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Sex Pheromones of Two Melittini Species, *Macroscelesia Japona* and *M. Longipes*: Identification and Field Attraction

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Abstract Two Melittini species, *Macroscelesia japona* and *M. longipes* (Lepidoptera: Sesiidae), are native to Japan, but occupy different localities as their host plants seldom grow together. The contents of the sex pheromone gland of adult females of both species, obtained after rearing larvae collected from the field, were investigated by gas chromatograph-electroantennogram detection (GC-EAD) and gas chromatograph-mass spectrometry (GC-MS) analyses. Two GC-EAD-active components were found in a crude extract of *M. japona* female pheromone gland, and identified as (2*E*,13*Z*)-2,13-octadecadien-1-ol (E2,Z13-18:OH) and (2*E*,13*Z*)-2,13-octadecadienal (E2,Z13-18:Ald). The average ratio of these two components was about 1:10. In the field, *M. japona* males were attracted to traps baited with E2,Z13-18:Ald alone, but the strongest attraction was observed with a 1:100 mixture of E2,Z13-18:OH and E2,Z13-18:Ald. The same two components were found in extracts of *M. longipes* females, but in a markedly different ratio. Male *M. longipes* were attracted most strongly to lures containing a 20:1 mixture of

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E2,Z13-18:OH and E2,Z13-18:Ald, although some males were also attracted to lures with E2,Z13-18:OH alone. Although the two species do not generally occur in sympatry, our data indicate that, in the event of overlap, cross attraction of the two species is unlikely.

Keywords (2*E*,13*Z*)-2,13-octadecadien-1-ol · (2*E*,13*Z*)-2,13-octadecadienal · Clearwing moth · Melittini · Reproductive isolation · Lepidopteran pheromones

Introduction

The Sesiidae is a family that includes over 1,000 diurnal species worldwide (Pühringer and Kallies, 2004). Adults of most species have wasp-mimic stripes (yellow or red bands with a black body), in a species-specific pattern that may be involved in mate recognition. In addition to visual cues, sesiids use female sex pheromones in mating (Bûda and Karalius, 1985; Karalius and Bûda, 1993). Since the original study on *Synanthedon pictipes* and *S. exitiosa* by Tumlinson et al. (1974), the sex pheromones of 15 species of Sesiidae have been identified (Ando, 2006). These identifications, however, have been mainly conducted in Europe and the United States, and information about Asian species is limited. About 30 yr ago, sex attractants for two agricultural pests, *S. hector* and *S. tenuis*, were discovered by a field screening test in Japan by using a synthetic pheromone of *S. pictipes* and related compounds (Yaginuma et al., 1976; Tamaki et al., 1977). However, no actual pheromone identifications were published on the Japanese species in this family until our recent study on *Nokona pernix* (Naka et al., 2006).

The Sesiidae are divided into two subfamilies, Sesiinae and Tintiinae, with the former subdivided into six tribes: Synanthedonini, Paranthrenini, Melittini, Sesiini, Osminini, and Cissuvorini. Pheromone studies worldwide have been carried out mainly with species in the tribes Synanthedonini and Paranthrenini. Species in each of the six tribes occur in Asia, but no species in the tribe Osminini are found in Japan (Arita and Ikeda, 2000; Pühringer and Kallies, 2004). The tribe Melittini is one of the largest tribes of Sesiinae, comprising six genera and 150 species in Asia, Africa, and the New World (Pühringer and Kallies, 2004). However, pheromones have been reported only for one species, *M. cucurbitae* (Klun et al., 1990). In Japan, six species in the tribe Melittini, two *Macroscelesia* species and four *Melittia* species, are known (Arita and Ikeda, 2000). Both *Macroscelesia* species are monophagous, feeding on different host plants in different habitats. Larvae of *M. japona* burrow into the stems of *Gynostemma pentaphyllum*, whereas larvae of *M. longipes* bore into the stems of *Actinostemma lobatum* (Arita and Ikeda, 2000). *G. pentaphyllum* grows commonly in Japanese coppices, whereas *A. lobatum* is an endangered species, growing in riverbeds. In Japan, almost all rivers are now enclosed between protective walls, virtually removing all habitats for *A. lobatum*. *M. longipes*, which feeds exclusively on *A. lobatum*, and has shown a consequent reduction in numbers.

