Journal of Chemical Ecology, Vol. 22, No. 8, 1996

OLEFINIC ACETATES, Δ-9,11-14:OAc AND Δ-7,9-12:OAc USED AS SEX PHEROMONE COMPONENTS IN THREE GEOMETRID MOTHS, *Idaea aversata*, *I. straminata*, AND *I. biselata* (Geometridae, Lepidoptera)

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(Received February 21, 1995; accepted April 10, 1996)

Abstract—Pheromone compounds so far identified from most geometrid moths consist of all-*Z* diene, triene, or tetraene hydrocarbons with chain lengths of C_{17} to C_{21}, and their monoepoxide derivatives biosynthesized from linoleic and linolenic acids. The present study reports the occurrence of olefinic acetates as sex pheromones in three species of Geometridae. (*Z*,*Z*)-9,11-tetradecadienyl acetate and (*Z*,*Z*)-7,9-dodecadienyl acetate found in female gland extracts of *Idaea aversata* elicited significant responses from conspecific male antennae in gas chromatography with electroantennographic detection (GC-EAD). In extracts of *I. straminata*, (*Z*,*E*)-7,9-dodecadienyl acetate, (*E*,*Z*)-7,9-dodecadienyl acetate, and (*Z*,*Z*)-7,9-dodecadienyl acetate were found, and the synthetic compounds elicited strong responses from conspecific male antennae. In the third species, *I. biselata*, only (*Z*,*Z*)-7,9-dodecadienyl acetate was found in the female extracts, and this compound elicited a strong EAD

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0098-0331/96/0800-1505$09.50/0

response from the conspecific male antenna. The identities of the pheromone components in *I. aversata* and *I. straminata* were further confirmed according to their characteristic ions after GC-MS analyses. Single sensillum recordings from *I. aversata* showed two types of pheromone-detecting sensilla present on the male antenna. One type contained two receptor neurons, one of which was specifically tuned to (*Z*,*Z*)-9,11-tetradecadienyl acetate, the other to (*Z*,*E*)-9,11-tetradecadienyl acetate. A second type contained one neuron responding to (*Z*,*Z*)-7,9-dodecadienyl acetate. The two types were clearly different also with respect to external morphology, the former being considerably longer and having a larger base diameter. Also in *I. straminata* two physiological types of sensilla could be distinguished. One type contained two neurons, one of which responded to (*Z*,*Z*)-7,9-dodecadienyl acetate, the other to (*Z*,*E*)-9,11-tetradecadienyl acetate. The second type contained one neuron, responding to (*Z*,*Z*)-7,9-dodecadienyl acetate. No correlation between external morphology and physiological response of the investigated sensilla was observed in *I. straminata*. In field tests, a two-component blend containing (*Z*,*Z*)-9,11-tetradecadienyl acetate and (*Z*,*Z*)-7,9-dodecadienyl acetate in a ratio of 10 : 1 was attractive to males of *I. aversata*. This two-component blend was also attractive to males of *I. straminata*, but in a ratio of 1 : 1. High numbers of male *I. biselata* were caught in traps baited with (*Z*,*Z*)-7,9-dodecadienyl acetate alone. The incorporation of deuterium labels into pheromone components after topical application of deuterium-labeled palmitic acid confirmed that the pheromone components of *I. aversata* could be synthesized from this precursor, as has been previously observed for acetate pheromone components of many other moth species. Our results suggest that an evolutionary reversal back to the production of palmitic acid-derived pheromone components has occurred within the geometrid subfamily Sterrhinae.

Key Words—Sex pheromone, *Idaea aversata*, *Idaea straminata*, *Idaea biselata*, (*Z*,*Z*)-9,11-tetradecadienyl acetate, (*Z*,*Z*)-7,9-dodecadienyl acetate, (*Z*,*E*)-7,9-dodecadienyl acetate, Lepidoptera, Geometridae, electroantennography, single cell recording, biosynthesis, phylogeny, evolution.

INTRODUCTION

The majority of pheromones of higher Lepidoptera consist of unsaturated acetates, alcohols, or aldehydes, and the carbon skeletons of these pheromone components can be biosynthetically derived from palmitic acid (hexadecanoic acid) or stearic acid (octadecanoic acid) by a combination of chain-shortening, desaturation, reduction, and acetylation reactions (Bjostad et al., 1987). A less common type of moth pheromone component includes unsaturated hydrocarbons and their epoxides, which can be derived from linoleic and linolenic acid (Rule and Roelofs, 1989). Such pheromone components occur mainly within the superfamilies Noctuoidea and Geometroidea (Arn et al., 1992). In Geometridae, sex pheromones have been identified for around 28 species, and sex attractants have been reported for an additional 55 species (Arn et al., 1992 and references therein). In most of these reports, sex pheromones and sex attractants were

all-*Z* diene, triene, or tetraene hydrocarbons with chain lengths from C_{17} to C_{21} and/or their corresponding monoepoxides. Three hemlock loopers utilize branched hydrocarbons as their pheromones (Gries et al., 1991, 1993a,b), and a few reports indicate that olefinic acetates and aldehydes can also be used as pheromones or attractants in some species of Geometridae, but only within the subfamily Sterrhinae. For example, (*E*,*Z*)-7,9-dodecadienyl acetate (*E*7,*Z*9–12:OAc) was reported to be attractive to males of *Idaea biselata* (Biwer et al., 1975), and compounds such as (*E*,*Z*)-7,9-dodecadienal (*E*7,*Z*9–12:Ald) and (*Z*)-7-dodecenyl acetate (*Z*7–12:OAc) have also been shown to be attractive for males of this species (E. Priesner personal communication and Ando et al., 1987). *Z*7–12:OAc, *E*7,*Z*9–12:OAc, and (*E*,*Z*)-7,9-dodecadienol (*E*7,*Z*9–12:OH) were reported as attractants for males of *I. imbecilla*, *I. rubraria*, and *I. trisetata*, respectively (Szöcs et al., 1987; Ando et al., 1977, 1987).

The type of sex pheromone components used in moths may be of taxonomic significance at the genus, subfamily, and family levels, and pheromone characteristics may be used for phylogenetic analysis of different taxa (Roelofs and Brown, 1982; Löfstedt et al., 1991; Löfstedt and Kozlov, 1996). The possibility that acetates are used as pheromone components in one geometrid subfamily, whereas other subfamilies appear to use branched hydrocarbons or unsaturated hydrocarbons and/or their corresponding epoxides as pheromone components, is intriguing and deserves to be studied in more detail. In the present study we confirm the occurrence of acetates as sex pheromone components in three geometrid moths, *I. aversata* (L., 1758), *I. straminata* (Borkhausen, 1794), and *I. biselata* (Hufnagel, 1767). Active components were detected by gas chromatography with electroantennographic detection (GC-EAD) and identified by gas chromatography and gas chromatography–mass spectrometry. The activity of synthetic pheromone components was confirmed by single sensillum recordings on male antennae and by field trapping.

