litogenic protein but be unresponsive to it. Many examples of such immune response (Ir) genes are now known, and many of these are linked to major histocompatibility loci such as *Ag-B* (*8*). (iii) An entirely nonimmunological mechanism could be involved.

At present there is not enough information to establish which of these mechanisms is correct, but the work of Kornblum (*3*) strongly supports possibility (ii). Sensitized lymph node cells from Lewis but not BN rats were able to react against guinea pig spinal cord in the irradiated hamster test. If possibility (ii) is correct, it is interesting to note how the EAE gene differs from *Ir-1* of mice. *Ir-1* maps inside the *H-2* region (*9*), whereas the EAE gene is separable from *Ag-B* by recombination. Second, the *Ir-1* locus of mice is known to cause stimulation in the MLC test (*10*), but this is not the case with the EAE gene. Cells of DA and BN.B4 do not stimulate each other in the MLC test, but those of BN.B4 and BN cause mutual stimulation in mixed culture (*11*). Since BN.B4 and BN have the same susceptibility to EAE, the locus associated with this disease is distinct from that causing MLC stimulation.

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12. We thank B. Koeberlein and A. Stever for technical assistance. Supported in part by NIH grants AI-09275 and CA-10097, and NSF grants NS-05572-09 and NS-05712-02. C.M.N. holds a postdoctoral fellowship from the Leukemia Society of America, Inc.

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Clarification of the Chemical Status of the Pink Bollworm Sex Pheromone

Abstract. *Propylure, 10-*n*-propyl-*trans*-5,9-tridecadienyl acetate, and deet,* N,N,*-diethyl-*m*-toluamide, were previously reported as the sex pheromone and a sex pheromone activator, respectively, of the pink bollworm. Neither chemical in three extracts of female moth abdomen tips could be detected by gas-liquid chromatographic analysis. These compounds, alone or in combination, exhibited little or no biological activity in the laboratory or in the field. Hexalure,* cis*-7-hexadecenyl acetate, a synthetic attractant for pink bollworm males, could not be detected in female moth abdomen tip extracts. The pink bollworm sex pheromone was identified as a mixture of* cis,cis *and* cis,trans *isomers of 7,11-hexadecadienyl acetate.*

Considerable confusion exists concerning the status of female sex pheromones (*1*) or sex attractants (*1*), of the pink bollworm moth, *Pectinophora gossypiella* (Saunders) (Gelechiidae). In 1966, Jones *et al.* (*2*) isolated and identified a compound, 10-*n*-propyl-*trans*-5,9-tridecadienyl acetate (propylure), from an extract made from whole bodies of virgin female moths. They reported that propylure caused a sexual response in caged male moths, thus qualifying it as a sex pheromone. However, their subsequent studies indicated that propylure was not attractive to male moths in the field (*3*). In 1968, Jones and Jacobson (*3*) stated that for attractancy in the field, propylure requires the simultaneous presence of a naturally occurring activator. The activator, which they found in methylene chloride extracts of female pink bollworm moths, was *N,N*-diethyl-*m*-toluamide. This compound is commercially available as the insect repellent "deet" and had been found earlier to be moderately attractive to male pink bollworm moths (*4*).

The claim that propylure was a sex pheromone for the pink bollworm was challenged by Eiter *et al.* (*5*) in 1967. They synthesized the compound independently and reported that it did not excite male moths in the laboratory. Jacobson (*6*) then presented data showing that as little as 15 percent of the *cis* isomer of propylure could completely mask the biological activity of the natural *trans* isomer. This masking, he explained, was the reason for the lack of activity of Eiter's preparation, which was a 1 : 1 mixture of *cis* and *trans* isomers.

At about the same time, through empirical screening of synthetic compounds, an unrelated chemical was found to be an attractant for pink bollworm males in the field (*7*). This compound, *cis*-7-hexadecenyl acetate, was called hexalure.

Because of this confused situation, we undertook a reinvestigation of propylure (*8*), deet (*9*), and hexalure (*10*), with regard to their possible presence in the female moths and their biological activities in the laboratory and the field.

The natural sex pheromone was characterized by determining its gas-liquid chromatographic (GLC) retention time, with the use of a hydrogen flame ionization detector in parallel with a cage of male moths. An ether extract of the abdomen tips of 200 adult virgin female moths, 2 to 4 days old (*11*), was filtered to remove particulate matter and a portion was used for quantitative bioassays. The ether was removed from the remainder by evaporation at room temperature with a stream of nitrogen. The residue was taken up in a known volume of carbon disulfide and analyzed by GLC (*12*). A "splitter" diverted nine parts of the effluent to the cage of male moths used as a biological detector while one part

Table 1. Gas-liquid chromatographic characteristics for hexadecyl acetate, hexalure, propylure, deet, and extracted pink bollworm sex pheromone.

