (*Z*)-9-tetradecenal (100 ng) as an internal standard. A 6890 N (Agilent) gas chromatograph, equipped with a DB-23 column (30 m × 0.25 mm, J&W Scientific, USA) was used to measure the amount of pheromone. The oven temperature was programmed at 80 °C for 1 min, then at 10 °C/min to 230 °C, and held for 8 min. Results were analyzed by two tailed *t*-test (unpaired) or non-parametric analysis (one-way

ANOVA followed by Tukey's multiple comparison test) using GraphPad Prism 6 (GraphPad Software, Inc., USA).

We also determined if tarsal compounds could be found on the surface of a glass Petri dish after males walked on the surface. Four *H. zea* males were placed in glass Petri dishes (100 × 22 mm) cleaned with hexane and left overnight. The internal surfaces of the Petri dish were washed with 4 ml hexane, and the hexane extract was concentrated and analyzed using the GC with mass-selective detector and DB-5 column as described above. This process was repeated four times.

Electroantennogram (EAG) Studies EAG responses of male and female *H. armigera* to 16:Ald and Z9–16:Ald were measured in New Zealand where *H. armigera* is available but *H. zea* is not. *Helicoverpa armigera* and *H. zea* utilize similar compositions of sex pheromone components and had similar levels of 16:Ald on the tarsi. Therefore we utilized *H. armigera* to conduct the EAG responses. Male and female pupae were placed in separate plastic containers, and adults were fed on 10 % sucrose. For EAG recording an antenna was isolated from 2- to 3-day-old moths by excising the antennal base with a micro-knife. The proximal end of the isolated antenna was connected to a glass capillary reference electrode (0.86 mm ID, A-M Systems Inc., USA) filled with electroconductive gel (Spectra 360®, Parker Laboratories Inc., USA). After cutting off a few terminal flagella subsegments, the distal end of the antenna was connected to another gel-filled glass electrode serving as the recording electrode. The position of the electrodes was controlled with micromanipulators, and Ag-AgCl junctions were used to maintain electrical continuity between the electrodes and the inputs of an EAG recording system that had a high input impedance preamplifier (Syntech, The Netherlands). The DC signals through the preamplifier were amplified further, digitized at a 100/s sampling rate, and processed with a PC-based signal processing system (IDAC-4, Syntech, The Netherlands) and software (Autospike 32, Syntech, The Netherlands). Once a stable contact was made between the electrodes and an antenna, as indicated by a stable baseline, the antenna was stimulated with a series of different doses of two compounds, hexadecanal (16:Ald) (synthesized by Peter Teal, USDA-ARS) and (Z)-9-hexadecenal (Z9–16:Ald) (Bedoukian, USA). Each compound was dissolved in hexane as 0.1 μg/μl and 1.0 μg/μl solutions. Hexane was used as a solvent control. Presentations of test chemicals to the antennae were similar to previous studies (Park and Baker 2002; Park and Hardie 2004). A 10 μl aliquot of each test solution was applied onto a piece of filter paper (5 × 30 mm; Whatman No 1, USA), and the filter paper strip was inserted into a glass Pasteur pipette (146 mm) after being evaporated for 10 s in air. The tip of the pipette was inserted into a small hole (2 mm diam, 10 cm from the outlet to the antennae) in a glass main airflow tube with a continuous, charcoal-filtered and humidified airflow (600 ml/min) over the antennal preparation. A 0.1-s pulse of charcoal-filtered airflow (10 ml/s) was injected through the wide end of the Pasteur pipette odor cartridge for stimulation, using an electronic airflow controller (CS-55, Syntech, The Netherlands). The wide end of the Pasteur pipette was covered with a piece of aluminum foil when not in use to reduce evaporation. Each odor stimulus cartridge was used no more than 10 times. The order of testing chemicals was random. The time interval between successive stimulation was at least 30 s. The responsiveness of antennae was analyzed by measuring the change of DC amplitude before and after odor stimulation. In each recording, the peak amplitude after odor stimulation was subtracted by the average amplitude before the stimulation. In total, 33–36 EAG recordings were made for each dose of each compound from 6 males, and 39–42 recordings from 6 females. Statistical analysis was carried out using ANOVA followed by Fisher LSD test or a *t*-test.