METHODS AND MATERIALS

Insects. Female adults of all three investigated species were collected at various sites in southern and south-central Sweden. Laboratory cultures were established from eggs laid by these collected insects. The larvae were reared on withered moist leaves from a number of plant species, predominantly willows (*Salix* spp.). The cultures were kept under a 16 L:18 D photoperiod at a temperature of 22°C and a relative humidity of 60%. Sex was determined at the pupal stage, and the emerging adults were kept individually in small cups under the same environmental conditions as the larvae.

Pheromone Gland Extraction. Females of all species started calling 3–4 h after darkness on the second day after emergence. The pheromone glands of calling females were excised and extracted for 30 min in redistilled hexane.

Electrophysiological and Chemical Analyses. For electroantennographic (EAG) recordings, excised male antennae were placed with the base in a capillary electrode filled with Beadle-Ephrussi Ringer, and grounded via an Ag–AgCl wire. The distal tip of the antenna was cut, and the exposed surface was placed in contact with a second electrode, similar to the indifferent electrode. The tip electrode was connected to a high-impedance DC amplifier with automatic baseline drift compensation.

Single sensillum recordings were performed utilizing the tip-cutting technique. Either excised male antennae or intact insects were used. Excised antennae were placed with the base in a capillary electrode as described above. Intact insects were restrained in holders cut from plastic disposable pipet tips, and the head and antennae were fixed in position with dental wax. A thin tungsten wire serving as ground electrode was inserted into the abdomen. Olfactory sensilla were cut by means of two glass knives, and a recording electrode was placed in contact with the cut surface of the sensillum. The recording electrode was a glass pipet with a tip diameter of about 4 μm, filled with Beadle-Ephrussi Ringer. The recording electrode was connected to a high-impedance AC amplifier via an Ag–AgCl wire. The recorded data was analyzed on a Macintosh computer with a Maclab analog/digital interface (M. C. Macknight, Analog Digital Instruments).

In both EAG and single sensillum experiments, the antenna was continuously flushed with charcoal-filtered and moistened air (0.5 m/sec) delivered through a glass tube (8 mm ID) ending 10 mm in front of the preparation. Stimulus cartridges consisted of Pasteur pipets containing a piece of filter paper (7 × 15 mm) on which the stimulus had been applied. In the dose–response tests, serial dilutions (10^{-2}–10^{2} μg in decadic steps) of the compounds dissolved in 10 μl of hexane were applied. Air (2 ml) was passed over the filter paper in the stimulus cartridge during 0.5 sec and injected into the airstream 150 mm upstream of the antenna.

A Hewlett Packard 5830 gas chromatograph (GC) equipped with a DB-wax column (30 m × 0.25 mm ID, J & W Scientific, Folsom, California) and an effluent split, allowed simultaneous flame ionization (FID) and electroantennographic detection (EAD) of the separated pheromone components. Hydrogen was used as carrier gas, and the effluent split ratio was approximately 1 : 1. Samples were injected in splitless mode. The injector temperature was 250°C and the split valve was opened 1 min after injection. The column temperature was maintained at 80°C for 3 min following the injection and then linearly increased to 230°C at 10°C/min. The outlet for the EAD was placed in a purified airstream flowing over the antennal preparation at a speed of 0.5 m/sec. Either female pheromone gland extracts or synthetic standard were injected into the column for the analysis. GC retention times of EAD-active components were converted to equivalent chain lengths (ECLs) relative to the retention times of

homologous, straight-chain acetates (7–22:OAc). The electrophysiological methods were identical to those described above.

Individual female pheromone gland extracts were also analyzed on a Hewlett-Packard 5880 GC equipped with the same column and under the same operating conditions as described above.

Mass spectrometry with electron impact ionization (70 eV) was performed on a HP 5970B GC-MS system equipped with a 59970B computer system, and interfaced with a HP 5890 GC. Gas chromatographic conditions for analyses were the same as those described above.

Biosynthesis Experiment. [16,16,16-$^2H_3$]hexadecanoic acid (D_3-16:COOH) (Larodan Fine Chemicals, Malmö, Sweden) dissolved in DMSO (4 μg/0.2 μl) was applied topically to the pheromone glands of 2- to 3-day-old female *I. aversata* with a 10-μl syringe. After 1 hr incubation, the pheromone glands were dissected and extracted for 30 min in 6 μl redistilled hexane containing 1 ng Z8–13:OAc as an internal standard. Deuterium-label incorporation from different precursors into pheromone components was determined by GC-MS in the selected ion monitoring mode. An acquisition program was designed in which diagnostic ions (DI) of unlabeled and D_3-labeled (DI+3) pheromone acetates were monitored simultaneously and the selected ions were changed at the appropriate time during the separation based on the retention times of the synthetic standards. This allowed selective detection of each of the pheromone components and their respective D_3-labeled analogs. Thus, *m/z* 164.20 and 167.20 were chosen for detection of unlabeled and deuterium-labeled Δ-7,9–12:OAc, respectively, whereas Δ-9,11–14:OAc was monitored by *m/z* 192.20 and 195.20.

Field-Trapping Tests. Field tests were conducted in the Hågadal and Nåsten regions of Uppsala in south-central Sweden from the end of June to the beginning of August in 1992 and 1993. Synthetic blends were prepared in hexane. Red rubber septa (Arthur H. Thomas Co. Catalog No. 1780-J07) were used as dispensers, and the traps used were delta traps. Traps were placed in woodland habitats, where all the species under investigation occur, and were hung on the tree twigs or placed in low bushes approximately 1 m above the ground. Within a replicate ($N = 5$), the traps were set at least 2 m apart. The traps were checked and the captured males removed once a week, and trap positions within a series were randomized to minimize the effects of habitat heterogenities. In a first experiment, from June 26 to August 1, 1992, 14 different blends of pheromone components were tested at 50 μg loading on the dispenser (details in Table 1). In a second field test from July 4 to August 21, 1993, different doses and ratios of those pheromone components that had been most attractive in the field test of 1992 were tested.

Chemicals. Z9,E11–14:OAc was prepared as described by Hall et al. (1975). The product was further purified by flash column chromatography on silica gel in 2% diethyl ether in hexane to remove any traces of the corresponding

TABLE 1. MALE CAPTURES OF *Idaea* SPECIES IN TRAPS BAITED WITH DIFFERENT COMBINATIONS OF SYNTHETIC ISOMERS OF 9,11-14:OAc AND 7,9-12:OAc IN THE VICINITY OF UPPSALA, SWEDEN (1992)[a]

Bait composition (μg)						Total male captures			
Δ-9,11-14:OAc			Δ-7,9-12:OAc						
ZZ	*ZE*	*EZ*	*ZZ*	*ZE*	*EZ*	*I. aversata*	*I. straminata*	*I. biselata*	*I. emarginata*
50	25.5	3.5	5			1 c	0 b	0 b	0 b
	25.5	3.5	5			0 c	1 b	0 b	1 b
50		3.5	5			12 a	0 b	0 b	0 b
50	25.5		5			3 c	0 b	0 b	0 b
50	25.5	3.5				0 c	0 b	0 b	0 b
50			5			7 b	0 b	0 b	0 b
50		3.5				0 c	0 b	0 b	0 b
50	25.5					1 c	0 b	0 b	0 b
50			50			1 c	5 ab	0 b	8 ab
50						1 c	1 b	0 b	0 b
			50			0 c	0 b	112 a	0 b
50			50	5		0 c	5 ab	5 b	69 a
50			50		5	0 c	9 a	0 b	11 ab
50			50	50	5	0 c	8 ab	0 b	17 a
Control						0 c	0 b	0 b	0 b

[a]Catch numbers within a column followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

alcohol and contained 1.2% *E*9,*E*11-14:OAc after Kugelrohr distillation (100°C/0.02 mm).