Although *G. pentaphyllum* and *A. lobatum* seldom grow together, it is possible that adults of the two moths might coincide as they have similar flight periods and their distributions may be in close proximity. With this in mind, we set about to determine whether the two closely related clearwing moths have different pheromone systems, as well as to increase the information on pheromone components used by Asian sessiid species. This report deals with the identification and field evaluation of the first pheromones from clearwing moths in the tribes Melittini.

Methods and Materials

Insects and Pheromone Extraction About 40 overwintering larvae of *M. japona* were collected in Maihara City, Shiga Prefecture, in November 2003 and vernalized in a refrigerator at 8±1°C without light for 2 mo. After vernalization, insects were kept in a plastic cage at 25±1°C under a 15L: 9D photoperiod until they emerged as adults. Adults were sexed based on their antennae (Arita and Ikeda, 2000). Under laboratory conditions, females exhibited the calling position irregularly during the photophase. Therefore, we observed the behavior of 1- or 2-d-old virgin females and excised their abdominal tips when a calling position was observed. Abdominal tips were placed individually in hexane (250 μl/female) for 5 min to extract the pheromone, before analysis by gas chromatography- electroantennographic detection (GC-EAD; Struble and Arn, 1984) and gas chromatography-mass spectrometry (GC-MS).

For the study on *M. longipes*, about 20 galls containing larvae were collected from a riverbed near Lake Sanaru in Hamamatsu City, Shizuoka Prefecture, in August 2003. The galls were kept at 25±1°C under a 15L-9D photoperiod until the adults emerged after 15–20 days. As *M. longipes* females showed a calling position 1–4 hr after the beginning of the photophase, their abdominal tips were excised 4 hr after the beginning of the photophase. Extracts were made and analyzed as for *M. japona*.

Chromatography and Derivatization of the M. japona Pheromone The crude extract of pheromone glands [10 female equivalents (FE)] was adsorbed on a microcolumn packed with Florisil® (100–200 mesh, Floridin Co., 200 mg). The column was successively eluted with 1 ml each of hexane and 5, 10, 20, and 50% diethyl ether in hexane. Each fraction was concentrated by a gentle nitrogen stream and an aliquot (1 FE) analyzed by GC-EAD. The 5% diethyl ether fraction (9 FE), which included an electroantennogram (EAG)-active aldehyde component, was treated with $NaBH_4$ (0.5 mg) in 2-propanol (0.1 ml) for 3 hr at room temperature. After mixing with water (1 ml), the crude products were extracted with hexane (0.5 ml) and analyzed by GC-MS.

Chemicals (2*E*,13*Z*)-2,13-octadecadienyl acetate (E2,Z13-18:OAc) was supplied by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). The sample included traces (<2%) of some unknown impurities, but not the corresponding alcohol. (2*E*,13*Z*)-2,13-octadecadien-1-ol (E2,Z13-18:OH) was prepared by the hydrolysis of E2,Z13-18:OAc with methanolic NaOH. Other geometric isomers of (2,13)-octadecadien-1-ol were synthesized from 1,9-nonanediol. Their synthesis and structure confirmation by NMR analysis have been reported in a previous paper (Naka et al., 2006). (2*E*,13*Z*)-2,13-octadecadienal (E2,Z13-18:Ald), was obtained by oxidation of E2,Z13-18:OH with pyridinium chlorochromate in CH_2Cl_2. ^{1}H NMR: δ (ppm) 0.90 (t, 3H, *J*=7.3 Hz), 1.2–1.4 (broad s, 16H), 1.50 (m, 2H), 2.02 (m, 4H), 2.33 (dt, 2H, J=7 and 7 Hz), 5.35 (m, 2H), 6.12 (dd, 1H, *J*=7.9 and 15.6 Hz), 6.85 (dt, 1H, *J*=6.8, and 15.6 Hz), 9.50 (d, 1H, *J*=7.9 Hz). ^{13}C NMR: δ (ppm) 14.0, 22.4, 26.9, 27.2, 27.9, 29.2, 29.3, 29.4, 29.5 (× 2), 29.8, 32.0, 32.8, 129.8, 129.9, 133.0, 159.1, 194.2.

