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27. In vitro transcribed RNAs were obtained by SP6 RNA polymerase transcription of respective S-α1 cDNA and R-α1 cDNA subcloned into psp73 transcription vector (Promega) according to manufacturer's specifications. Equality of S- and R-α1 transcripts were ascertained by densitometric quantitation of full-length nondegraded transcripts on Northern blot analysis with random-primed ^{32}P-labeled α1 cDNA probe (*14*). *Xenopus* oocyte expression experiments were done as described (*16–18*) with 25 ng of SS- and RR-specific in vitro transcribed α1 RNA with equimolar β1 in vitro transcribed RNA in 50 nl of ddH_2O. Microinjected oocytes were apportioned for $^{86}Rb^+$ uptake assays and immunoprecipitation with the α1-specific monoclonal antibody, MCK-1 (*21*). Immunoprecipitation was done as described (*16, 17*) with the following modifications: 12 hours after microinjection, unhealthy oocytes were removed; only intact oocytes were incubated in fresh modified Barth saline with L-[^{35}S]methionine (1 mCi/ml; specific activity, 1146 Ci/mmol) for 60 hours with one media change; before membrane isolation, oocytes were again checked for integrity; oocyte membranes were isolated by sucrose step-gradient centrifugation; membranes were incubated with MCK-1 antibody overnight at 4°C; affinity-purified goat antibody to mouse immunoglobulin G (CAPPEL) was used for precipitation of the antibody-antigen complexes.
28. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y. Tyr.
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Sexual Role Reversal in Mate-Finding Strategies of the Cabbage Looper Moth

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The mate-finding behavior of the cabbage looper moth *Trichoplusia ni* (Lepidoptera: Noctuidae) includes both female- and male-produced sex pheromones used in distinct mate-finding strategies. Both sexes release multicomponent pheromones attractive to the opposite sex. Male pheromone is comprised of *d*-linalool, *m*-cresol, and *p*-cresol released from abdominal hair pencils. Males exposed to host plant odor or to the female sex pheromone (*Z*)-7-dodecen-1-ol acetate are more attractive to females, suggesting stimulation of male pheromone release.

THE MATE-FINDING BEHAVIOR OF the cabbage looper moth *Trichoplusia ni* (Hübner) has been depicted as a system typical of other insects, particularly other moths. The stationary calling female produces a potent species-specific male attractant (*1–3*), and males release possible aphrodisiac pheromones during courtship interactions (*2, 4*). This pheromone communication system was one of the first studied and has served as a model for research on insect sex pheromones. However, we discovered an alternate mate-finding strategy in this species that involves attraction of females to males (*5*). Observations of cabbage loopers in field cages confirmed that male attraction to females and female attraction to males constitute separate mate-finding strategies. Female visitation at cages of males occurred principally at dusk, whereas male visitation at cages of females occurred in the fourth to ninth hours of the 10-hour night (*6*).

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We report here the identification of a male cabbage looper pheromone isolated from hair pencils that is attractive to females. We show that males release this pheromone in response to female pheromone and that males in an airstream of host odor or female pheromone are more attractive to females. Such behavior, as well as male attraction to host plants and scent-marking on host plants, suggests a resource-based mating strategy, with host plants as natural sexual rendezvous sites. We have identified a long-range sex attractant from a male moth and documented host plant kairomonal enhancement of male attractiveness in *Lepidoptera*.

Because we previously isolated material attractive to females from male cabbage looper hair pencils (*5*), hexane extracts of male cabbage looper hair pencils were fractionated with capillary gas chromatography (GC). Flight tunnel bioassays of GC fractions showed that maximum attraction of unmated females required the combination of three fractions. Attraction response rates were 0, 0, 13, and 44% for fractions 1, 2, 3, and the three fractions combined respectively ($n = 45$), with attraction to combined fractions significantly higher (Duncan's new multiple range test, $P \leq 0.05$, after arcsine transformation of the data). Spectroscopic analyses of compounds in these fractions resulted in the identification of the active components as *d*-linalool, *m*-cresol, and *p*-cresol (*7*). Bioassay results confirmed that all three compounds were required to entice the cabbage looper moths to fly upwind for contact with the pheromone dispenser. Thirty-seven percent of 35 moths were attracted to contact the dispenser baited with all three compounds, compared to none for cresols, 9% for *d*-linalool, and 34% for hair pencil extract. The configuration of *d*-linalool was established via chiral derivitization techniques (*7*).

Initial attempts to collect pheromone released by males were unsuccessful. We hypothesized that males may be stimulated to release pheromone by host plant chemicals, by female pheromone, or by both. In a previous study of cabbage looper moth attraction to cabbage plants [*Brassica oleraceae* (L.)] (*8*), males arriving at plants called and scent-marked. Also, male cabbage loopers exhibit full hair pencil displays upon approaching calling females (*9, 10*), possibly releasing the same pheromone blend we isolated from these hair pencils. Experiments were conducted to determine if male pheromone release is stimulated by host plant odor or by female pheromone and if the pheromone released is the attractant we isolated from extracts of hair pencils.

