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Sex Attractant of an Arctiid Moth (*Utetheisa ornatrix*): A Pulsed Chemical Signal

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Summary. The sex attractant pheromone produced by the female of the moth *Utetheisa ornatrix* was shown to contain *Z*, *Z*, *Z*-3, 6, 9-heneicosatriene. The compound, whose structure was confirmed by synthesis, proved active in electroantennogram and field bioassays. Pheromone emission occurs discontinuously, in the form of short pulses (pulse repetition rate = 1.5 ± 0.2 pulses/s). It is argued that such temporal patterning – which had not previously been demonstrated for an airborne chemical signal – can provide close-range orientation cues to the male moth as it seeks out the female.

Introduction

Sex attraction in flying insects is a more complicated process than previously thought. The mediating pheromones, to the extent that they are known, are blends rather than single compounds, which, when broadcast downwind, form aerial plumes that are spatially patterned along their length as a result of concentration changes of the components. To an approaching mate, such a patterned trail may offer sequential cues, which in lepidopteran males, for example, are said to trigger successive behavioral stages in the courtship sequence (e.g., landing, wing fanning, 'hair penciling,' and copulation) (Roelofs and Cardé 1977). We have discovered that, contrary to frequent assumption, an aerial pheromonal plume may also be patterned temporally. We found this to be the case in the arctiid moth *Utetheisa ornatrix*, the female of which releases its sex attractant in the form of discrete pulses rather than in a continuous stream. We here describe this phenomenon, together with the results of our investigation of the chemistry of the pheromone, and of the morphology of the pheromonal gland, which is anomalous and seemingly specialized for discontinuous release of its products.

Material and Methods

Utetheisa ornatrix has a broad range, extending through North America east of the Rockies, and southward into Brazil, Argentina, and Chile (Pease 1968). Distinctly aposematic in appearance as a consequence of its pink, yellow, black, and white wing markings, it often flies in the daytime and is extremely conspicuous when doing so. It is commonly abundant around stands of its leguminous larval foodplants (*Crotalaria* spp.). Our studies were done with *Utetheisa* from Florida, taken in patches of *Crotalaria mucronata*, mostly at or near the Archbold Biological Station, Lake Placid, Highlands County. Tests were done with wild specimens in the field, and with individuals raised at the Archbold Station or at Cornell University, on either of two larval diets: (a) a natural diet consisting of seeds from fresh or previously frozen pods of *Crotalaria mucronata*, and (b) a semisynthetic pinto-bean based diet (Miller et al. 1976), devoid of *Crotalaria*.

Observations of courtship in the field in darkness or near-darkness were effected with an infrared-sensitive silicon diode television system (Conner and Masters 1978), which enabled clear visualization of the events on a conventional video monitor. The necessary infrared illumination was provided by two 40-W incandescent bulbs, installed in darkroom lamp housings outfitted with infrared filters (transmittance 1% below 900 nm; and 0.03% below 700 nm). Pheromone emission by the female ('scenting behavior') was found to be inhibited by diffuse visible light, but could also be observed by using the infrared video viewing technique.

Sex attractant from scenting virgin females was collected by the technique of Byrne et al. (1975): air from a glass enclosure housing females was passed into a trap containing preconditioned Porapak Q (Supelco, Inc., Bellefonte, PA) as chemical absorbent. The attractant was eluted from the Porapak Q with redistilled *n*-pentane.

Electroantennograms (EAGs) were done by conventional procedure (Roelofs 1977). Gas-liquid chromatography (GLC) was carried out with a Varian 2100 instrument. Collection of GLC fractions for EAG analysis was effected by use of a 2.4-m × 2-mm glass column packed with 2% OV-17 on 100/120 Gaschrom Q (column temperature program: 50°–200 °C at 10 °/min with an initial 8-min hold). The column effluent was split (10% to detector, 90% for collection), and consecutive 2-min fractions were collected for EAG testing in capillary tubes chilled with dry ice (Roelofs 1977).

Infrared spectra were taken with a Perkin-Elmer model 237 diffraction grating spectrophotometer calibrated against the 1,601-cm^{-1} band of polystyrene. Ultraviolet spectra were obtained with a Cary model 14 recording spectrophotometer, nuclear magnetic

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resonance (NMR) spectra with a Varian (A60A or EM390) or a Brucker (HX90 or HX270) instrument, and gas chromatographic–mass spectral (GC-MS) data with a Finnegan 3300 gas chromatograph–mass spectrometer coupled with a Systems Industries 150 computer.

Field bioassays of synthetic compounds and isometric mixtures were carried out by use of Pherocon 1C Stickytraps (Zoecon Corp., Palo Alto, CA) modified by removal of the covering upper halves. As such, the traps consisted of open trays suspended by wire hangers, coated on their upper surface with a potent glue, in which the attracted moths became caught. The trays were hung from nylon lines strung between trees upwind of a *C. mucronata* patch. They were spaced 10 m apart in sets of three, five, or six, at about 1 m above ground. The compound or mixture to be tested was applied in hexane solution to a rubber cup (rubber serum stopper, Fisher Scientific Co., Rochester, NY) glued to the center of the trap. For each test, traps consisted of one control trap baited with two caged virgin females (in lieu of a treated rubber cup), one control trap baited with hexane only, and one to four traps baited with hexane solution(s) of the test compound(s). The natural density of *Utetheisa* in the *Crotalaria* patch at the time of testing was low, necessitating the occasional addition to the population of males collected from nearby. Tests in which the control traps baited with females caught no males were disregarded.

An AMR-1000 instrument was used to take scanning electron micrographs of glands dehydrated in ethanol and critical-point dried.