Liquid phase	Relative retention time (hydrogen flame)				
	Hexadecyl acetate	Hexalure	Propylure	Deet	Extracted pheromone
Apiezon L	1.00	0.83	0.51	0.15	0.80
SF-96	1.00	.86	.62	.15	.78
NPGA	1.00	1.01	.71	.44	1.03
Carbowax 20M	1.00	1.05	.83	.87	1.24
QF-1	1.00	.91	.63	.60	.86

Table 2. Mean percentage response by male pink bollworm moths exposed to various odors in a bioassay apparatus. Means ± S.E. are based on ten replicates, with ten moths for each replicate. Quantities of female extracts or of the various chemicals are given in terms of the amount of material placed on the copper-disk evaporation substrate.

Amount	Response (%)
Female abdomen extracts	
0.1 female equivalent	76 ± 7
0.01 female equivalent	65 ± 7
0.001 female equivalent	52 ± 20
0.0001 female equivalent	27 ± 12
Hexalure	
100 μg	38 ± 15
10 μg	37 ± 13
1 μg	30 ± 9
0.1 μg	14 ± 8
Deet	
100 μg	5 ± 2
Propylure	
100 μg	5 ± 2
1 μg	2 ± 2
Propylure plus deet	
100 μg each	0
Untreated control	
None	1 ± 1

went to a hydrogen flame detector (*13*). An active peak (biological detector), corresponding to about 5 ng of pheromone per female (hydrogen flame detector), was found, with the use of five columns having different polarities (Table 1). On all columns, the retention time of the natural pheromone was significantly greater than that for propylure or deet. Retention characteristics for the natural pheromone were also different from those of hexalure. Neither propylure, deet (*14*), nor hexalure could be detected in the female extract. We also searched for these three compounds in an extract made from 1.2 million pink bollworm moths of mixed sexes that had been kept under Dry Ice for several months (*15*) and in an extract made from the abdomen tips of 83 field-collected female moths (*16*). The natural pheromone, but none of the three other chemicals, was found in these extracts.

To further clarify the situation, we determined the relative biological activities of propylure and deet, separately and in combination, as well as hexalure, female extracts, and living females. These tests were carried out in the laboratory and in cotton fields in southern California.

Laboratory evaluations were conducted in a bioassay device (*17*). Known quantities of test chemicals were placed on individual copper disks. An air flow passed over each disk, evaporating some of the chemical and carrying it to an assay cage containing ten male moths. When two chemical samples were compared simultaneously (for example, propylure and deet), they were not mixed, but were placed on two separate copper disks inserted simultaneously into the air flow entering the male assay cage. The biological responses, consisting of the percentages of previously resting males that became activated, are given in Table 2. When the various chemicals were compared in terms of amounts placed on the copper disks, propylure and deet, alone or in combination, exhibited little biological activity compared to hexalure or to the extract of female moth abdomen tips. Male sexual behavior, including copulatory attempts, occurred in response to the extracts frequently, to hexalure infrequently, but never to propylure or deet (or both).

For field evaluations, samples were exposed from dusk to dawn in Stikem-lined traps (*18*). Living virgin females were used as the lure in certain of the traps. Female abdomen tip extracts (5 or 50 female equivalents) were placed on square sections (14 by 14 mm) of filter paper and pinned to corks, which were placed in the traps. These extract samples were replaced by fresh samples each day. All other samples were placed as pure liquids in stainless steel 25-mm-diameter planchets, or in Teflon tubes having cross-sectional areas 1/10 or 1/100 that of the steel planchets. Thus, chemical evaporation rates equivalent to those from 1, 1/10, or 1/100 of a steel planchet could be obtained. The traps were supported on stakes at plant height (approximately 1 m above the ground). They were separated from each other by at least 50 m, and their positions were rearranged at random each night for the 17-night duration of the experiment. Planchets were kept under refrigeration during the day and were refilled with fresh chemical every third day. The results of the field evaluations (Table 3) agree with those found in the laboratory. Propylure alone and propylure plus deet attracted very few males. The highest concentration of pure deet showed a low, erratic biological activity. Hexalure, female abdomen tip extracts, and living females attracted higher numbers of males than propylure or deet, or both.

Subsequent analysis revealed the natural pheromone to be a mixture of geometrical isomers of 7,11-hexadecadienyl acetate (*19*). Field-trap evaluations based on five replications of each chemical at a rate of evaporation equivalent to 0.1 steel planchet gave the following mean captures of male moths: *cis*-7,*cis*-11-hexadecadienyl acetate, 0; *cis*-7-*trans*-11-hexadecadienyl acetate, 0; a 1 : 1 mixture of these two compounds, 22.2 ± 5.2 (S.E.); hexalure, 0.4 ± 0.1; and ten virgin females, 6.4 ± 1.1. A more complete assessment of ratios of chemicals constituting this isomeric-mixture sex pheromone, for which we propose the name, "gossyplure," is in progress.