Results

Compounds on Tarsi Hexadecanal (16:Ald) was found on the tarsi of adult legs in four heliothine species (Fig 1). This was identified by comparison of retention times and mass spectra with those of an authentic standard (Fig S1). Smaller amounts (400–550 ng/male; 200–350 ng/female) of octadecanal (18:Ald) also were found on all tarsi of *H. zea*, *H. armigera*, and *H. assulta*. A large amount of 16:Ald was found on the fore, mid, and hind legs of *H. armigera*, *H. assulta*, *H. zea*, and *H. virescens* (Table S1).

To determine the specific location of 16:Ald we divided *H. zea* legs into tarsi and the remainder of the leg (coxa, trochanter, and tibia) and then extracted these with hexane. Most of the 16:Ald (> 90%) was detected from the tarsi (Fig. 2). The whole body with legs removed was extracted with hexane, but did not contain 16:Ald. Average amounts of 16:Ald from six tarsi per male were similar between

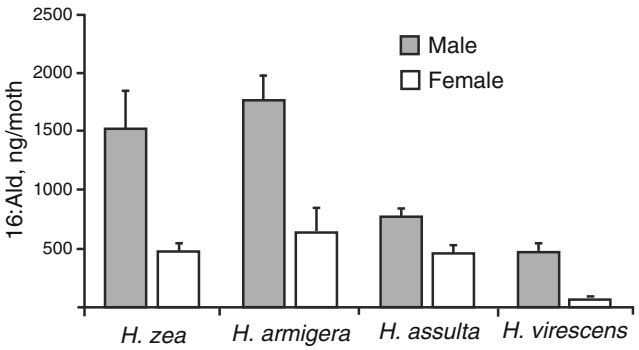

Fig. 1 Amounts of 16:Ald from heliothine male and female tarsi. Bars indicate mean + SEM. Statistical significance between male and female tarsi within a species was measured with a two-tailed *t*-test. *Helicoverpa zea* ($P < 0.001$), *Helicoverpa armigera* ($P = 0.005$), *Helicoverpa assulta* ($P = 0.012$), *Heliothis virescens* ($P < 0.001$); $N \geq 6$ for all measurements

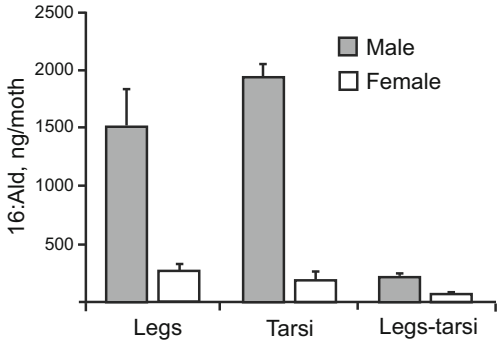

Fig. 2 Amounts of 16:Ald from whole legs, tarsi, and legs minus tarsi of *Helicoverpa zea* males and females. Bars indicate mean + SEM. Differences between males and females in each comparison was significant at $P < 0.001$ (two-tailed t-test); $N \geq 6$ for all measurements

H. zea (approx 1500 ng/male) and *H. armigera* (approx. 1700 ng/male). These values are approximately three times (2.8–3.2) the amounts found in females. In male *H. assulta*, the amount of 16:Ald (approx 770 ng) was about half that found in *H. zea* and *H. armigera*, with females having about 1.7-fold lower amounts than males. In addition, we investigated *H. virescens* females, which produce the same pheromone components as the *Helicoverpa* species. The 16:Ald amounts found in *H. virescens* males were relatively small (approx. 470 ng/male) with a high male to female ratio (8.2:1) (Fig. 1).