Procedures and Results

Courtship, Field Observation

Initial tests with single females (virgins of known postpupal age), caged in small screened containers placed outdoors beside stands of *C. mucronata*, showed that courtship activity takes place at dusk in near-darkness. By placing ourselves closely beside such cages, it was possible to keep visual track of approaching males. As is clear from Fig. 1, which summarizes data obtained on 32 evenings at two different times of the year (43 tests, 20 females), the daily period of female attractiveness is relatively short (28.6 ± 1.6 min) and in each season rather precisely timed relative to sunset (52.8 ± 1.5 min and 39.1 ± 1.8 min after sunset in June-July and January, respectively). Females were attractive daily, but only for the first consecutve evenings after their emergence ($\bar{x} = 4.6 \pm 0.7$ days; $n = 10$ females, tested daily until they failed to attract males for two consecutive evenings). Males invariably approached from downwind, indicating that female attractiveness was indeed attributable to a conventional pheromonal 'scent.'

Infrared video viewing of single uncaged females that were placed outdoors on upright perches enabled clear observation of the approaching males and of the events that led to copulation. Since these events will be the subject of a separate paper, they will only be summarized here. The males locate the females with relatively little close-range maneuvering. Upon

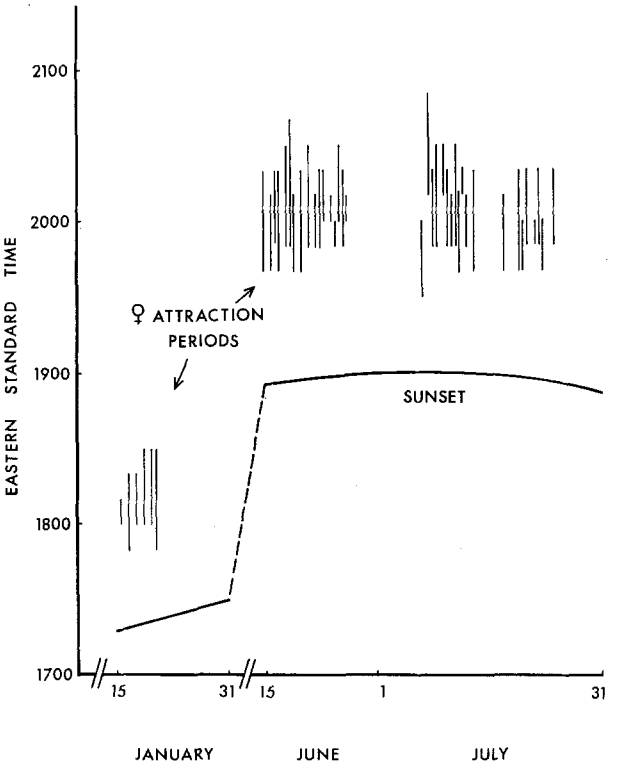


Fig. 1. Duration of periods of female attractiveness. Each *vertical line* represents the length of time that a caged virgin female attracted males. The *solid curve* indicates sunset time at the Archbold Biological Station (81°20′W; 27°11′N) where the observations were made. Details in text

contacting a female, a male hovers beside her and abruptly thrusts his abdomen toward her head and antennae, sometimes repeatedly, while simultaneously everting from his abdominal tip a pair of brushlike glandular devices (coremata). In response to these thrusts, the female raises her wings, whereupon the male lands beside her and mates with her. Only a few seconds transpire (6.9 ± 2.4, $n = 10$) between first contact of the sexes and genital engagement. Pairs remain in copula for several hours, or even throughout the night.

Isolation and Characterization of the Sex Attractant

Efforts to trap the sex attractant on Porapak Q met with success. Trappings were taken during the evening hours when the caged females could be expected to be scenting. GLC/EAG analysis of the *n*-pentane extract of several Porapak trappings (total female equivalent = 75 female scenting periods) revealed maximum EAG activity (1.6 ± 0.4 mV, 2.5 ± 0.5 mV, and 3.0 ± 0.2 mV; $n = 5$) with the three fractions (24–26, 26–28, and 28–30 min) bracketing the principal GLC peak (27.4-min retention time) (Fig. 2). The total quantitative equivalent of this peak in the extract was 2.4 µg (32 ng/female/scenting period). Extracts of a Porapak Q trapping made

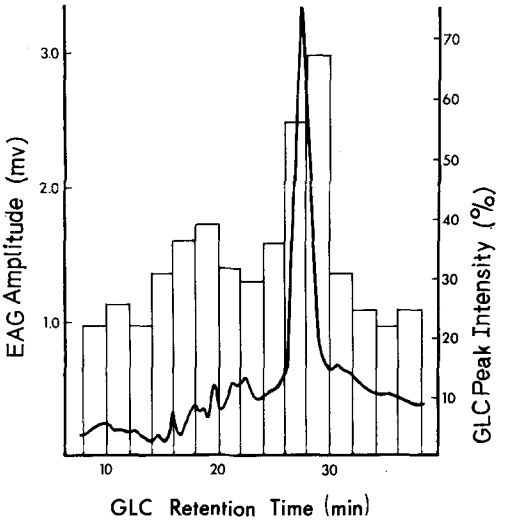
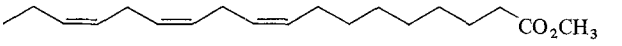


Fig. 2. Electroantennogram (*EAG*) bioassay of the volatile material released by scenting virgin females (75 female scenting-period equivalents), collected on Porapak Q and separated by gas-liquid chromatography (*GLC*). The *curve* gives the GLC trace of a pentane extract of the Porapak sample, while the *bars* of the histogram give the EAG activity (mean of single responses of five male antennae) of consecutive 2-min fractions of the extract. Details in text

at midday did not contain the peak, and, accordingly, showed less EAG activity in the 24–30 min range.