Analytical Instruments The GC-EAD apparatus and conditions have been reported (Naka et al., 2006). Briefly, an HP-5890 Series II gas chromatograph, (Hewlett-Packard, Wilmington, DE, USA) with a DB-23 capillary column (0.25 mm×30 m, J&W Scientific, Folsom, CA, USA) was used for the analyses. The oven temperature was maintained at

100°C for 2 min, programmed at a rate of 20°C/min to 175°C, then at 6°C/min to 220°C, and held at this temperature for 15 min. The effluent from the column was split at a ratio of 1:1 into two lines, one of which led to a flame ionization detector (FID) and the other to the EAD. GC-MS analyses were conducted in electron impact ionization mode (EI, 70 eV) using an HP-5973 mass spectrometer interfaced to an HP-6890 gas chromatograph, using the same column and conditions as for the GC-EAD analyses. The ion source temperature was 230°C. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL Alpha 500 Fourier transform spectrometer at 500.2 and 125.7 MHz, respectively, for $CDCl_3$ solutions containing TMS as an internal standard.

Field Tests Attraction of *M. japona* males to synthetic lures was examined at coppices in Ibaraki Prefecture. Test I was conducted in Chikusei and Tsukuba cities from June 30 to July 12, 2003; test II was conducted in Tsukuba City from June 25 to July 10, 2003; and tests III and IV were performed in Tsukuba City from June 17 to July 16, 2004. Test chemicals were absorbed onto a white rubber septum (Sigma-Aldrich Co., St. Louis, MO, USA), which was placed in the center of a sticky board trap (Takeda Chem. Co. Ltd., Tokyo, Japan). Traps were placed 1.5 m above the ground and at least 10 m from each other. Numbers of captured males in each trap were counted every few days. After counting, the traps were rotated to eliminate any positional effect (Latin square design; Perry et al., 1980).

Trials testing *M. longipes* synthetic pheromone were conducted over 4 d in mid-August, 2004 at the riverbed of an oxbow in Fujimi City, Saitama Prefecture. As *M. longipes* is an endangered species, we did not capture males with a sticky trap but recorded their behavior around rubber septa baited with synthetic chemicals. Each septum was fixed to a riverside plant with a safety pin, 50 cm above the ground.

Statistical Analyses The EAG data that used 1 ng of synthetic pheromone compounds (see Fig. 3a and c) were analyzed by one-way ANOVA. Means that were significantly different were separated by Tukey–Kramer's HSD test (Sokal and Rohlf, 1995). For the EAG-dose data, using synthetic E2,Z13-18 compounds and for the field trials, the data were log-transformed before analysis by one-way ANOVA. Means that were significantly different were separated by Tukey–Kramer's HSD test. The level of significance in all tests was set at 5%.

Results

Identification of Pheromone Components of M. japona Male *M. japona* antennae were stimulated by two components, I and II (Fig. 1a). Component I eluted from a Florisil® column with 10% ether in hexane, indicating an alcohol, whereas component II eluted with 5% ether in hexane, indicating an acetate or aldehyde. The mass spectrum of component I (Fig. 1b, c) showed M^+ at m/z 266 (relative intensity: 1.4%), $[M\text{-}H_2O]^+$ at m/z 248 (10.1%), and a series of fragment ions derived from straight-chain compounds, indicating octadecadien-1-ol. The spectrum is similar to that of synthetic E2,Z13-18:OH, which is easily distinguished from spectra of 3,13-octadecadien-1-ol by the relative intensity of M^+ and $[M\text{-}H_2O]^+$ (Naka et al., 2006). The retention time (R_t) of Component I was the same as that of authentic E2,Z13-18:OH, but different from those of the three other geometric isomers [(2*E*,13*E*)-isomer, R_t 11.30 min; (2*Z*,13*E*)-isomer, R_t 11.46 min; (2*Z*,13*Z*)-isomer,