Z9,Z11-14:OAc was prepared by Wittig reaction of propyl (triphenylphosphonium) bromide with potassium *t*-butoxide in tetrahydrofuran and the tetrahydropyranyl ether of (Z)-11-hydroxy-2-undecenal. The latter was prepared by coupling of propargyl alcohol with the tetrahydropyranyl ether of 8-bromooctan-1-ol using lithium amide in liquid ammonia, hydrogenation of the resulting acetylenic alcohol over Lindlar catalyst in ethanol at 0°C, and oxidation of the allylic alcohol with activated manganese dioxide in toluene. The aldehyde was used without further purification and gave 9,11-14:OAc isomers *ZE*:*EZ*:*ZZ*:*EE* in 6:7:87:<0.5 ratio after removal of the tetrahydropyran protecting group with *p*-toluenesulfonic acid in methanol and acetylation with acetic anhydride in pyridine. This material was further purified by liquid chromatography on silica gel impregnated with 10% silver nitrate eluted with a

gradient of diethyl ether in hexane to give a product containing *ZE*:*EZ*:*ZZ*:*EE* isomers of 9,11-14:OAc in 2.3:0.8:96.9: <0.5 ratio after Kugelrohr distillation (120°C/0.02 mm).

*Z*7,*Z*9-12:OAc was prepared by reduction of (*Z*)-9-dodecen-7-ynyl acetate (Brückner et al., 1988) with activated zinc in methanol–water (1:1) (Boland et al., 1987). At best, only 72% conversion was achieved even after 6 days at room temperature. The product, which was separated from unreacted starting material by liquid chromatography on silica gel, eluted with 5% diethyl ether in hexane to give material containing *ZE*:*EZ*:*ZZ*:*EE* isomers of 7,9-12:OAc in 0.7:0.6:98.7: <0.1 ratio after Kugelrohr distillation (90°C/0.03 mm).

(*Z*)-9-Dodecen-7-ynyl acetate was isomerized in sunlight with a catalytic quantity of iodine in hexane solution to give a mixture of the *E* and *Z* isomer in 61:39 ratio. This mixture was reduced with activated zinc as above and the resulting mixture of *Z*7,*E*9-12:OAc and *Z*7,*Z*9-12:OAc was separated by chromatography on a silver ion exchange column eluted with methanol at atmospheric pressure (cf. Houx et al., 1974). Fractions containing the *Z*7,*E*9-12:OAc only were combined to give material containing less than 0.3% of any other isomers after Kugelrohr distillation (86°C/0.01 mm).

GC analyses of the different isomers of these two conjugated acetates were conducted on a polar CP Wax 52CB column (25 m × 0.32 mm ID; Chrompack UK), helium carrier gas at 0.5 kg/cm^{-2}, oven temperature 70°C for 2 min, then programmed at 20°C/min to 120°C and then at 4°C/min to 230°C. The order of eluted synthetic compounds was *ZE*-, *EZ*-, *ZZ*-, *EE*-7,9-12:OAc and 9,11-14:OAc, respectively.

Scanning Electron Microscopy. To examine the possible correlation between the external morphology of the pheromone-sensitive sensilla and their response specificity, antennae of *I. aversata* and *I. straminata* were fixed in 70% ethanol and subsequently dehydrated in absolute ethanol, critical point-dried, mounted with epoxy glue on specimen holders, and coated with gold/palladium (Polaron E5500 high resolution sputter) before examination in a Jeol JSM-T330 scanning electron microscope, operated at 15 kV.

RESULTS

Chemical Analyses. In *Idaea aversata*, analysis of female pheromone gland extracts by GC-EAD showed that two compounds elicited strong responses from a conspecific male antenna (Figure 1B). ECLs of the two active peaks were 1390 and 1596, respectively. By comparison of these retention indices to those of synthetic compounds, these two compounds were tentatively identified as (*Z*,*Z*)-7,9-dodecadienyl acetate (*Z*7,*Z*9-12:OAc) and (*Z*,*Z*)-9,11-tetradeca-

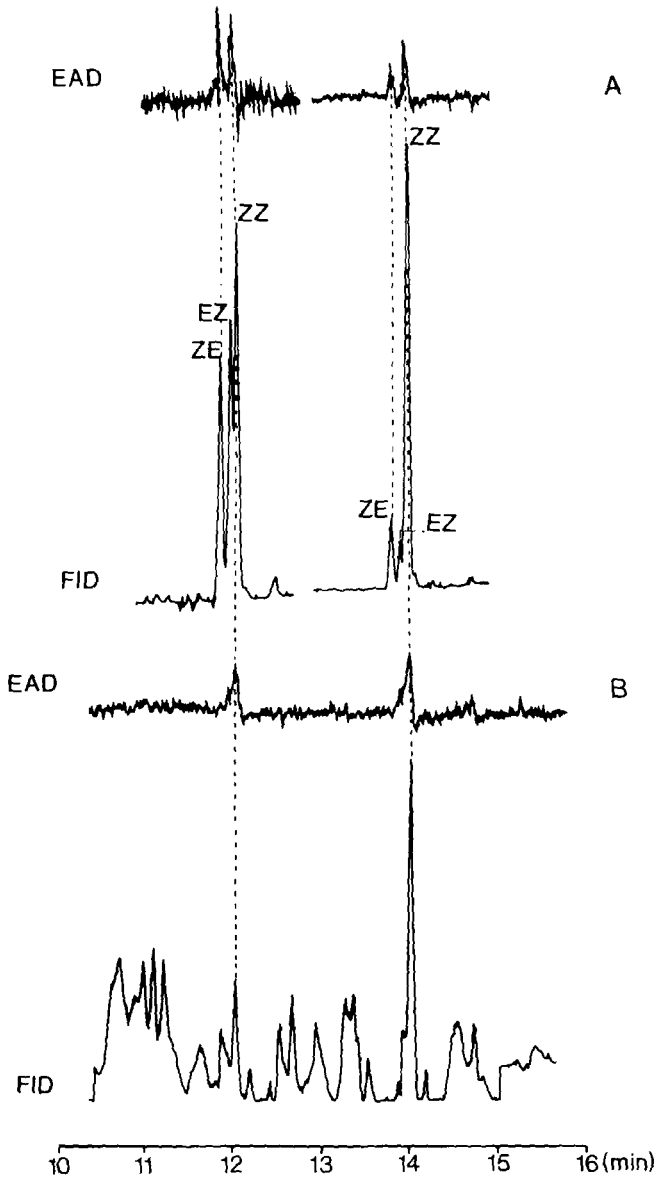

FIG. 1. GC-EAD analyses using antennae of male *Idaea aversata*. (A) Responses to the mixture of *Z*7,*E*9-, *E*7,*Z*9-, and *Z*7,*Z*9-12:OAc and the mixture of *Z*9,*E*11-*E*9,*Z*11-, and *Z*9,*Z*11-14:OAc, respectively. (B) Responses to female gland extracts.