Chemical efforts were directed at the identification of the compound associated with the principal GLC peak. Solvent extraction of various body portions of freshly emerged virgin females had shown strong EAG activity to be associated only with extracts of the abdominal tips. Consequently, a carbon disulfide extract was made of 283 abdominal tips, severed from virgin females during the evening scenting period. The extract was passed through a short Florisil column to remove fatty acids. The solvent was evaporated, and the volatile components were separated from glycerides and other polar lipids by preparative GLC (15% OV-275 on Chromsorb P AW DCMS, 1.8 m × 2 mm ID, 40°–200 °C at 10 °/min). The volatile components were then further divided into seven fractions of ca. 2 min each (6% Carbowax 20 on 100/120 Gas Chrom Q, 2.4 m × 2 mm ID, 20 ml N_2/min, 100°–165 °C at 8 °/min) before collection of the major EAG-active fraction (R_t = 13.7 min, 90 µg). This fraction appeared as a single compound on two analytical GLC columns (OV-17 and Carbowax 20). Its retention time fell between those of the C_{21} and C_{22} paraffins, and its electron capture and chemical ionization mass spectra indicated it to be a hydrocarbon with a molecular weight of 290, corresponding to the molecular formula $C_{21}H_{38}$, a hydrocarbon with three sites of unsaturation.

Hydrogenation of this compound (4 µg, 10% Pd/C, in ether at room tempature) gave a product whose GLC retention time and EI mass spectrum matched that expected for *n*-heneicosane (n-$C_{21}H_{44}$). Since the pheromone showed no ultraviolet absorption maximum above 210 nm, the three double bonds were seen to be nonconjugated. The mass spectrum of this triene was similar to that of methyl linolenate (Holman and Rahm 1966) ((I), methyl-9Z, 12Z, 15Z-octadecatrienoate) in the low mass region ($m/e < 180$); this portion of the ester's spectrum is largely characteristic of the hydrocarbon moiety of the molecule.



(I)

The high-field ^1H NMR spectrum of the triene also suggested a close relationship to (I). Two triplets (3H each) correspond to the terminal methyl group of an alkane (δ 0.88) and a homoallylic methyl group (δ 0.98). The remainder of the spectrum closely resembled that of (I), with allylic methylene (δ 2.07) and doubly allylic methylene (δ 2.81) absorptions of equal intensity (4H), and olefinic absorption at δ 5.37 (6H). These observations suggested that the hydrocarbon is an unbranched C_{21} triene, with a series of three methylene-interrupted double bonds starting at C_3 (II).

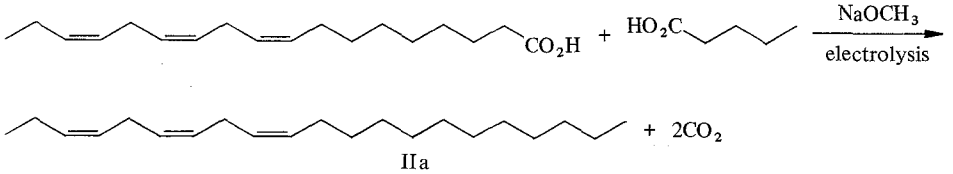
$$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_{10}CH_3 \qquad \text{(II)}$$

This deduction received support from a more-detailed consideration of the mass spectral data. According to several authors (Blumer et al. 1970; Karunen 1974; Youngblood et al. 1971), the positions of the outer double bonds in methylene-interrupted polyenes can be determined from mass spectral evidence alone. The main diagnostic ions to be expected for the postulated 3,6,9-heneicosatriene structure, (II), should appear at *m/e* 108 and 234. In fact, the mass spectrum of the triene showed both these diagnostic fragments. The peak at *m/e* 108 (64%) corresponds to $[CH_3CH_2(CH{=}CH)_3H]^+$, and is characteristic of methylene-interrupted polyenes with one outer double bond at C-3. The *m/e* 234 (2.5%) ion corresponds to $[H(CH{=}CH)_3(CH_2)_{10}CH_3]^+$, indicative of an outer double bond at C-12 (counting from the other end of the molecule). Taken together, this pair of fragment ions establishes the positions of both ends of the double-bond system, and confirms the triene structure to be that given in formula (II). This structure, as well as the Z,Z,Z-configuration of the natural pheromone, was substantiated by synthesis.

Synthesis of the Sex Attractant and Its Stereoisomers

A crossed Kolbe reaction between linolenic acid and valeric acid provided a one-step route to 3Z, 6Z, 9Z-heneicosatriene (IIa), as shown in Eq. (A).

Linolenic acid (2 g, 7.0 mmol), and valeric acid (1.4 g, 14.0 mmol), dissolved in a methanolic solution of sodium methoxide (from 0.1 g, 4.4 mmol of sodium) in methanol (50 ml) was placed in an electrolysis cell equipped with a cold-finger condenser. A current of 1 A (Princeton Applied Research Model 173 Poten-



IIa

(A)

tiostat/Galvanostat, coupled with a Model 175 Universal Programmer) was maintained across the concentric cylindrical platinum electrodes for 20 min after the pH of the solution reached 10–11 (2.5 h). The solution was neutralized with acetic acid and evaporated under reduced pressure. Water was added, and the solution extracted with ether. The ethereal extract was washed with base and dried over anhydrous $MgSO_4$. Filtration and vacuum evaporation of the ether yielded 1.3 g of oil, which was passed through a Florisil column with CS_2 to remove remaining acids. High molecular weight contaminants were removed by medium pressure liquid chromatography (silica gel, 1.5 cm × 100 cm), and the desired product (234 mg, 14%), as well as a small amount of $C_{17:3}$ (52 mg, 3%), were isolated by preparative GLC (3% OV-17 on 100/120 Gas Chrom Q, 1.8 m × 6 mm ID, 60 ml He/min, 180 °C, $R_t C_{17:3}$ – 3 min, $C_{21:3}$ – 23 min).