Changes in Amounts of 16:Ald during Development The amount of 16:Ald on the tarsi of *H. zea* gradually increased from the first scotophase of newly emerged adult males, and reached a maximum in 4-d-old moths (Fig. 3). Typically, *H. zea* adults start to emerge 2–3 h before lights off until the early scotophase, and at that time the newly emerged male is not sexually mature. It is not until the second scotophase that *H. zea* males are ready for mating. The sibling species, *H. assulta*, has the highest mating rate during the second scotophase after emergence (Cho and Boo 1988), which is similar to *H. zea*. Unlike males, the amount of the tarsal 16:Ald in females was relatively high on the first day after emergence, and then decreased with age (Fig. 4). There is clearly a different production pattern between male and female *H. zea*. The other parts of male and female legs contained much lower amounts of 16:Ald (Fig. S2, S3).

Effects of Decapitation Decapitation of 2–3-d-old males resulted in maximum amounts of 16:Ald at 48–72 h post-decapitation (approx 3000 ng/male, Fig. 5), which were similar to the intact males at the same age (scotophase: approx 2900 ng and photophase: approx 400 ng, Fig. 3). This result indicates that head removal did not affect 16:Ald production in the tarsi. For females, however, 48–72 h after decapitation (Fig. 5) the average amount (approx 100 ng/female) of 16:Ald was almost twice that of the same aged normal females (scotophase ~40 ng and photophase ~44 ng). The higher amounts of 16:Ald found in decapitated females could be due to higher production or to lower amounts being released from the tarsi. In the other leg segments, 16:Ald amounts were much lower than in tarsi, but similar patterns were observed between decapitated males and females (compare Fig. 5 and Fig. S4).

EAG Responses Male antennae strongly responded to the female sex pheromone component Z9–16:Ald, whereas female antennae showed significantly lower responses at both 1 and 10 μg doses (Fig. 6a). In contrast, 16:Ald elicited EAG responses from females and males that were not significantly different in magnitude (Fig. 6b).

SPME Collections Analysis of volatiles in the headspace above tarsi of male *H. zea* collected on an SPME fiber showed the presence of 16:Ald and 18:Ald (Fig S5). This demonstrated that 16:Ald found on the tarsi is released into the environment.

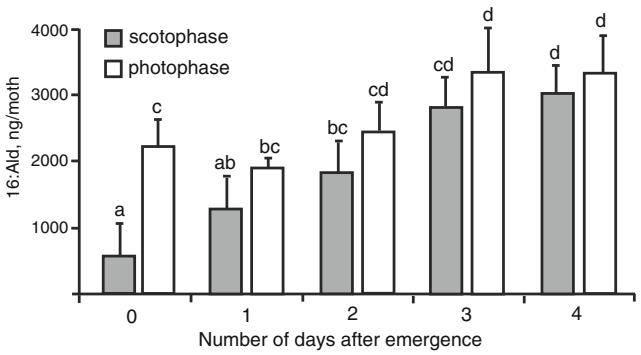

Fig. 3 Amounts of 16:Ald from tarsi of *Helicoverpa zea* males taken during the scotophase and photophase at the indicated days after adult emergence. Bars indicate mean + SEM; bars with the same letters are not statistically different at $P \leq 0.05$ using one-way ANOVA followed by Tukey's multiple comparison test; $N \geq 5$