dienyl acetate (Z9,Z11-14:OAc). Coupled GC-MS analysis of the same extracts supported the assignment of chemical structures to these two active compounds. The mass spectrum of the first eluting compound included ions at *m/z* 224 (M) and *m/z* 164 (M − 60), typical of a dodecadienyl acetate with conjugated double bonds. The spectrum of the second active peak included ions at *m/z* 252 (M) and *m/z* 192 (M − 60), indicating a tetradecadienyl acetate with conjugated double bonds. In separate GC-EAD analyses of synthetic standards, a mixture of Z7,E9-, E7,Z9-, and Z7,Z9-12:OAc produced responses to the *ZE* and *ZZ* isomers, while Z9,Z11-14:OAc containing small amounts of Z9,E11- and E9,Z11-14:OAc produced responses to the former two compounds (Figure 1A).

In *I. straminata*, combined GC-MS analyses of female pheromone gland extracts confirmed the occurrence of Z7,Z9- as well as Z7,E9- and E7,Z9-12:OAc in the females. A peak with the retention time of synthetic Z9,Z11-14:OAc was found in several gas chromatographic analyses of female extracts. When mixtures of the geometric isomers of 7,9-12:OAc and 9,11-14:OAc were tested in the GC-EAD experiments, the dodecadienyl acetates were more active than the tetradecadienyl acetates, with the Z7,Z9-12:OAc being the most active stimulus (Figure 2).

In *I. biselata*, only one active peak was found when female extracts were analyzed by GC-EAD with a conspecific male antenna as a detector (Figure 3B). The retention time of the active compound corresponded to that of synthetic Z7,Z9-12:OAc. Analysis of a synthetic sample confirmed the activity of Z7,Z9-12:OAc (Figure 3A).

Male Antennal Morphology. In *I. aversata*, sensilla trichodea of two clearly different size classes were present, as is evident from Figure 4. A large type (type I) measured ca. 70 μm in length with a diameter at the base of 3 μm. A smaller type (type II) was approximately 50 μm long and measured 2 μm at the base. The type I sensilla were much more prominent, being almost perpendicular to the antennal surface. Intermediates between the two categories were rare. In *I. straminata* the sensilla also showed a variation in size, but the differences were less pronounced.

Electrophysiology. The biological activity of the synthetic pheromone compounds was tested by EAG in all three species while single sensillum recordings were performed in two species. EAG data (Figure 5) showed that antennae of male *I. aversata* responded strongly to Z9,E11-14:OAc, Z9,Z11-14:OAc, and Z7,Z9-12:OAc. Significant responses were obtained when the male antennae of *I. straminata* and *I. biselata* were stimulated by each of the four isomers of 7,9-12:OAc. Antennae of *I. straminata* responded also to Z9,Z11-14:OAc and those of *I. biselata* showed some response to all four isomers of 9,11-14:OAc. The strongest response from male antennae of both species was elicted by Z7,Z9-12:OAc.

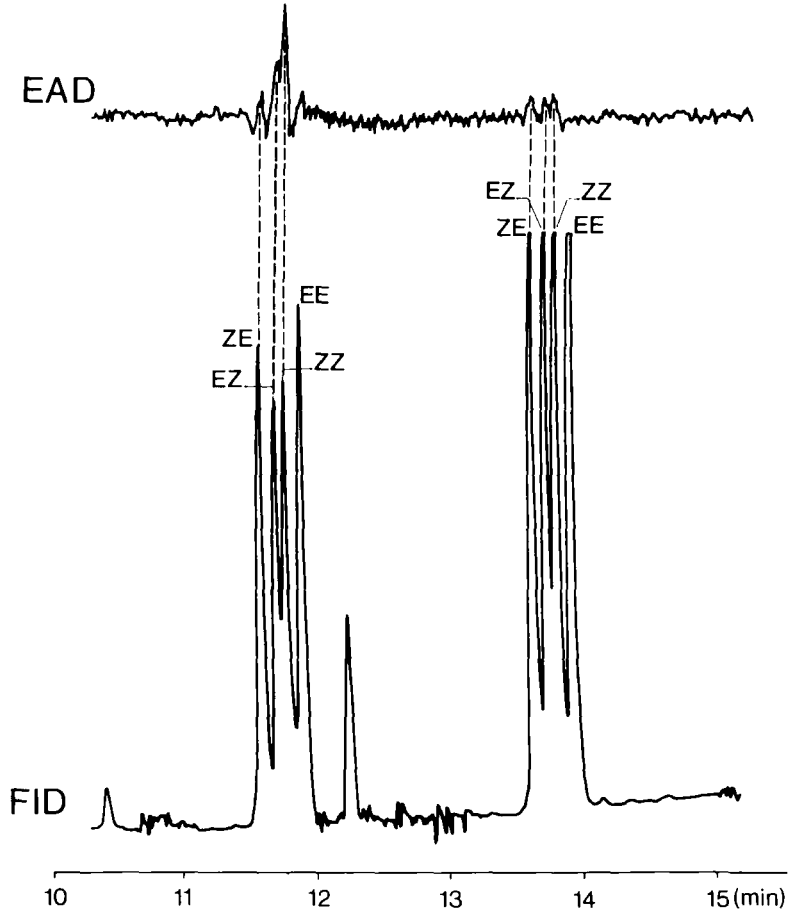

FIG. 2. GC-EAD analysis displaying male *Idaea straminata* antennal response to the mixture of four isomers of 7,9-12:OAc and four isomers of 9,11-14:OAc.

Single sensillum recordings were conducted on sensilla trichodea present on the male antennae of *I. aversata* and *I. straminata*. Recordings were made from 35 sensilla of males of *I. aversata*. All these sensilla displayed one or two receptor neurons responding to pheromone compounds. Two physiological types could be distinguished. Type I ($N = 30$) contained two neurons. One, characterized by a large action potential (spike) amplitude (neuron A), responded to Z9,*E*11-14:OAc, and the other, characterized by a smaller spike amplitude (neuron B) responded to Z9,Z11-14:OAc. Type II ($N = 5$) contained only one