The following major peaks were observed in the $C_{21:3}$ (% base peaks): 234 (3.4), 135 (9.3), 121 (13.4), 108 (56.2), 95 (37.7), 93 (42.0), 91 (22.2), 80 (65.4), 79 (100), 67 (63.2), 67 (20.4), 55 (36.0), 43 (34.9), 41 (44.3); and in the $C_{17:3}$: 178 (8.1), 135 (6.0), 121 (8.9), 108 (3816), 95 (28.2), 93 (39.7), 91 (23.4), 80 (51.9), 79 (100), 67 (60.2), 55 (31.2), 43 (19.5), 41 (40.3). ^{1}H NMR $C_{21:3}$(δ, 270 MHz, $DCCl_3$): 0.88 (3H, *t*, J = 6.6 Hz), 0.98 (3H, *t*, J = 7.4), 1.27 (18, s), 2.07 (4H, m), 2.82 (4H, m), 2.82 (4H, *t*, J = 5.88), 5.37 (6H, m). ^{1}H NMR $C_{17:3}$ (δ, 90 MHz, $DCCl_3$): 0.93 (6H, m), 1.27 (10H, s), 2.04 (4H, m), 2.78 (4H, *t*, J = 5 Hz), 5.33 (6H, m). The infrared spectra of the $C_{21:3}$ and $C_{17:3}$ had no absorption at 10.3 μ, indicating that all double bonds had retained their original *Z* configurations.

A more convenient synthesis of (IIa) was achieved via the coupling (Johnson and Dutra 1973) of lithium di-*n*-propylcuprate with 9*Z*, 12*Z*, 15*Z*-octadecatrien-1-ol *p*-toluenesulfonate (III). This tosylate was readily prepared from linolenic acid, and the entire synthesis, as outlined in Eq. (B), gave the desired triene in excellent yield.

Methyl linolenate, prepared by treatment of 4.00 g of linolenic acid with ethereal diazomethane, was reduced with lithium aluminum hydride in ether at room temperature, under argon. The resultant alcohol was treated with 1 equivalent of *p*-toluenesulfonyl chloride in pyridine at 0 °C. The reaction mixture was worked up in the usual way, and the desired tosylate ((III), 5.90 g) was purified by chromatography over silica gel (hexane:ether, 9:1).

An ethereal solution of (III) (5.90 g, 14.1 mmol, 20 ml ether) was added to an ethereal solution of lithium di-*n*-propylcuprate (30 mmol in 50 ml of ether) at –20 °C. The reaction mixture was stirred at this temperature for 5 h, and then quenched by stirring with saturated aqueous ammonium chloride. The ether layer was separated, washed twice with saturated aqueous sodium chloride, and dried over anhydrous $MgSO_4$. Evaporation of the ether and chromatography of the residual hydrocarbon (silica gel; pentane) gave 3.68 g of pure triene corresponding to an 88% overall yield of (IIa) from linolenic acid.

The preparation of the *E*, *E*, *E* isomer of (IIa), along with mixtures of *E*, *E*, *Z* and of *E*, *Z*, *Z* isomers, was effected by using the technique for equilibrating alkene stereoisomers, described by Litchfield et al. (1965). A sample of (IIa) (24.2 mg, 0.083 mmol) was treated with 3*M* HNO_3 (60 μl) and 2*M* $NaNO_2$ (90 μl) at ca. 40 °C for 1 h. Nitrogenous by-products were removed by passing a hexane solution of the recovered product through a Florisil column. The resultant hydrocarbon mixture was resolved into four spots (R_f = 0.32; 0.46; 0.59; and 0.69) on analytical thin-layer chromatographic (TLC) plates [Uniplate Precoated TLC plates, Silica Gel GF, 250 μ, spray saturated with 15% $AgNO_3$ in aqueous ethanol, dried at 120 °C, developed with hexane-ether (95:35) and visualized with 0.1% methanolic 2,7′-dichlorofluorescein]. These four materials were isolated by preparative TLC and were individually purified by passage (in hexane) through a short Florisil column.

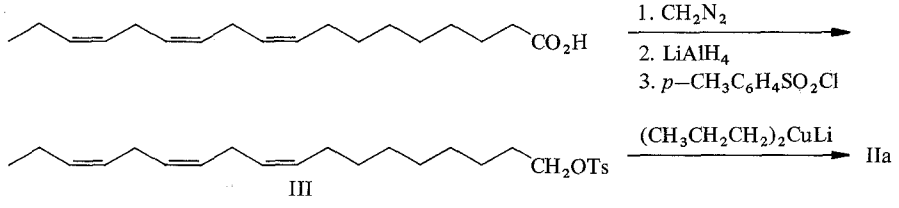
In the 10.3-μ region of the infrared spectrum, the fraction with R_f = 0.32 showed no absorption, confirming its identity with the original sample of (IIa) and with the natural pheromone, with which this fraction cochromatographed. The R_f = 0.46; 0.59; and 0.69 fractions showed increasing intensity of absorption at 10.3 μ, and are therefore considered to be the *E*, *Z*, *Z* (as a mixture), *E*, *E*, *Z* (as a mixture), and *E*, *E*, *E* isomers, respectively.