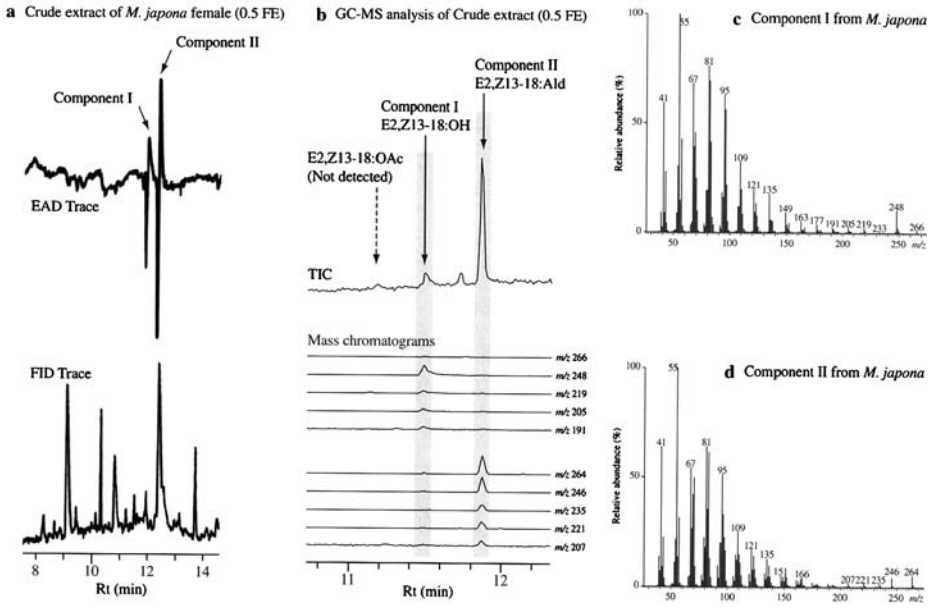

Fig. 1 Analysis of the sex pheromone components of *Macroscelesia* japona by GC-EAD and GC-MS. (a) GC-EAD analysis of pheromone extract (0.5 FE). (b) GC-MS analysis of extract (0.5 FE); total ion chromatogram (TIC) and mass chromatograms monitoring diagnostic ions at *m/z* 266 (M^+ of E2,Z13-18:OH), *m/z* 248 ($[M-18]^+$ of alcohol and $[M-60]^+$ of acetate), 219, 205, and 191 of E2,Z13-18:OH and E2,Z13-18:OAc, and *m/z* 264 (M^+), 246 ($[M-18]^+$), 235, 221, and 207 of E2,Z13-18:Ald. E2,Z13-18:OAc was not detected in the crude extract. (c) Mass spectrum of Component I (E2,Z13-18:OH). (d) Mass spectrum of Component II (E2,Z13-18:Ald)

R_t 11.67 min]. The mass spectrum of component II showed M^+ at m/z 264 (4.4%) and $[M-H_2O]^+$ at m/z 246 (4.4%), indicating an octadecadienal (Fig. 1c, d). This spectrum corresponded closely to that of synthetic E2,Z13-18:Ald. Furthermore, $NaBH_4$ reduction of the 5% ether fraction produced E2,Z13-18:OH, indicating that Component II was E2,Z13-18:Ald. Ten GC-MS injections (1 FE each) of different extracts allowed determination of the mean amounts of the two components: 0.3±1.2 ng/female for E2,Z13-18:OH and 4.2±5.4 ng/female for E2,Z13-18:Ald. The two components were in a ratio of roughly 1:10, although there was considerable variation among samples. No other candidate pheromone chemicals, such as E2,Z13-18:OAc, could be detected (Fig. 1b).

Identification of Pheromone Components of M. longipes Antennae of *M. longipes* males exhibited consistent EAG responses to two compounds with the same retention times as components I and II of *M. japona* (Fig. 2a). GC-MS analysis of the pheromone extract (Fig. 2a–d) confirmed that extract of *M. longipes* pheromone gland contained E2,Z13-18:OH and E2,Z13-18:Ald. Three GC-MS injections (1 FE each) of *M. longipes* extract gave a mean titer of E2,Z13-18:OH of 16.8±8.0 ng/female. E2,Z13-18:Ald was detected in one sample (0.4 ng/female), but not in the other two.