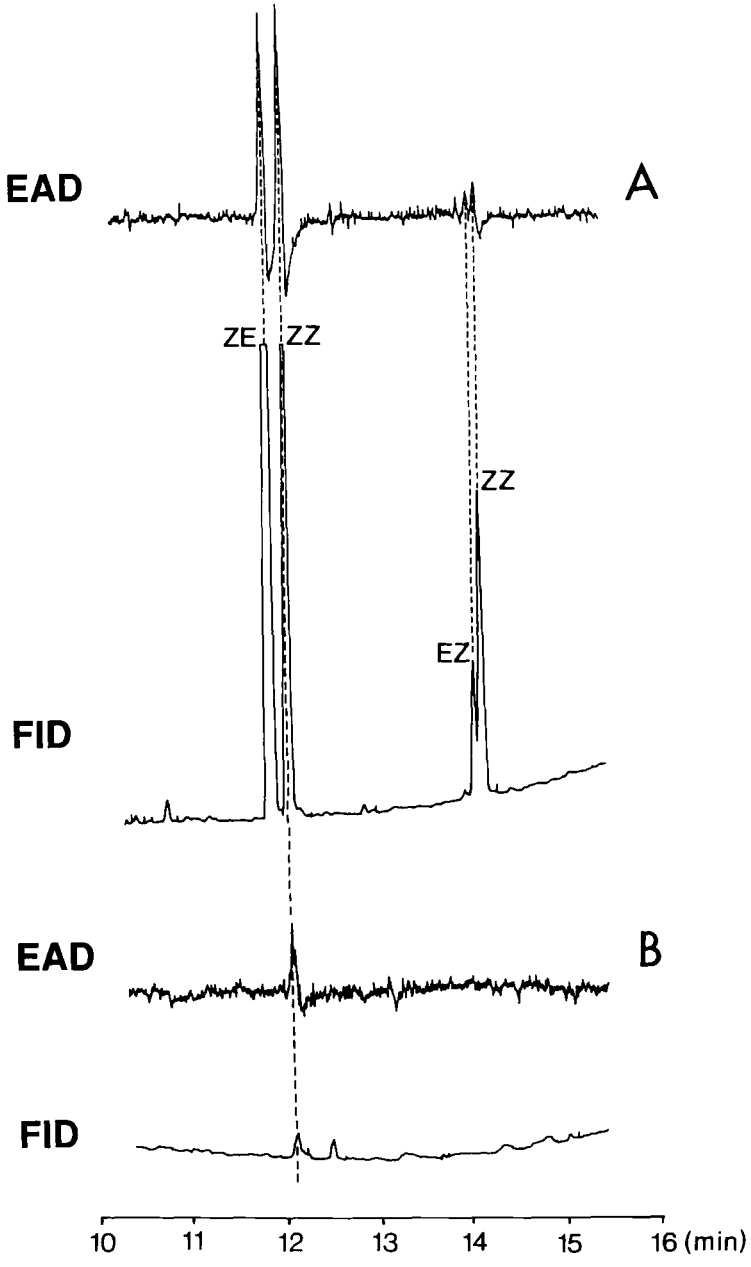

FIG. 3. GC-EAD analyses using antennae of male *Idaea biselata*. (A) Responses to the mixture of *Z*7,*E*9- and *Z*7,*Z*9–12 : OAc and *E*9,*Z*11- and *Z*9,*Z*11–14 : OAc. (B) Responses to female gland extracts.

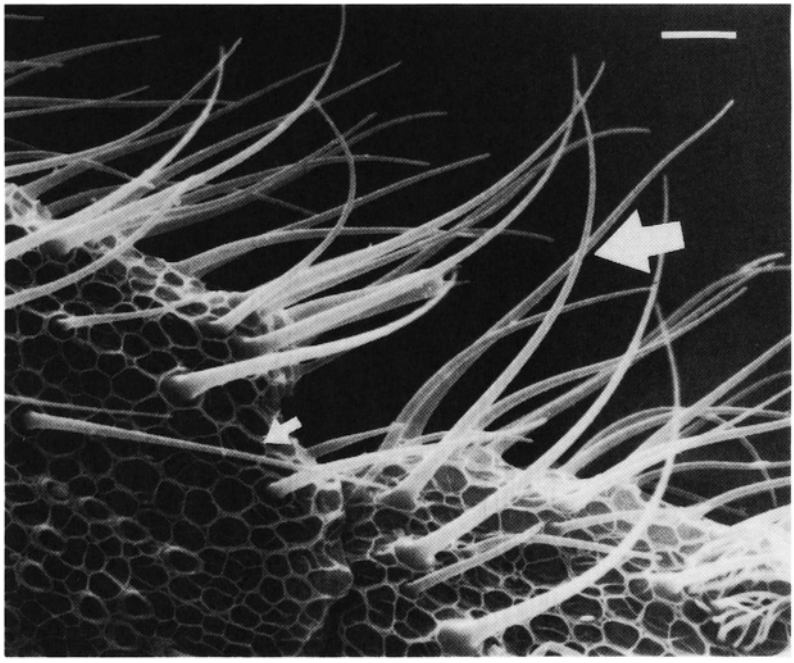

FIG. 4. The surface of male antennal segments of *Idaea aversata*, showing sensilla trichodea type I (large arrow) and type II (small arrow). Scale bar = 10 μm.

physiologically active neuron, which was specifically tuned to Z7,Z9–12:OAc. Representative recordings from these three cell types are shown in Figure 6a–c. The spike amplitudes of the two receptor neurons present in sensillum type I were quite similar, and only a thorough, computer-aided investigation of the recordings yielded an unambiguous identification (Figure 6b, inset). Type I sensilla were found among the long morphological type, while type II sensilla were found among the shorter. Dose–response tests showed that all three receptor neuron types responded to their respective key stimulus with equal sensitivity (Figure 7a–c).

In *I. straminata*, two physiologically different types of sensilla could also be distinguished. Type I (N = 17) contained two neurons, one of which responded with a large spike amplitude (neuron A) to Z7,Z9–12:OAc, and one characterized by a smaller spike amplitude responding to Z9,E11–14:OAc (neuron B). Type II (N = 13) contained one activated neuron, responding to Z7,Z9–12:OAc. Recordings from all three receptor neuron types are shown in Figure 8a–c. No correlation between external morphology and physiological specificity was established in this species. In dose–response tests, the two Z7–Z9–12:OAc

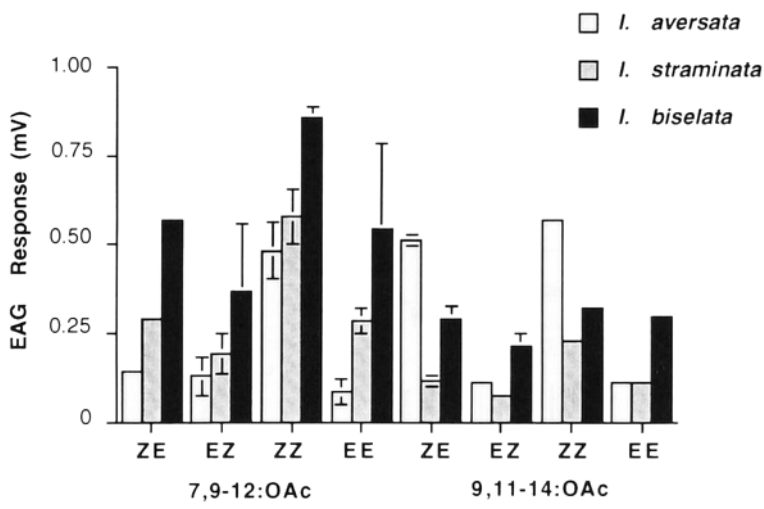

FIG. 5. EAG responses of males of *Idaea aversata*, *I. straminata*, and *I. biselata* to synthetic compounds. The blank response (air) was subtracted from each actual response. Vertical bars indicate the standard error of the mean. $N = 2$–3.

receptor neurons responded with equal sensitivity (Figure 7d). However, the *Z*9,*E*11–14:OAc receptor showed a significantly lower response.