Bioassays: Electroantennograms and Field Tests

The four synthetic products – the all-*Z* heneicosatriene, its all-*E* isomer, and the two isomeric mixtures (synthesized by the crossed Kolbe reaction) – were tested for EAG activity. Significant activity (analysis of variance, $n = 6$, $P < 0.01$) was shown by the all-*Z* isomer only (Fig. 3).

The all-*Z* isomer also proved active in the field tests with Pherocon sticky traps. An initial series of tests was carried out with isomeric products synthesized by the crossed Kolbe reaction. In these tests (Fig. 4), traps baited with live females caught 4.7 ± 0.9 males/trap/night ($n = 11$), while those baited with the all-*Z* isomer (500 and 1,000 μg/lure) caught 1.3 ± 0.3 males/trap/night ($n = 10$). Control traps baited with hexane alone ($n = 11$), and those baited with 100 μg or less of the all-*Z* isomer ($n = 10$), were consistently ineffective. Two of the other three isomeric samples – the all-*E* isomer and the *E*, *Z*, *Z* isomeric mixture – also proved unattractive (1,000 μg/lure; $n = 2$ for each sample). The third isomeric sample was lost through breakage in the mail.

Limited additional data were obtained the following August with all four isomeric products, synthesized by the lithium cuprate coupling technique. The all-*Z* isomeric sample proved active again, but only at the relatively high dosage of 5,000 μg/lure (n = 2; 2 and 4 males/trap/night). Lower dosages of the sample (500 and 1,000 μg/lure; $n = 2$ for each) were



(B)

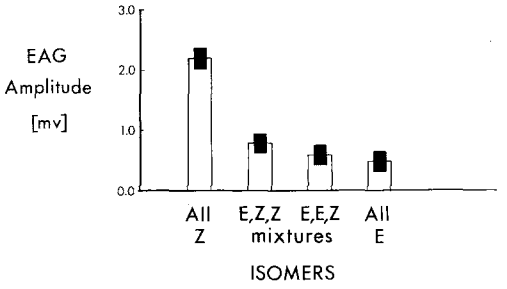


Fig. 3. Electroantennogram bioassay of *Z,Z,Z*-3,6,9-heneicosatriene and its stereoisomers. *Bars* give means of six responses from separate male antennae; *black bars* indicate one standard error on each side of mean (Control responses to puffs of air: $\bar{x}=0.4\ \text{mV}\pm0.06$; $n=6$)

ineffective. The other isomeric products, tested at high dosages, were ineffective (all-*E* isomer, 5,000 µg/lure; *E, Z, Z* isomeric mixture, 15,000 µg/lure; *E, E, Z* isomeric mixture, 15,000 µg/lure; $n=2$ for each). Live females were attractive as before (2 males/trap/night; $n=2$), and hexane lures were unattractive ($n=2$).

We are uncertain why the all-*Z* isomer synthesized by the lithium cuprate technique should have proven relatively less active. Efforts were made to shield all synthetic samples against oxidative degradation, but the precautions might have been variably successful, causing the samples to differ somewhat in purity by the time they were field tested.

Pulsed Emission of the Sex Attractant

Laboratory observations of abdominal tips of females that were scenting in darkness revealed a 'throbbing' action, which at close range was seen to consist of a rhythmic extrusion of two terminal abdominal segments (segments 8 and 9) ordinarily concealed by the female (Fig. 5A and B). Measurements made of the temporal course and rate of this extrusion in seven females are summarized in Fig. 6. Onset of the movement is evidently abrupt, while its cessation at the end of the scenting period is more gradual. During first 90 min of the scenting period, extrusion rates are relatively constant, in the range of 0.82 to 2.1 extrusions/s ($\bar{x}=1.57\pm0.06$). Examination with the scanning electron microscope revealed the presence of two small orifices, situated dorsally in the membranous region of the abdomen (between segments 8 and 9) that protrudes during pulsation (Fig. 5C). Dissection revealed that these orifices are the outer openings of a pair of slender tubular glands (Fig. 5G) that lie longitudinally beneath abdominal tergites 7 and 8 and extend forward almost to the 6th abdominal segment. The excised glands were found to be potently stimulative in EAG tests, unlike the surrounding tissues, indicating that the glands are indeed the source of the sex attractant.

The supposition that during scenting the female actually emits the pheromone in a sequence of pulses was confirmed experimentally. Females that were visibly scenting were carefully placed in an airstream (33 cm/s) 6 cm upwind from an EAG preparation. With each of 10 females tested, the EAG had a characteristic wave form, consisting of a series of transient depolarizations (Fig. 7). The pulse repetition rate was somewhat variable, but in all cases fell within the known range of the abdominal extrusion frequency. Stimulation by a continuous pheromonal signal of comparable duration (all-Z-3,6,9-heneicosatriene placed on filter paper held in the airstream) produced a sustained depolarization (Fig. 7).