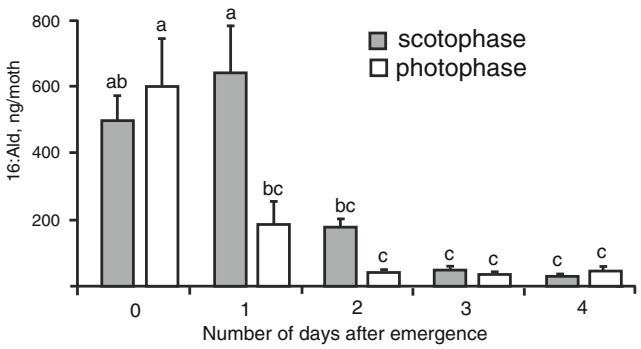

Fig. 4 Amounts of 16:Ald from tarsi of *Helicoverpa zea* females taken during the scotophase and photophase at the indicated days after adult emergence. Bars indicate mean + SEM; bars with the same letters are not statistically different at $P \leq 0.05$ using one-way ANOVA followed by Tukey's multiple comparison test; $N \geq 5$

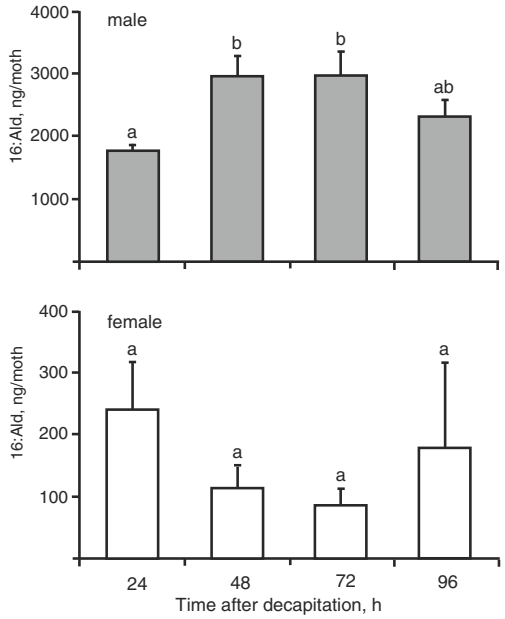

Fig. 5 Amounts of 16:Ald from tarsi of *Helicoverpa zea* males and females taken at the indicated times after decapitation. Bars indicate mean + SEM; bars with the same letters are not statistically different at $P \leq 0.05$ using one-way ANOVA followed by Tukey's multiple comparison test; $N \geq 5$

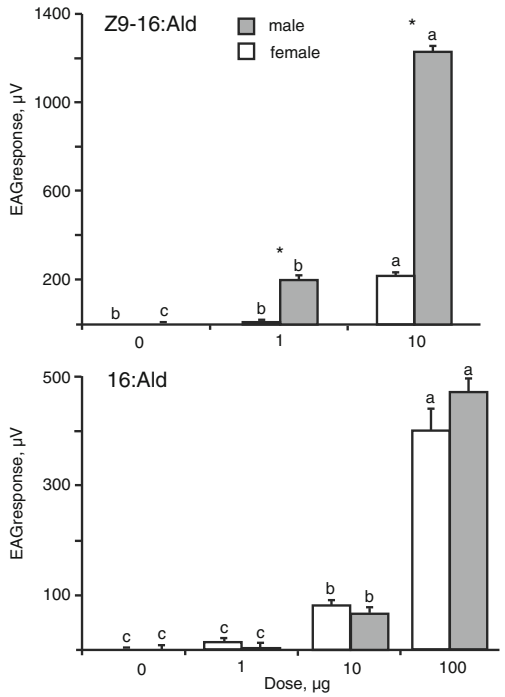

Fig. 6 EAG responses of male and female *Helicoverpa armigera* to Z9–16:Ald and 16:Ald (bars indicate mean + SEM, males $N = 33–36$, females $N = 39–42$). Different letters above the bars indicate significant differences among different doses within each sex (ANOVA followed by Fisher LSD, $P = 0.001$), and asterisks indicate significant differences between males and females for the given dose and compound (t-test, $P = 0.001$). There was no significant difference between males and females at all doses of 16:Ald