Biosynthesis Experiments. When D_3-16:COOH was applied to the glands of *I. aversata*, there was significant incorporation into both pheromone compounds, *Z*7,*Z*9–12:OAc and *Z*9,*Z*11–14:OAc, as shown by selected ion monitoring. The D_3 analog eluted approx. 0.05 min before the unlabeled components (Löfstedt et al., 1986).

Field Tests. The results of the first field trapping test including 14 different blends of the potential pheromone compounds (Table 1), showed that males of *I. aversata* were attracted by baits containing two compounds, *Z*9,*Z*11–14:OAc and *Z*7,*Z*9–12:OAc in a ratio of 10:1, but the attraction of males was significantly increased when *E*9,*Z*11–14:OAc was included in the blend. Males of *I. straminata* were also attracted to the two component blend active to *I. aversata*, but with the compounds present in a different ratio (1:1). Such baits also trapped males of *I. emarginata*, and high captures of males of this species were obtained by adding 5 μg of *Z*7,*E*9–12:OAc to the two-component mixture. High numbers of male *I. biselata* were attracted to the bait containing only *Z*7,*Z*9–12:OAc, and adding any of the other isomers of either 9,11–14:OAc or 7,9–12:OAc reduced attractiveness significantly.

When a mixture of the two compounds *Z*9,*Z*11–14:OAc and *Z*7,*Z*9–

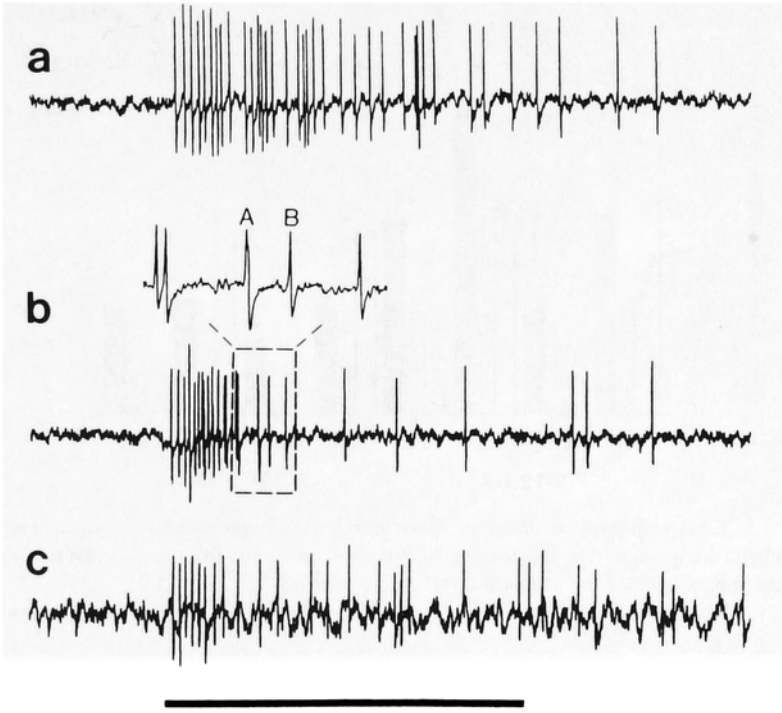

FIG. 6. Physiological responses recorded from receptor neurons present in sensilla trichodea on the male *Idaea aversata* antenna. (a) Response of the large-spike-producing neuron in the type I sensillum trichodeum to Z9,E11-14:OAc. (b) Response of the small-spike-producing neuron, present in the same sensillum type, to Z9,Z11-14:OAc. Inset shows the amplitude difference between the two receptor neurons present in sensillum trichodeum type I. (c) Response of the only receptor neuron firing in the type II sensillum trichodeum to Z7,Z9-12:OAc. Horizontal line under the records indicate duration of stimulation.

12:OAc in different ratios was tested again, the catches were low but the results confirmed those of the previous year (Table 2).

The dose-response test of Z7,Z9-12:OAc showed that *I. biselata* males responded in high numbers to baits with doses in the range of 5-500 μg. Reducing the loading further to 0.5 μg gave significantly lower catches (Table 3). No males of *I. emarginata* were trapped by baits containing 100 μg of Z7,E9-12:OAc. The attraction of males of *I. biselata* to 100 μg of Z7,Z9-12:OAc was not significantly affected by the addition of 10 μg Z7,E9-12:OAc (Table 3).

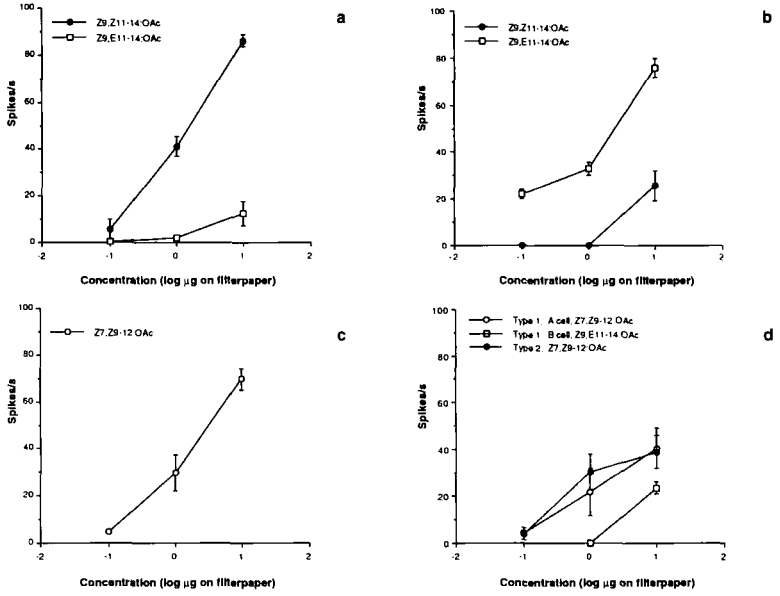

FIG. 7. Dose-response relationships for the different types of receptor neurons in *Idaea aversata* (a–c), and *I. straminata* (d). (a) *I. aversata* sensillum type I: responses of A cell to Z9,Z11-14:OAc and Z9,*E*11-14:OAc. (b) *I. aversata* sensillum type I: responses of the B cell to Z9,Z11-14:OAc and Z9,*E*11-14:OAc. (c) *I. aversata* sensillum type II: response of the single activated receptor neuron to Z7,Z9-12:OAc. (d) *I. straminata* sensillum type I and II: responses to Z7,Z9-12:OAc and Z9,Z11-14:OAc.