Table 3. Mean numbers of male pink bollworm moths captured per night in traps baited with living virgin females, female extracts, or various chemicals. Means ± S.E. are each based on 17 nightly replicates.

Amount	Moths captured (mean ± S.E.)
Ten living females	
	194 ± 30
Female abdomen tip extracts	
50 female equivalents	171 ± 27
5 female equivalents	124 ± 88
Hexalure	
1.0	79 ± 20
0.1	57 ± 17
0.01	3 ± 1
Deet	
1.0*	28 ± 12
0.1	2 ± 1
0.01	0
Propylure	
1.0	0
0.1	1 ± 0
0.01	0
Propylure plus deet	
1.0 + 1.0	3 ± 1
0.1 + 1.0	1 ± 1
1.0 + 0.1	0
Untreated control	
None	0

* These figures represent relative evaporation rates for each chemical, with 1.0 being the rate from a stainless steel planchet 25 mm in diameter. Hexalure and propylure evaporate from this planchet at about 0.1 μg/min.

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References and Notes

1. A diverse group of chemicals is collected together under the term "sex pheromones." These chemicals are produced by either males or females and stimulate one or more behavioral reactions in the opposite sex, leading either directly or indirectly to mating. Because the most obvious of the behavioral reactions is attraction, the term "sex attractants" has been used in the past, synony-

mously with "sex pheromones." Most authors now use "sex attractants" to denote chemicals which are similar in biological activity to sex pheromones in that they attract only one sex, but which have not been identified as occurring in the opposite sex.
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8. We used a sample of propylure synthesized by A. I. Meyers and E. W. Collington, *Tetrahedron* **27**, 5979 (1971). We thank Dr. Meyers for this sample. Our GLC analysis (*12*) of the sample on a 2 percent Apiezon L column (240 by 0.2 cm, inside diameter) at 130°C and a flow rate of nitrogen of 76 ml/min ($N = 2700$ theoretical plates) showed the presence of six compounds with relative retention times of (percentage composition based on peak areas): 0.66 (0.1 percent); 0.80 (0.1 percent); 0.85 (0.1 percent); 0.91 (0.9 percent); 1.00 (97.3 percent); and 1.14 (1.5 percent). Thus the sample of propylure was 97.3 percent *trans* with the maximum single minor component being 1.5 percent. However, the most probable peak representing the *cis* isomer of propylure is the one with a relative retention time of 0.91 (0.9 percent). Under identical conditions, the relative retention times of *cis*- and *trans*-7-hexadecenyl acetates were 1.70 and 1.82, respectively (the retention time of *cis* isomer was 137 minutes).
9. Purchased from Aldrich Chemical Co. and redistilled before use to 99 percent purity.
10. The hexalure was obtained from Farchan Division, Story Chemical Corporation, and used without further purification. Gas-liquid chromatography, nuclear magnetic resonance, and thin-layer chromatography indicated that the sample consisted of more than 95 percent 7-hexadecenyl acetate (of which more than 90 percent was the *cis* isomer).
11. The moths used for extracts, bioassays, and lures in traps were obtained from larvae reared on a modified wheat-germ diet [P. L. Adkisson, E. S. Vanderzant, D. L. Bull, W. E. Allison, *J. Econ. Entomol.* **53**, 759 (1960)], using the individual rearing method of R. Patana, *U.S. Department of Agriculture, Agricultural Research Service, Production Research Report* (1969). The sexes were separated as pupae, which were placed for emergence into 4-liter paper cartons with screen tops. The cartons were placed in rearing units at 28°C under a light : dark cycle of 14 hours of light and 10 hours of darkness, synchronized with that occurring in the field. Rearing units holding males and females were kept in separate rooms.
12. We used an F & M Hewlett-Packard dual channel gas chromatograph, model 402, equipped with an all-glass inlet system and glass columns (50 by 0.2 cm, inside diameter). The columns were operated at 135° to 150°C, with nitrogen flow rates of 50 to 70 ml/min. The liquid phases (Table 1) were absorbed on 80–100 mesh AW, hexamethyldisilazane-treated Chromosorb W.
13. The two legs of the splitter were heated and adjusted in size so that the effluent arrived at the hydrogen flame detector and the biological detector simultaneously. At the biological detector, an air purge of 5000 ml/min picked up the effluent and circulated it through the 1000-ml volume container enclosing the cage of male moths. Details of our splitting technique have been described [L. K. Gaston, T. R. Fukuto, H. H. Shorey, *Ann. Entomol. Soc. Amer.* **59**, 1062 (1966)].
14. Adult female pink bollworms had been reported to contain 0.06 to 0.60 mg of deet each (*3*). Since females weigh 12 to 20 mg, deet should make up 0.3 to 5 percent of their total body weight. Our GLC method can detect 5 ng, but we could find no deet at this level.
15. We thank W. O. Ridgway for shipments of frozen pink bollworm moths originating from a mass rearing facility of the U.S. Department of Agriculture, Phoenix, Ariz.
16. Pupae were collected by hand from rosetted cotton blooms in the field. The pupae were separated according to sex (*11*). Emerged female moths were held for 3 to 5 days before their abdomen tips were removed and extracted with ether.
17. R. Bartell and H. H. Shorey, *J. Insect Physiol.* **15**, 33 (1969).
18. R. K. Sharma, R. E. Rice, H. T. Reynolds, R. M. Hannibal, *J. Econ. Entomol.* **66**, 377 (1973).
19. The purified biologically active material showed two partially overlapping peaks in GLC analysis (4 percent diethylene glycol succinate, at 175°C). Collection of this active material, followed by reductive ozonization, gave three compounds: 1-pentanal, 1,4-butanedial, and 7-acetoxy-heptanal. The two compounds constituting the biologically active material had relative retention times of 1.40 and 1.46 (*n*-hexadecyl acetate = 1.00). The *cis*-7,*trans*-11 and *cis*-7,*cis*-11 synthetic isomers of 7,11-hexadecadienyl acetate had relative retention times of 1.41 and 1.47, respectively. The two active peaks were collected separately. The infrared spectrum of the earliest collected active peak (1.40) showed absorption at 966 cm^{-1} (*trans*-double bond) and was identical to the spectrum of synthetic *cis*-7,*trans*-11-hexadecadienyl acetate. The infrared spectrum of the later collected active peak (1.46) showed no absorption in the 960 cm^{-1} region and was identical to the spectrum of synthetic *cis*-7,*cis*-11-hexadecadienyl acetate. Gas-liquid chromatographic analysis by coinjection of the earliest collected active peak with synthetic *cis*-7,*trans*-11-hexadecadienyl acetate gave a single peak; likewise, coinjection of the later collected active peak with *cis*-7,*cis*-11-hexadecadienyl acetate gave a single peak. These synthetic compounds were prepared and supplied by Farchan Division, Story Chemical Corporation.
20. This is paper No. 40 of a series entitled "Sex pheromones of Lepidoptera." We thank S. U. McFarland, S. Eubanks, and B. Wilk for assistance. Supported in part by grants from the Rockefeller Foundation and Cotton Incorporated.