EAG Activities of Synthetic Compounds EAG responses of male antennae to synthetic compounds known to be pheromone components of Sesiidae were determined for the two

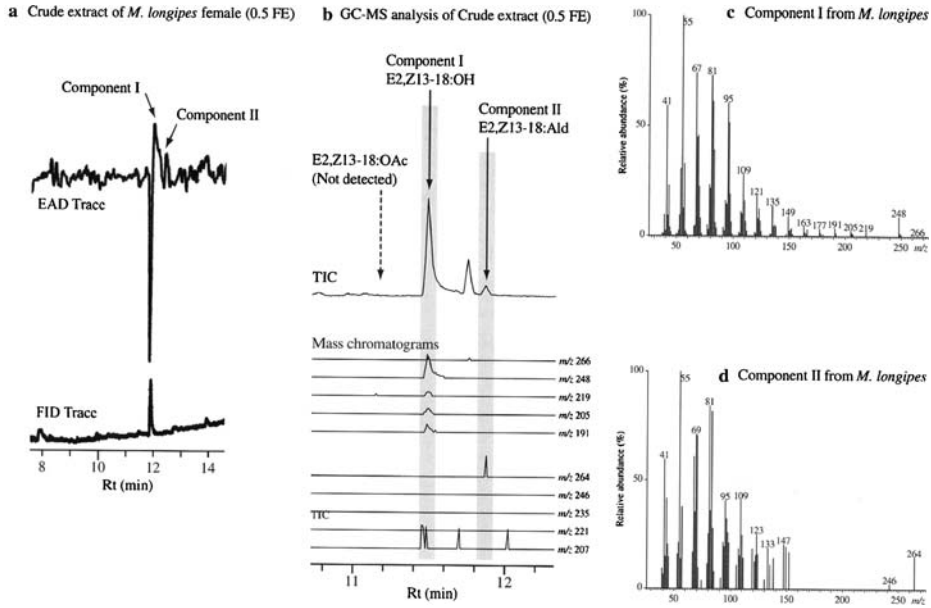

Fig. 2 Analysis of the sex pheromone components of *Macroscelesia* longipes by GC-EAD and GC-MS. (a) GC-EAD analysis of pheromone extract (0.5 FE). (b) GC-MS analysis of extract (0.5 FE); total ion chromatogram (TIC) and mass chromatograms monitoring diagnostic ions at *m/z* 266 (M^+ of E2,Z13-18:OH), *m/z* 248 ($[M-18]^+$ of alcohol and $[M-60]^+$ of acetate), 219, 205, and 191 of E2,Z13-18:OH and E2,Z13-18:OAc, and *m/z* 264 (M^+), 246 ($[M-18]^+$), 235, 221, and 207 of E2,Z13-18:Ald. E2,Z13-18:OAc was not detected in the crude extract. (c) Mass spectrum of Component I (E2,Z13-18:OH). (d) Mass spectrum of Component II (E2,Z13-18:Ald)

Macroscelesia species (Fig. 3). *M. japona* antennae showed the greatest response to E2, Z13-18:Ald, the more abundant component in the pheromone gland of females, and a moderate response to E2,Z13-18:OH, the less abundant compound (Fig. 3a). Responses to both compounds were greater than responses to all other compounds tested (ANOVA, $df=6$, $F=21.50$, $P<0.01$). There were no significant differences in response of antennae with respect to dose of three 2,13-dienyl compounds. However, the relative response to the three different compounds was the same at all doses (E2Z13-18:Ald> E2Z13-18:OH> E2Z13-18:OAc) (Fig. 3b). *M. longipes* antennae showed the greatest EAG response (greater than to all other compounds tested; ANOVA, $df=6$, $F=9.90$, $P<0.01$) to E2,Z13-18:OH, the most abundant compound in the female pheromone gland extract (Fig. 3c). Again, there were no significant differences in response to antennae with respect to dose of the three 2,13-dienyl compounds. However, EAG responses to E2,Z13-18:OH were greater than responses to the other two compounds at all three doses tested (Fig. 3d).

Field Attraction of M. japona Males Of the single-component lures baited with one of the 2,13-dienyl compounds, only traps baited with E2,Z13-18:Ald caught male *M. japona* (Test I, Table 1). When different amounts of E2,Z13-18:OH or E2,Z13-18:OAc were added to E2,Z13-18:Ald, surprisingly, only traps baited with both E2,Z13-18:OAc and E2,Z13-18:Ald caught males (Test II, Table 1). As treatments with E2,Z13-18:OAc appeared to shut down trap capture to all other blends (including to that of E2,Z13-18:Ald alone), further tests were conducted in which the effect of the alcohol component was evaluated in a field