DISCUSSION

Our chemical analyses, electrophysiological assays, and field tests support the identification of tetradecadienyl and dodecadienyl acetates as pheromone components in the genus *Idaea*. Thus we were able to confirm previous reports on such similar compounds acting as sex attractants for certain geometrid moths (cf. Arn et al., 1992). Conjugated tetradecadienyl and dodecadienyl acetates, alcohols, and aldehydes have earlier been reported as sex pheromone components in a number of species belonging to the Pyralidae, Tortricidae, and Noctuidae families (Arn et al., 1992).

Relatively small numbers of insects were available for this work, and the identifications are by no means complete. In *I. aversata*, Z7,Z9-12:OAc and Z9,Z11-14:OAc, were identified in extracts of female glands by their GC retention times and mass spectra, and these components caused strong EAG responses in GC-EAD analyses of the female extract. In the field test, a 1:10 mixture of

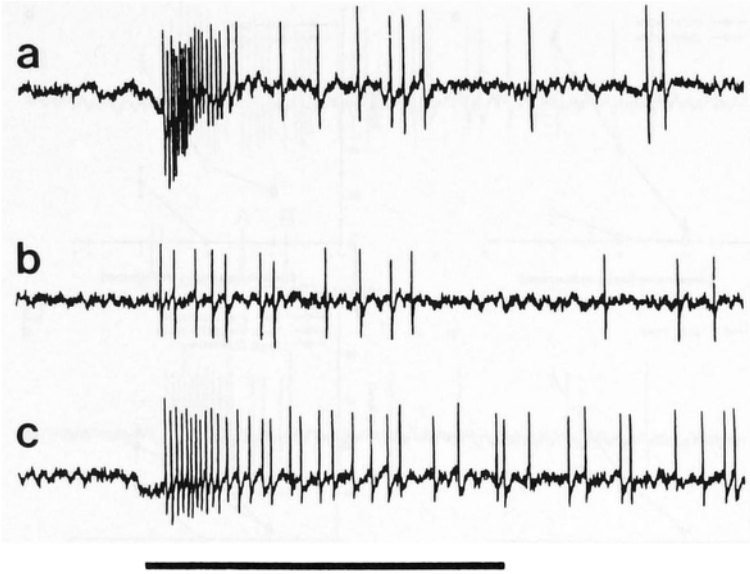

FIG. 8. Physiological responses recorded from receptor neurons present in sensilla trichodea on the male *Idaea straminata* antenna. (a) Response of the large-spike-producing neuron in the type I sensillum trichodeum when stimulated with Z9,E11-14:OAc. (b) Response of the small-spike-producing receptor neuron, present in the same sensillum type, to Z7,Z9-12:OAc. (c) A strong response was elicited by the same compound in the neuron present in the sensillum type II. Horizontal line under the records indicate duration of stimulation.

TABLE 2. MALE CAPTURES OF TWO *Idaea* SPECIES IN TRAPS BAITED WITH DIFFERENT RATIOS OF SYNTHETIC COMPOUNDS, Z9,Z11-14:OAC AND Z7,Z9-12:OAC IN THE VICINITY OF UPPSALA, SWEDEN (1993)[a]

		Total male captures	
Z9,Z11-14:OAc (μg)	Z7,Z9-12:OAc (μg)	*I. aversata*	*I. straminata*
50	0.5	8 a	0 b
50	5	10 a	1 b
50	25	5 a	0 b
50	50	0 b	5 a

[a]Catch numbers in each column followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

TABLE 3. MALE CAPTURES OF *Idaea biselata* IN TRAPS BAITED WITH DIFFERENT DOSES AND DIFFERENT RATIOS OF SYNTHETIC COMPOUNDS, *Z*7,*E*9–12:OAc AND *Z*7,*Z*9–12:OAc IN THE VICINITY OF UPPSALA, SWEDEN (1993)[a]

*Z*7,*E*9–12:OAc (μg)	*Z*7,*Z*9–12:OAc (μg)	Total male catches
	500	101 a
	50	95 a
	5	93 a
	0.5	15 b
100		5 b'
	100	90 a'
10	100	116 a'

[a]Catch numbers followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

these two compounds was attractive to male moths, and their attractiveness seemed to be increased by addition of *E*9,*Z*11–14:OAc. For *I. straminata*, the presence of *Z*7,*Z*9–12:OAc in female pheromone gland extracts was confirmed by GC-MS, and a peak at the retention time of *Z*9,*Z*11–14:OAc was observed in GC analyses. *Z*7,*Z*9–12:OAc was the most active of the isomers of 7,9–12:OAc and 9,11–14:OAc in GC-EAD analyses, and in field tests a 1:1 mixture of *Z*7,*Z*9–12:OAc and *Z*9,*Z*11–14:OAc attracted male moths. Biwer et al. (1975) and Szöcs et al. (1987) reported that males of *I. biselata* are attracted to *E*7,*Z*9–12:OAc and according to E. Priesner (personal communication referred to in Arn et al. 1992), *Z*7–12:OAc is also attractive to this species. In the present study, GC-EAD analyses showed that both *Z*7,*E*9–12:OAc and *Z*7,*Z*9–12:OAc elicited strong male antennal responses, but only the latter compound was identified from female extracts. *Z*7,*Z*9–12:OAc alone attracted a high number of male moths in the field, and no increase of male catches was observed after addition of *Z*7,*E*9–12:OAc. Since *E*7,*Z*9–12:OAc was not found in female extracts of *I. biselata*, this compound was not tested in our field tests.

The overall catches of male *I. aversata* and *I. straminata* were rather low, although the chemical analyses and electrophysiological data from EAG, GC-EAD, and single sensillum recordings of both species were mutually corroborating. The low catches could be due to the poor weather during the period of field tests, especially in 1993. Missing minor components, suboptimal ratios between pheromone components, or the presence of minor impurities in the synthetic blends with antagonistic effects on male attraction may more likely have contributed to the observed low attraction, since we observed quite a few males flying around the traps. Compounds with conjugated double bonds are

known to isomerize on the rubber septa used as pheromone dispensers (Brown and McDonough, 1986). The composition of the blends released from the septa may, after a short time, deviate significantly from what was applied to the septa.

Among eight synthetic compounds tested, the suggested major pheromone components elicited the highest EAG responses in all three species assayed. The responses of male sensilla trichodea to the female sex pheromone in Lepidoptera are well known, but the single sensillum recordings reported in the present paper are the first published on geometrid male responses to female sex pheromone components such as Z9,Z11-14:OAc,Z7,Z9-12:OAc, etc. A response pattern often encountered in various moth orders was found (Den Otter, 1977; Hansen, 1984; Priesner et al., 1984; Hansson et al., 1987, 1990): a large spike amplitude cell responding to the main pheromone component in the female pheromone gland extracts and a small spike amplitude cell responding to the minor component. The B neuron in type I sensilla of male antenna of *I. aversata* has a smaller spike amplitude responding to Z9,*E*11-14:OAc, which acts as a behavioral antagonist as demonstrated in the field bioassay. Z9,*E*11-14:OAc has not been found in pheromone gland extracts of female *I. straminata*, but the presence of a receptor specific for this compound in males suggests that its behavioral activity should be further investigated.