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Pavlovian Conditioning with Heat Reinforcement Produces Stimulus-Directed Pecking in Chicks

Abstract. *In a cooled chamber, chicks approached and pecked a small disk whose illumination preceded heat lamp activation, even when pecks prevented heat lamp onset. These behaviors did not occur when the disk and heat stimuli were randomly presented. Approach and contact of conditioned stimuli may develop even though these behaviors are not (i) evoked by the reinforcing stimulus, (ii) necessary for reinforcer reception, or (iii) ever followed by the reinforcer.*

Pavlovian conditioning involves the presentation of stimulus events irrespective of an organism's behavior. As a result of this experience, a formerly ineffective stimulus [conditioned stimulus (CS)] may come to evoke a response whose form often resembles that elicited by the reinforcing stimulus [unconditioned stimulus (US)].

Unlike conventional Pavlovian procedures that restrict the subjects' locomotor behavior, recent conditioning experiments have permitted subjects unrestrained access to CS's (*1–3*). Under these circumstances, subjects not only evidence similar conditioned responses (CR's) and unconditioned responses (UR's), but they frequently draw near to the CS and they may even contact it. For example, Jenkins and Moore (*3*) found that freely moving pigeons exhibited conditioned "eating" and "drinking" behaviors to visual CS's which preceded food and water US's, respectively. Not only were skeletal components of the UR (such as licking and swallowing) elicited by the small visual CS, but they were integrated into a complex sequence of skeletal behaviors beginning with approach and culminating in physical contact with the CS.

Most reports of approach and contact of CS's have involved appetitive US's that, like food and water, must be approached and physically contacted in order to be received (*4*). The possible importance of these obligatory skeletal behaviors to the development of CS approach and contact has not yet been extensively investigated. To this end, thermal stimulation was used as the US in the present study. This reinforcer requires no instrumental behavior for its reception; accordingly, conditioned approach and contact of a CS could not readily be attributed to a similar preconsummatory sequence for the heat US. Furthermore, most previous experiments have utilized CS's and US's that often give rise to similar or compatible behaviors (*5*). Birds frequently peck at small visual features. They also peck at kernels of grain and thrust their beaks into puddles of water. Would birds also peck a small visual CS if the subsequent US evoked highly dissimilar behaviors? Here again, thermal stimulation is an interesting US. The stereotypical behaviors evoked in chicks by heat (such as immobility and wing extension) are not only topographically different from stimulus-