Butyrate Esters and Hydrocarbons All tarsi contained a variety of hydrocarbons that were identical to those identified from the forelegs of *H. zea* and *H. virescens* (Fig S6A) (Böröczky et al. 2008). Böröczky et al. (2008) also identified a series of butyrate esters from the forelegs of *H. zea* and *H. virescens*. We found that the butyrate esters are present in the tarsi of *H. zea* and *H. armigera*, and in lower amounts on the femur and tibia (Fig S7). Extraction of whole bodies of males minus the legs determined that the butyrate esters are found only on the legs. The butyrate esters are secondary esters of straight-chain alcohols with 25, 27, 29, and 31 carbons. In the 29-carbon series, at least four additional butyrate esters with a methyl branch were present, with the position of the methyl group unresolved based on the mass spectra (Fig S6B). Using a hydrocarbon as an internal standard, 2-d-old *H. zea* males had 16.4 ± 4.3 μg ($N = 3$) total butyrates on their 6 tarsi. In contrast, the same aged *H. zea* females had 1.7 ± 0.5 μg ($N = 3$) total butyrates on their 6 tarsi and only the 25-carbon butyrate was present. In contrast the total amounts of hydrocarbons found on the tarsi were equal between males (16.8 ± 3.6 μg) and females (16.3 ± 3.0 μg).

Males deposit the butyrate esters along with the hydrocarbons as they walk on the surface of glass Petri dishes. The hydrocarbons and butyrate esters extracted from the glass surface and the tarsi were found in similar ratios (Fig S8), indicating that these compounds originated from the tarsi and not from the scales that are almost continuously shed by moths kept in a confined space.

Discussion

Long-range sex pheromones in moths usually are produced by females and attract conspecific males. If the pheromone is a multicomponent blend, typically it is formed in species-specific ratio. The major sex pheromone component identified from heliothine moths is Z11–16:Ald, except for *H. assulta* and *H. gelotopoeon*, which use Z9–16:Ald as the major component (Table S2). The major sex pheromone component is the most abundant volatile compound found in the pheromone glands, and is the principal active compound for male attraction. Minor sex pheromone components are lower in abundance but are in most cases essential for male attraction. A variety of minor components, including acetates, alcohols, and aldehydes, have been identified from heliothines and some play a role in sex pheromone activity. A common minor compound found in sex pheromone glands is 16:Ald found in all heliothine moths investigated so far (Table S2), and sometimes in a relatively large amount compared to other minor components (Cork et al. 1992; El-Sayed 2014; Klun et al. 1980a, b). The role of 16:Ald in attraction of males has been investigated in *H. virescens* (Groot et al. 2010; Klun et al. 1980a; Ramaswamy et al. 1985) and *H. assulta* (Cork et al.

1992; Sugie et al. 1991). In *H. virescens*, 16:Ald was found to be the most consistent of the five minor components to elevate the behavioral activity of males in flight-tunnel tests (Vetter and Baker 1983). However addition of 16:Ald in field tests did not affect attraction of *H. virescens* males (Groot et al. 2010). The exact role of 16:Ald in heliothines, including *H. zea* (Klun et al. 1980b), is still unclear.

Here, we describe the tarsi of adult legs as a new source of 16:Ald in four species of heliothine moths. Surprisingly large amounts, in some cases microgram levels, of 16:Ald were found from all six legs total per individual male, and in lower amounts in females of all four species. This is the first time that a known female sex pheromone component has been identified from the legs of nocturnal moths. Recently, a sex pheromone was identified from male mesothoracic tarsi but not from the other legs in the diurnal moth, *P. archon* (Frerot et al. 2013). We found that amounts of 16:Ald and 18:Ald were similar among all legs of *H. zea*.