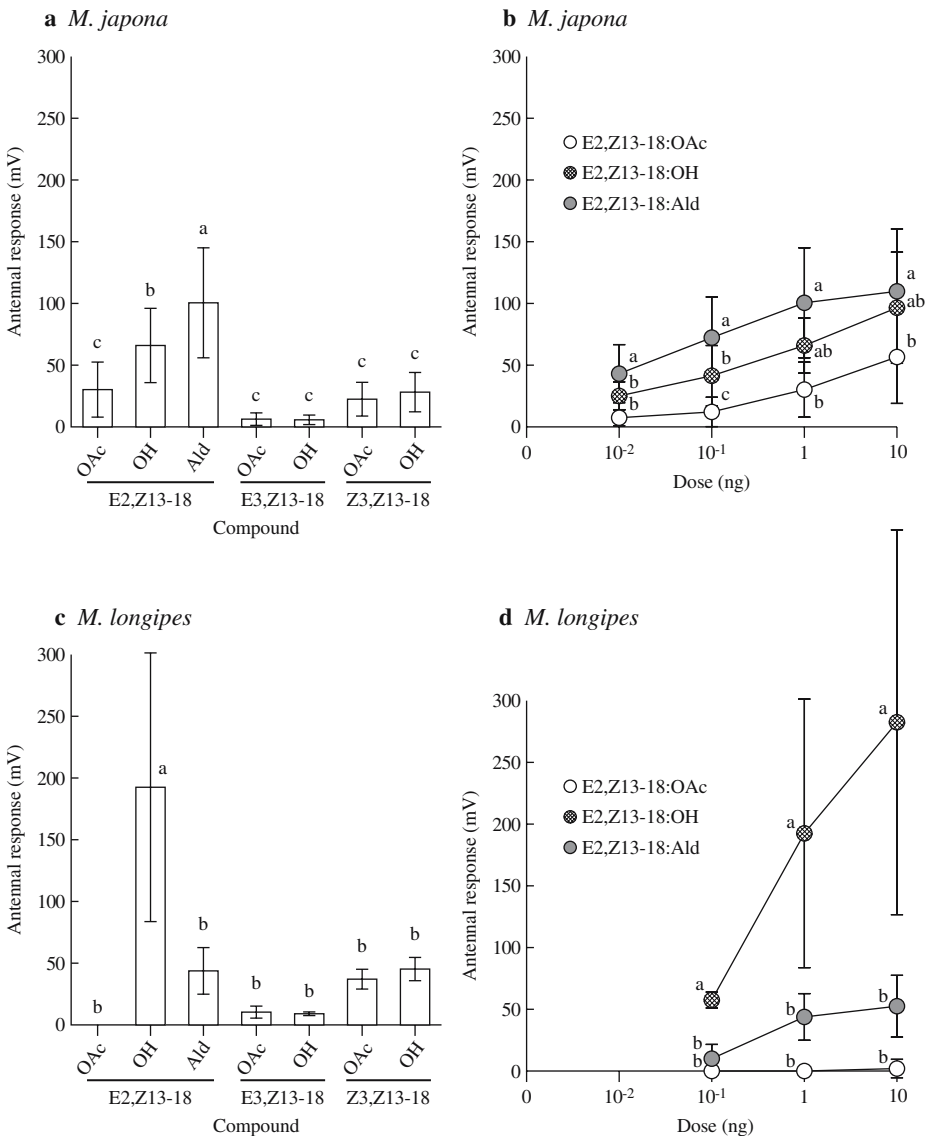

Fig. 3 EAG responses of *Macroscelesia japona* and *M. longipes* males. (a) EAG responses of antennae of *M. japona* males to synthetic standards (1 ng). (b) Responses of antennae of the *M. japona* males to various doses of (2*E*,13*Z*)-2,13-octadecadienyl compounds. (c) EAG responses of antennae of *M. longipes* males to synthetic standards (1 ng). (d) Responses of antennae of the *M. longipes* males to various doses of (2*E*,13*Z*)-2,13-octadecadienyl compounds. Responses are the means of at least ten antennae of *M. japona* and four antennae of *M. longipes*. Values followed by a different letter are significantly different at $P<0.05$ (Tukey–Kramer's HSD test) in (a)–(c). In (d), values followed by a different letter are significantly different at $P<0.05$ (Tukey–Kramer's HSD test) for a given dose of the different compounds

about 4 km from the field testing mixtures of the aldehyde and acetate. Traps baited with lures containing both E2,Z13-18:Ald and E2,Z13-18:OH caught significant numbers of males (Test III, Table 1), with the greatest numbers (significantly greater than for all other treatments) being caught in the 1:100 (E2,Z13-18:OH: E2,Z13-18:Ald) blend. Again binary