The sex attractant glands have some interesting features. They are devoid of compressor muscles and are overlain by glandular epithelium only (Fig. 5I

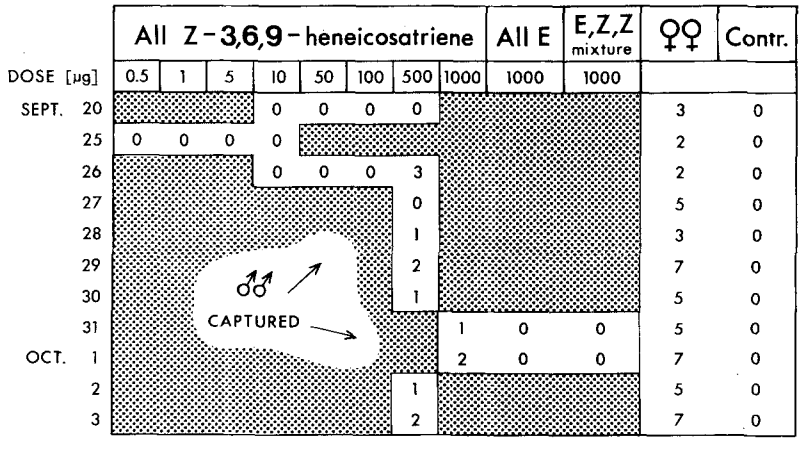


Fig. 4. Field-trapping data (summer 1977), given in numbers of males caught per trap. Nature of 'bait' used in traps is indicated in *column headings*. Trapping dates are listed along *left margin*

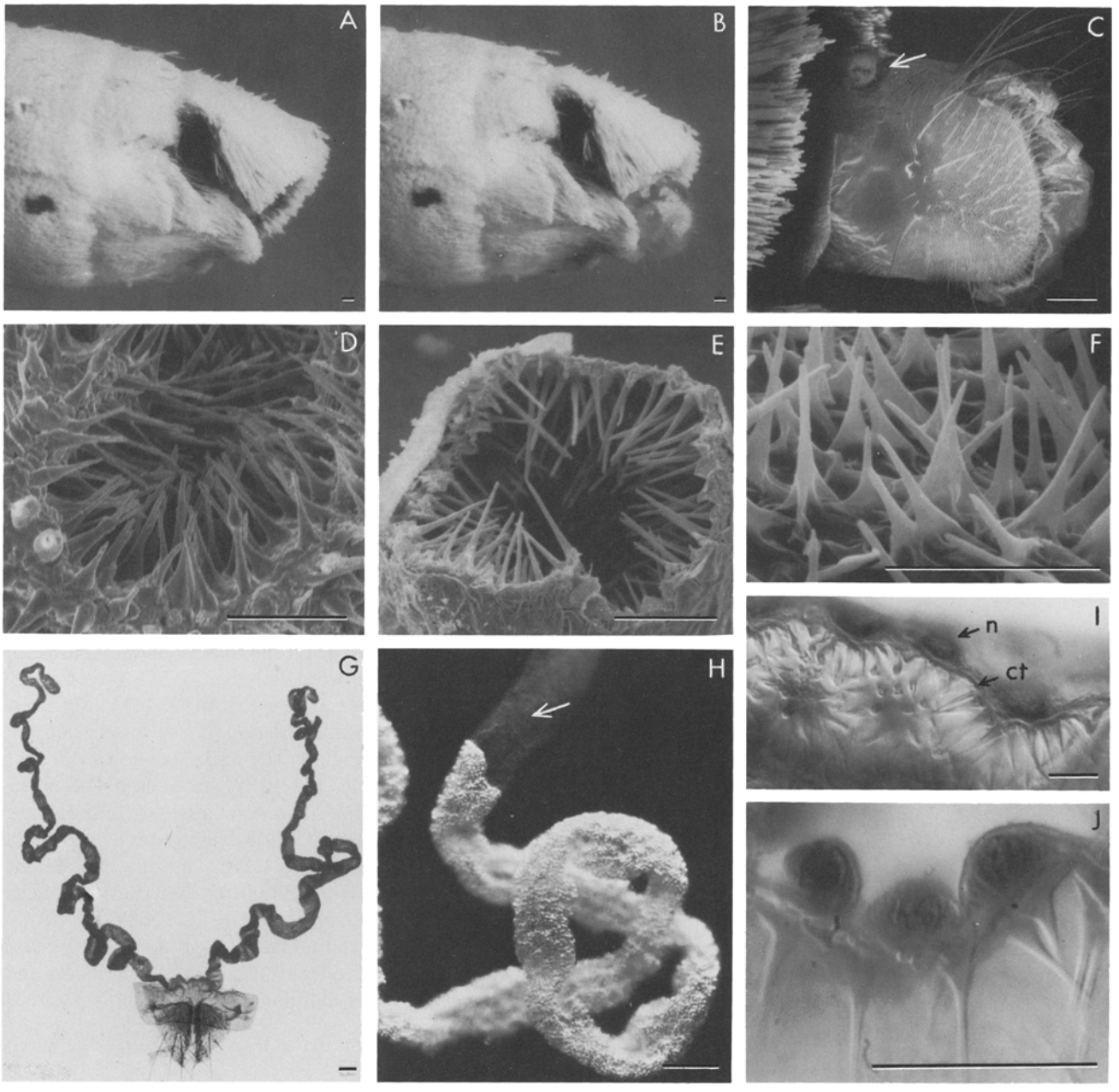


Fig. 5. **A** and **B** Abdominal tip of scenting female, shown with region of gland openings exposed (**B**) and retracted (**A**). **C** Detail of the extruded portion of the abdominal tip, with the left gland opening shown (*arrow*). **D** Enlarged view of the gland opening. **E** View into lumen of a gland fractured transversely at a distance from the opening. **F** Closeup view of spines that line the glands. **G** Pair of glands, excised together with the portion of the abdominal tip on which they open. **H** Portion of freshly excised gland, glistening from the air trapped within. Some of the fluid in which the gland was mounted has begun to infiltrate the gland and displace the air (*arrow*). **I** Whole mount of a gland (fixed in Bouin's solution and stained with Ehrlich's hematoxylin) seen in optical cross section by Normarski interference contrast microscopy. The spatial relationship of the glandular epithelium (n=nuclei), cuticular lining of gland (*ct*), and internal spines is shown. **J** Similar to preceding, showing detail of gland wall. *Reference bars:* **A**, **B**, **C**, **G**, **H**=100 μm; **D**, **E**, **F**, **I**, **J**=20 μm. **C–F** are scanning electron micrographs

and J). Internally they are densely beset with spines (Fig. 5E and F) and hence at most only partly compressible. They are not replete with fluid, but on dissection appear to be filled with air. Compression of freshly excised glands under fluid results in bubble emission from the gland openings; as fluid seeps into the glands, they lose their glistening appearance (Fig. 5H). The openings themselves are also internally braced by spines and hence presumably not totally occlusible (Fig. 5D).