The use of sex pheromones and sex attractants for taxonomic and phylogenetic analysis should be carried out with caution since considerable diversity exists in the placement of functional group, double-bond positions, and chain lengths of pheromone components among species of the same genus (Renou et al., 1988). Roelofs and Bjostad (1984) have postulated that the elucidation of pheromone biosynthesis is a more useful criterion than comparison of the pheromone components themselves for determining evolutionary relationship. The sex pheromones used by the great majority of lepidopteran species so far investigated consist of unsaturated straight-chain aliphatic acetates, aldehydes, or alcohols with chain lengths of 12–18 carbons. Previous research on the biosynthesis of these pheromone components has shown that they are typically synthesized de novo from acetyl-CoA and malonyl-CoA in the pheromone glands, which produce palmitic or stearic acid via the fatty acid cycle. These acids in turn serve as precursors for a number of different acyl intermediates that can be generated by various combinations of desaturation and chain shortening (Bjostad et al., 1987). Δ11-Desaturation seems to account for the largest number of unsaturated pheromone components, and this type of pheromone component is widely distributed among ditrysian moths (Löfstedt, 1991). In females of *Spodoptera littoralis*, the sex pheromone component Z9,*E*11-14:OAc is synthesized by chain-shortening of palmitic acid to tetradecanoic acid, which, by action of a *E*11 desaturase and subsequently a Z9 desaturase, is converted into (*Z*,*E*)-9,11-tetradecadienoate (Martinez et al., 1991). Reduction and acetylation of this

compound finally leads to formation of the tetradecadienyl acetate. The incorporation of deuterium-label into pheromone acetates after applying D_3-palmitic acid onto the pheromone gland of *I. aversata* shows that palmitic acid can serve as a precursor for pheromone biosynthesis in this species. However, further experiments are needed to clarify whether or not the pheromone biosynthesis of *Idaea* species involves the same pathway as that found in *S. littoralis*.

The biosynthesis of hydrocarbon pheromone components, such as those used by most species of geometrids, has only been investigated in the arctiid moth, *Phragmatobia fuliginosa* (Rule and Roelofs, 1989). Its pheromone component, (Z,Z,Z)-3,6,9-heneicosatriene is derived from chain elongation of linolenic acid to docosatrienoic acid, presumably followed by decarboxylation. Thus, biochemical evidence suggests that two very different biosynthetic pathways are used for the production of the two major compound classes used as pheromone components in Geometridae, of which the less common pathway is similar to the route employed in most other ditrysian moths.

The higher classification (phylogeny) of Geometridae is poorly resolved. Among the commonly recognized subfamilies Archiearinae, Oenochrominae (s.str.), Geometrinae, Ennominae, Larentiinae, and Sterrhinae, it is quite likely that at least the latter is polyphyletic (M. Scoble, personal communication). There is no reason to view "Sterrhinae," including *Idaea*, as the sister group to the rest of the Geometridae. The subfamily that has often been considered as the most primitive within Geometridae is the Archiearinae. Where larvae are known, the prolegs are all present (although somewhat reduced), which is unlike the typical, and characteristic, geometrid situation. However, this view is not based on any particularly detailed analysis, and Archiearinae may eventually be shown to belong within the Ennominae (M. Scoble, personal communication). There are less than a dozen species of Archierinae in the world and nothing is known on the composition of their pheromones. Ennominae include about half of the species of Geometridae. Oenochrominae are polyphyletic; true Oenochrominae are probably confined to the Australian region, and this group may, as with Archiearinae, eventually be subsumed into the Ennominae. Again the association of Geometrinae with Ennominae may be close. So the picture may be one group of Archiearinae, Oenochrominae (s.str.), Geometrinae, Ennominae; another group including the Larentiinae; and a few independent groups from the "Sterrhinae." To these groupings can be added various genera from the Oenochrominae (s.l). The phylogenetic situation is clearly involved and unresolved (M. Scoble, personal communication).

According to the relationships between the chemical structures of identified sex pheromones or sex attractants reported and the taxonomic position of species in Geometridae, Szöcs et al. (1991) drew a distribution pattern showing that methylene-interrupted polyenic hydrocarbons and their oxygenated derivatives

are predominant in Oenochrominae, Larentiinae, and Ennominae, and olefinic acetates and derivatives are used by species in Sterrinae and Geometrinae. The limited information on pheromones of Geometridae currently available can obviously not be used to resolve the phylogeny of the family. However, it is interesting to discuss the possible ancestral state of pheromones in geometrids as this could shed some light on how pheromone communication systems may change in evolutionary time. Minet (1991) suggested that the superfamily Geometroidea should include: Geometridae, Sematuridae, and Uraniidae. Pheromones of Sematuridae and Uranidae are not known. He identified Drepanoidea (i.e., Drepanidae and Epicopeiidae) as the sister group to Geometroidea. All sex attractants reported from Drepanidae are similar to the pheromones identified from *Idaea* (Arn et al., 1992). Thus, it appears likely that the common ancestor of Geometroidea and Drepanoidea possessed the ability to produce unsaturated acetate pheromone components from palmitic acid, and this type of pheromone most likely belongs to the groundplan of all ditrysian moths (Löfstedt and Kozlov, 1996). In general it is more likely that the presence of a complex apomorphic (derived) character in many entities (species, genera, families, etc.) is the result of common heritage rather than independent evolution, as parallel/convergent evolution would depend on similar functional selection pressure operating on all the taxa under consideration. Thus, we propose that the multistep biosynthetic machinery required to produce pheromone components derived from linoleic/linolenic acids has evolved once in the evolution of Macrolepidoptera, but the production of the actual pheromone components has been subsequently and repeatedly turned on and off in the evolution of certain taxa within Geometroidea (and Noctuoidea, where such compounds occur in several families such as Noctuidae, Arctiidae, and Lymantriidae). This hypothesis would be supported if the production of "the other type of pheromone component" could be induced in "nonproducing taxa" by the artificial administration of critical "missing" precursors. Finally, identification of pheromones from certain key groups among Macrolepidoptera, as well as better resolved phylogenies based on all relevant characters, are critical to allow a rigorous reconstruction of pheromone evolution in higher Lepidoptera. With respect to geometrids, the presumed primitive geometrid subfamily Archiearinae and the sister groups of Geometridae (Sematuridae and Uraniidae), for which information on pheromone composition is scanty or missing, deserve special attention.

Acknowledgments—We thank A. Fohrman for assistance with insect rearing; L. Holmberg for field assistance; and Drs. M. Scoble, J. Dugdale, and J. Byers for helpful discussions. We also thank Dr. E. Buschmann (BASF) for a gift of (Z)-9-dodecen-7-yl acetate and Dr. R. Robinson (NRI) for assistance with synthesis. This study was financially supported by the Swedish research councils (NFR and SJFR) and the Bank of Sweden Tercentenary Foundation. N. Ryrholm is grateful to the Foundation for Zoological Research at Uppsala University for supporting the field tests.

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