Table 1 Attraction of *M. japona* males to lures baited with synthetic (2*E*,13*Z*)-2,13-octadecadienyl compounds[a]

	Content of C18 compounds (μg)			Mean number of catches (Mean ± SD)[a]
	E2,Z13-18:OH	E2,Z13-18:Ald	E2,Z13-18:OAc	
Test I:	0	1,000	0	21.7±4.0
	1,000	0	0	0
	0	0	1,000	0
	0	0	0	0
Test II:	0	1,000	0	0
	100	1,000	0	0
	300	1,000	0	0
	0	1,000	100	15.8±4.1[a]
	0	1,000	300	10.5±6.4[a]
	0	0	0	0
Test III:	0	1,000	0	1.0±1.0b
	100	1,000	0	13.0±7.0[a]
	50	1,000	0	2.3±3.2[b]
	100	1,000	0	0.3±0.6[b]
	300	1,000	0	0
	500	1,000	0	0
	0	0	0	0
Test IV:	0	1,000	0	0.7±1.2b
	0	1,000	10	2.7±3.8[a b]
	0	1,000	50	5.0±3.6[a b]
	0	1,000	100	6.0±4.4[a b]
	0	1,000	300	8.0±3.6[a b]
	0	1,000	500	11.0±2.6[a]
	0	0	0	0

[a] Test I was performed in two fields (N=3); Chikusei City (a private coppice under Kokai-Ohashi Bridge) and Tsukuba City (coppices in NIAES) from June 30 to July 12, 2003. Test II was performed in Tsukuba City (N=4); coppices in NIAES, NIES and Yukari-no-Mori from June 25 to July 10, 2003. Tests III and IV were performed in the same fields as Test II (N=3) from June 17 to July 16, 2004.

[b] Values followed by a different letter are significantly different at the 5% level by Tukey-Kramer test.

blends of E2,Z13-18:Ald and E2,Z13-18:OAc caught significant numbers of male *M. japona* (Test IV, Table 1).

Field Attraction of M. longipes Males Because *M. longipes* males fly slowly, their behavior around synthetic lures could readily be observed in the field. Two behaviors were observed: approaching the lure and touching the lure with forelegs. Males only approached lures containing E2,Z13-18:OH (Table 2). About half of the approaching males touched the lures. The greatest numbers of males that approached lures did so to binary blends of E2,Z13-18:OH and E2,Z13-18:Ald; the numbers that approached the 1,000:50 and 1,000:300 lures were significantly greater than the number that approached the lure containing only E2,Z13-18:OH. Greater numbers of males touched the 1,000:50 lure (E2,Z13-18:OH: E2,Z13-18:Ald) than touched any of the other lures. The numbers of males that approached or touched the binary blend of E2,Z13-18:OH and E2,Z13-18:OAc were not significantly

Table 2 Attraction of *M. longipes* males to lures containing synthetic (2*E*,13*Z*)-2,13-octadecadienyl compounds[a]

Content of C18 compounds (μg)			Mean number of attracted males (Mean ± SD)[a]	
E2,Z13-18:OH	E2,Z13-18:Ald	E2,Z13-18:OAc	Touched	Attracted
1,000	0	0	6.3±1.7[b c]	3.0±1.8[b c]
1,000	50	0	13.8±3.3[a]	9.8±2.2[a]
1,000	100	0	13.0±6.1[a b]	5.3±2.9[b]
1,000	300	0	13.3±2.5[a]	5.5±1.3[b]
1,000	0	100	5.5±2.4[c]	3.0±1.4[b c]
0	1,000	0	0	0
0	0	1,000	0	0
0	0	0	0	0

[a] Test was performed in a riverbed of the Bin-Numa oxbow (Fujimi City, Saitama Prefecture) for 4 d between August 12 and August 23, 2004.

[b] Mean number of 4 days. Values followed by a different letter are significantly different at the 5% level by Tukey–Kramer test.

[c] Attracted: males attracted to within 5 cm. Touched: males touched the lure with their forelegs.

different from the numbers that approached or touched the lure containing only E2,Z13-18:OH (Table 2).