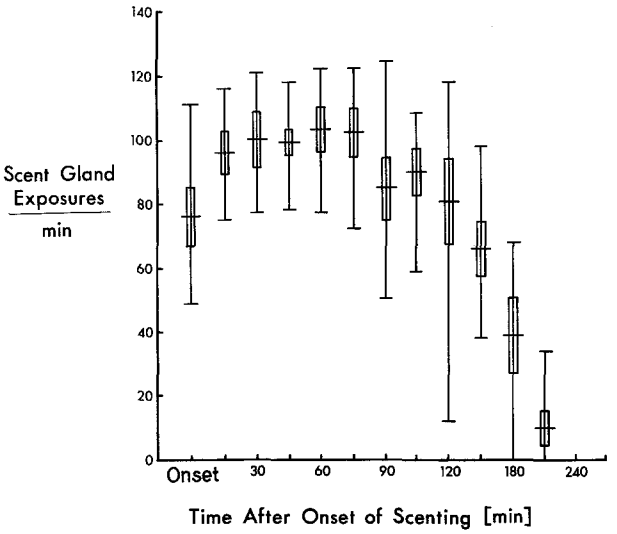


Fig. 6. Frequency of scent gland exposures (=extrusion frequency) plotted as a function of time after onset of scenting behavior. Observations were made on seven females confined in glass enclosures (still air) under infrared illumination. *Transverse lines* give the means, *vertical lines* the ranges, and *vertical bars* one standard error on each side of mean

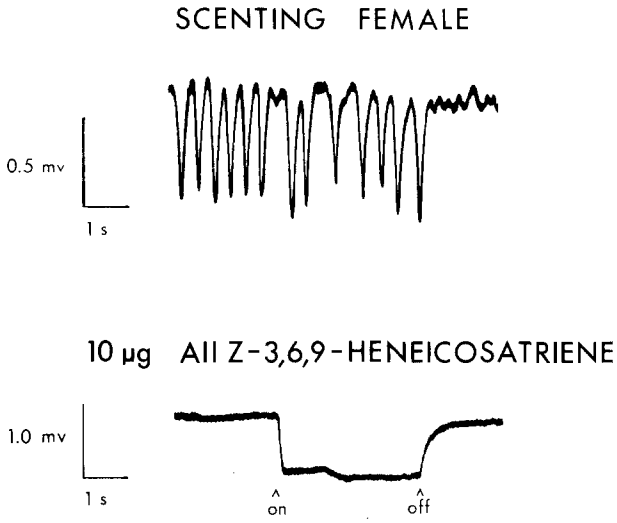


Fig. 7. Temporal characteristics of the pheromone signal emitted by the female. *Top trace* shows the electrical output of a male antenna placed 6 cm downwind of a scenting female (wind speed= 33 cm/s). *Bottom trace* is the electrical output elicited in a male antenna by a continuous pheromonal source (10 µg of *Z,Z,Z*-3,6,9-heneicosatriene on a strip of filter paper placed in the airstream in substitution of the female)

Effect of Diet on Sex Attractant Production

In order to determine whether the moth's production of pheromone depends on larval intake of *Crotalaria*, extraction was made of abdominal tips of two groups of 10 females raised on the pinto-bean based semisynthetic diet. Both extracts were found to contain *Z, Z, Z*-3,6,9-heneicosatriene, in the amount of 0.99 and 0.70 µg/female, respectively (GLC). The amount detected in a comparable extract of 10 females raised on *Crotalaria mucronata* was 0.72 µg/female.

Evidence for Additional Component(s) in the Sex Attractant

Visual inspection of the GLC/EAG data (Fig. 2) suggested that the 16–18 and 18–20 min chromatographic fractions were also EAG active. Indeed, the EAG activity (1.6 ± 0.2 mV and 1.8 ± 0.2 mV; $n=5$) of these fractions (combined for statistical analysis) proved significantly greater than background activity (analysis of variance, $P<0.01$). The quantities of material associated with these fractions were too small for isolation and characterization.

Evidence was obtained for the presence in the extracts of the female abdominal tips of a labile hydrocarbon closely related to the identified pheromonal constituent. Efforts are under way to characterize and synthesize this compound, a C_{21} tetraene.

Discussion

In certain basic respects, sex attraction in *Utetheisa* is conventionally mothlike. The attractant pheromone is produced by the female, it is emitted by virgins at fixed daily periods commencing shortly after pupal emergence, and it recruits males from downwind. Moreover, its identified chemical component is a simple aliphatic compound, as pheromone constituents usually are in moths. But *Utetheisa* pulses its pheromone, and in so doing resorts to a delivery system that has so far no known parallel in aerial chemical communication mechanisms.