The three *Helicoverpa* species are closely related, but found in different geographical areas. *Helicoverpa zea* lives only in the New World, whereas *H. armigera* and *H. assulta* are found in Asia, Europe, and Australia (Matthews 1991). The sex pheromones of *H. armigera* and *H. zea* have the same major (Z11–16:Ald) and minor (Z9–16:Ald) components, but *H. assulta* has the opposite ratio. *Heliothis virescens* is found in the Americas and uses Z11–16:Ald as the major sex pheromone component with Z11–16:OH as an isolating component. All of these heliothines have retained the production of Z11–16:Ald or Z9–16:Ald and 16:Ald in the female pheromone gland, and in this study the tarsi also were found to contain16:Ald. In addition, 18:Ald and a series of butyrate esters along with hydrocarbons were found on the tarsi.

Biosynthesis of sex pheromones in *Helicoverpa* and *Heliothis* species is regulated by pheromone biosynthesis activating neuropeptide (PBAN), released from the subesophageal ganglion during the scotophase to act on the pheromone gland (reviewed by Rafaeli 2009). Decapitation did not reduce the tarsal amounts of 16:Ald, indicating that the production of 16:Ald in legs is not under PBAN control via the central nervous system. The 16:Ald is made from reduction of palmitic acid in the pheromone glands (Choi et al. 2002). Future research will determine where the aldehydes are being biosynthesized; whether in tarsal excretory cells or in other parts of the body and transported to the tarsi.

The tarsi of other insects can produce compounds that aid in the tarsal adhesion to a substrate. These compounds have been identified as hydrocarbons in beetles (Attygalle et al. 2000; Geiselhardt et al. 2009) and a complex of fatty acids, glucose, amino acids in a locust (Votsch et al. 2002). A study on the tarsal morphology and attachment ability of male and female codling moths, *Cydia pomenella*, indicated that the arolia and pretarsal claws are involved in attachment of adult moths to smooth surfaces (Al Bitar et al. 2009, 2010). Although it appears that the arolium of *C. pomenella* has secretory capability, these studies did not identify chemicals on the tarsi. The tarsi of heliothine moths are relatively long (3–6 mm), and the chemicals we identified may not be involved in chemical adhesion, or they may have multiple functions. The hydrocarbons may be involved in forming adhesive fluids for attachment since they are found in similar amounts in males and females. However, a 10-fold difference in the amount and composition of the butyrate esters, and in some cases the aldehydes, was found between males and females, suggesting a different function, possibly chemical communication.

The EAG response indicated that female *H. armigera* detected 16:Ald and therefore could respond to the 16:Ald produced by male tarsi. The large amount of 16:Ald produced by males could be used for chemical communication between males and females, and possibly for male selection by female heliothine moths. In *H. virescens*, several male hair-pencil volatiles produced larger EAG responses by females compared to males, implying that these volatiles could be behaviorally relevant in females selecting males for mating (Hillier et al. 2006; Hillier and Vickers 2007).

Sex pheromones in nocturnal female moths have evolved as a chemical signal to find and select conspecific males from a long range. Many male sex pheromones have been found from either simple scales or from hair pencils associated with various male organs such as abdomen, thorax, legs, and wings that are involved in short-range mating behaviors (Baker et al. 1981; Dussourd et al. 1991; Heath et al. 1992; Jacquin et al. 1991; Nishida et al. 1982; Teal and Tumlinson 1989). However, scent organs in male Noctuidae are not diverse, but consistently found in hair pencils located in the 9th abdominal sternite. Saturated or unsaturated 16-carbon fatty alcohols or acetate esters are produced from hair pencil glands in mature adult males (Birch et al. 1990; Teal and Tumlinson 1989). Heliothine moths release these compounds by displaying abdominal hair pencils during courtship. These pheromones make females quiescent for successful acceptance, or, possibly, repel competing males of *H. virescens* and *H. subflexa* (Hillier et al. 2007; Hillier and Vickers 2004, 2011).

The biological role(s) of aldehydes (16:Ald and 18:Ald) and butyrate esters present on the tarsi of male heliothine moths remains unknown. We propose that some or all of these compounds function as short-range signals in conjunction with the hair pencil bouquet to facilitate courtship and female acceptance, but further research is required to test this hypothesis.

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