Discussion

GC-EAD, GC-MS analyses of pheromone extracts, combined with field studies, revealed E2,Z13-18:OH and E2,Z13-18:Ald as common pheromone components of two *Macroscelesia* species, *M. japona*, and *M. longipes*. The major component of the former species was aldehyde and that of the latter was alcohol. These compounds were identified by comparison with chemical data of known compounds, not by direct chemical reaction, because of the limited number of available insects. All the dienyl compounds known as Sesiidae pheromones or attractants have a C_{18}-chain skeleton unsaturated at the 3- and 13-positions or the 2- and 13-positions (Ando, 2006). The GC-MS data of the alcohol component of both *Macroscelesia* species strongly indicated a 2,13-dienyl structure (Naka et al., 2006). Configurations of the two double bonds were determined by comparing R_t values of the natural component and the four geometric isomers. The second component was indicated as an aldehyde by its elution time on the DB-23 column. The double-bond positions and configurations of the aldehyde component from *M. japona* females were determined by reduction by $NaBH_4$ to produce the same dienyl alcohol as the other pheromone component. To assist in pheromone identifications of other sessiid moths, we intend to synthesize the four geometric isomers of 2,13-18:Ald.

Compounds with the C_{18} 2,13-dienyl skeleton have been reported as sex pheromones in several species in the lepidopteran families of Sesiidae, Cossidae, and Tineidae. All these compounds have a (2*E*,13*Z*)-configuration; no 2,13-dienes with other configurations have been found in pheromone gland extracts of female moths. (Ando, 2006; El-Sayed, 2006). In the Sesiidae, E2,Z13-18:OH and E2,Z13-18:Ald have been identified only from *Melittia cucurbitae* (Klun et al., 1990) and *Sesia apiformis* (Francke et al., 2004), respectively,

whereas E2,Z13-18:OAc has been identified in a number of species. Interestingly, *Melittia cucurbitae* is taxonomically close to *Macroscelesia* spp.

The reproductive isolation of sibling species, through use of different blends of common sex pheromone components, has been reported in many species of moths (Löfstedt et al., 1991; Ishikawa et al., 1999). Our study revealed that two *Macroscelesia* species produce the same two pheromone components, E2,Z13-18:OH and E2,Z13-18:Ald, but apparently in a different ratio. In accord with this, males of the two species appear to respond optimally to blends similar to those produced by their respective females: 1:100 for *M. japona* males and 20:1 for *M. longipes* males (Tables 1 and 2). Although single-component blends that are attractive to the other species were tested in the respective habitats of the two species, no *M. japona* males approached lures in the habitat of *M. longipes*, and no *M. longipes* males were captured in traps in the test for *M. japona* males. This result suggests that geographical overlap of the species is not common (at least in the areas tested), and precludes any possible hybridization of the two species. However, if there were any geographical overlap, our data suggest that there would be little, if any, cross attraction between the species.

Although E2,Z13-18:OAc was not detected in the pheromone glands of the two *Macroscelesia* species, we were interested in its activity with respect to males of the two species. The acetate appeared to have little effect on *M. longipes* males. However, in the case of male *M. japona*, addition of this compound to E2,Z13-18:Ald gave increased trap captures relative to the latter compound alone. Field Tests II and IV indicated that E2,Z13-18:OAc may have acted as a substitute for E2,Z13-18:OH, the minor pheromone component of *M. japona*. Substitution of a minor component by other analogs has been observed in other lepidopteran species (Linn et al., 1984; Sugie et al., 2003; Inomata et al., 2005). However, in field Test II, the blends of the aldehyde and acetate caught significantly greater numbers of moths than the natural blends of the aldehyde and alcohol, with trap catches to the latter blends being completely suppressed. This preferential response to an unnatural blend over a natural one is perplexing, and suggests that *M. japona* males could be attracted to another species that produces a blend of E2,Z13-18:Ald and E2,Z13-18:OAc. To date, no species using this mixture as a sex pheromone has been identified, and the mixture in our field trials attracted no other species. However, considering the diversity of Sesiidae, and the apparent limited variety of chemicals used by this family as pheromone components, it is possible that some species secrete such a mixture. Further studies on a greater number of species of Sessiidae are necessary to identify such species.

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