Although temporal patterning plays a major role in acoustical and visual signaling systems, it had hitherto been considered only as a theoretical possibility in pheromonal communication (Bossert and Wilson 1963; Bossert 1968). On mathematical grounds, it was argued that chemical pulsation could be of communicative value only over short distances, since even under optimal conditions (that is, moderate steady wind speeds) amplitude fluctuations in the signal would essentially be undetectable at distances greater than a few meters downwind from the periodic source. But close to the source, chemical discontinuities may indeed be discernible, as we were able to confirm electrophysiologically by EAG recordings from *Utetheisa* males, and visually by simulation of a pulsed

Fig. 8. Simulation of a temporally patterned chemical signal. Air laden with a visible marker (titanium tetrachloride) is pulsed at the rate of 2 'puffs'/s from the glass capillary shown at *left* (wind speed ≅ 11 cm/s). The pulses evidently remain discrete over the distance (60 cm) spanned by the photograph

pheromone plume (Fig. 8). What, then, might be the adaptive value of chemical pulsation in *Utetheisa*? In our view, the temporal pattern may serve as a short-range orientation cue to the male as he attempts to locate the female, rather than as a contributive diagnostic cue that helps him identify her as his conspecific mate. Female identification might be effected solely on the basis of the unpatterned chemical stimulus (witness the attractiveness of our pheromone traps) at distances beyond the range where pulsation is detectable. But pulsation could come into play as the male flies upwind to within proximity of the female, and is confronted with the ultimate and potentially difficult task of pinpointing her quickly and precisely.

Temporal patterning could facilitate goal localization in at least two ways. First, it could signify to a male that he is in fact close to a female and in need therefore of shifting from upwind flight to an alternative form of orientation, lest he overshoot his mark. To a moth such as *Utetheisa*, which lives in dense localized populations where clustered females could occasionally be envisioned to scent in groups, proximity cues could be of special importance since they would make the individual females of a 'chorus' discernible to their prospective mates.

A second possibility is that in a pulsed plume the male's antennae function more effectively. As is well known, chemoreceptors subjected to ongoing stimulation tend to adapt to the chemical input. Such desensitization is potentially preventable through intermittent nonstimulation, the very condition encountered by the *Utetheisa* male when it is near the female. Males of moth species that orient along continuous chemical plumes tend to zigzag in and out across their intended path (Marsh et al. 1978). The behavior is said to be indicative of the moth's periodic need to depart from the plume in order to retain ongoing chemical sensitivity to it. Within a pulsed chemical plume, ongoing sensitivity could presumably be maintained without, or with only lessened, need to deviate from the direct line of flight.

To what extent these factors, as well as some others that can be imagined (as for example detection of Doppler shifts, or of time of arrival differences of the puffs at the two antennae), are actually operative in close-range mate localization in *Utetheisa* remains open to experimental testing. It is intriguing, however, that chemical pulsation may be more widespread in moths than hitherto suspected. As pointed out by Kettlewell (1946) and others (Roelofs and Cardé 1971) and already noted in connection with his theoretical calculations by Bossert (1968), a number of female moths show the rhythmic extrusion of terminal abdominal segments during scenting that is the visible concomitant of pulsing. In cases where such extrusion rates have been measured, they were found to be close to that of *Utetheisa*. A further possibility is that pulsation is of economic benefit to scenting females, but this might be so only if the savings accrued through decreased output of pheromone are not offset by other factors, such as the muscular effort expended in pulsing the peromone.

One wonders how precisely pulsed emission is effected by the peromone glands. The fact that these glands are tubular, seemingly air-filled, partly compressible, and elastically reexpansible, suggests that they might ventilate mechanically in the manner of lungs. We propose that pulsation is achieved simply by rhythmic changes in abdominal blood pressure. Such changes might be the natural concomitant of the visible abdominal motions that characterize the scenting process, and they could effect a regular compression and decompression of the glands. The cuticular lining of the glands, including the collective surface of the inner spines, is envisioned as being ordinarily

coated with a thin film of liquid pheromone. Air drawn into the gland during gland decompression would tend to become saturated with pheromone vapor by contact with the film, and on subsequent compression would be expelled as a pheromonal puff. Ongoing 'inhalation' and 'exhalation' would generate the discontinuous plume. Critical to this postulated mechanism are the internal spines of the glands. In addition to providing the appropriately large evaporative surface, they could serve to prevent localized collapse of the glands during glandular compression, and to effect elastic reexpansion of the glands during decompression.

The fact that pheromone pulsation might not be an isolated phenomenon has applied implications. Pheromonal traps such as are widely used in insect pest control programs do not presently incorporate into their physical design the capacity to pulse the attractants. The possibility that discontinuous emission could improve trapping efficiency should clearly be explored.

The finding that *Utetheisa* females produce the same pheromonal material whether or not they are raised on normal *Crotalaria* diet was to be expected. It had been claimed on the basis of work with other moths that sex attractant composition varies with diet (Hendry et al. 1975), but the claim has been discredited (Miller et al. 1976). Nonetheless, we have evidence that in the *Utetheisa* male, normal sexual performance is contingent upon *Crotalaria* intake. Males that were raised on our semisynthetic pinto-bean diet were found to have a reduced chance of gaining acceptance by females. Under such conditions, they fail to produce a corematal pheromone, also present in other *Utetheisa* species, that serves for seduction of females and is derived from pyrrolizidine alkaloids in *Crotalaria* (Conner 1979). This obligate dependence on ingested plant metabolites for pheromone production is reminiscent of a comparable dependence in male danaid butterflies, which also produce aphrodisiac pheromones from pyrrolizidine alkaloids (Edgar et al. 1973; Pliske and Eisner 1969; Schneider et al. 1975).

The apparent presence in the *Utetheisa* secretion of one or more additional components should come as no surprise, since existing evidence indicates that pheromones are generally mixtures rather than single compounds. We intend eventually to characterize at least the C_{21} tetraene, since this material can be isolated in sufficient quantity.

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