In flight tunnel experiments, noncompetitive comparisons were made of unmated female *T. ni* responses (upwind-oriented flight and contact) to males, potted cabbage

plants, and males on cabbage plants. Attraction to cages containing five males and a plant was significantly greater than to five males in a cage or to a plant in a cage (Table 1). In a second experiment, odors from males, from a plant, or from both males and a plant in glass holding chambers were piped into the flight tunnel. Females were tested for responses to airflow from over five males, from over a cabbage plant, or from over males exposed to cabbage odor. Again, more females were attracted to volatiles from the combination of males and a plant than to either alone (Table 1). In a third experiment, comparisons were made of female attraction to male odor, to the major component of the female sex pheromone [(*Z*)-7-dodecen-1-ol acetate (*Z*7-12:Ac)] (*3*), and to males exposed to *Z*7-12:Ac. Significantly more females were attracted to the five males exposed to *Z*7-12:Ac than to the unexposed males or to only *Z*7-12:Ac (Table 1). We interpret the significantly higher female response rates to males exposed to either plant odor or *Z*7-12:Ac as an indication of stimulation of male pheromone release by plant odor or *Z*7-12:Ac.

We then collected volatile compounds emitted by groups of five males (*11, 12*). Collections were made from males (n = 11), from males exposed to airflow from over a cabbage plant (n = 5), from males exposed to airflow containing *Z*7-12:Ac (n = 5), and from males exposed to both cabbage odor and *Z*7-12:Ac (n = 5). Linalool, *p*-cresol, and *m*-cresol were present in all collections made from males exposed to *Z*7-12:Ac and to the combination of *Z*7-12:Ac + cabbage odor (Table 2). Males released significantly more pheromone in response to *Z*7-12:Ac and cabbage odor than to either *Z*7-12:Ac or cabbage odor alone. The absence of linalool and cresols (<200 pg) from males exposed to cabbage odor suggests that the high female response rates to plant odors and males may be a result of synergism between plant odor and male pheromone or the result of additional pheromones emitted by calling or scent-marking males exposed to host plants. In the laboratory, male *T. ni* scent-marking occurred in response to foliage of cotton, cabbage, soybean, and celery, but not in response to wet filter paper or the nonhost plant *Setcreasea purpurea*.

Most of the sex pheromones studied have been female-produced male attractants, which are consistent with contemporary sexual selection reasoning in that males have more to gain and females more to lose from increased expenditures of time and energy on sexual activities, including search behavior. Other mate-finding strategies are known where males attract females. However, such cases are considered exceptional, resulting from unusual selection pressures such as male shortages, male control of resources, signaling risks, or opportunities for female mate choice at male aggregations (*13*).

Female cabbage looper moths may be attracted to male pheromone for mate-choice opportunities if male cabbage looper moths aggregate, as do *Estigmene acrea* (*14*), or for access to resources. Effects of host stimuli on sex pheromone behavior in cabbage looper moths, whether stimulation of male pheromone release or synergizing of female attraction to males, suggests that females could obtain greater access to hosts by responding to male pheromone. Such access may offset female investments in time and energy for search behavior and explain this exception to researchers' expectations of Lepidoptera female signaling and male searching. Females may also gain resources directly from male spermatophores, as suggested for other Lepidoptera (*15*).

Perceived limitations to the widespread use of sex pheromones in pest control are due largely to sexual competition among males reducing treatment efficiency (*16*). Past attempts to trap-out populations with female pheromones as male lures or as mating disruptants were often ineffective, presumably because of high ratios of sexually active males to females and the resultant competition for mates. Attractants for females, like those reported here for the cabbage looper moth, should be more effective when used as trap lures in obtaining pest population reductions, by direct removal of their reproductive potential, particularly if mated females are also susceptible. The possibility of pheromonal or kairomonal triggering of male pheromone release provides an initial experimental framework for searching for such male-produced female attractant pheromones in other pest moths and in other insects.

Table 2. Male pheromone components (mean ± SD) collected from airflow over male *T. ni* (n = 5) exposed to odors, in 15-min collections. Amounts followed by the same letter are not significantly different by Duncan's new multiple range test, P = 0.05.

Treatment	Male pheromone components (ng)			n
	d-linalool	*p*-cresol	*m*-cresol	
Control	0a	0	0	11
Cabbage	0a	0	0	5
*Z*7-12:Ac	5.0 ± 2.3b	0.3 ± 0.2	0.1 ± 0.1	5
*Z*7-12:Ac + cabbage	9.0 ± 4.3c	0.6 ± 0.3	0.2 ± 0.1	5

Table 1. Comparisons of percentages of unmated female *T. ni* responding to a cabbage plant, male *T. ni*, or both, in a cage in the flight tunnel, and to cabbage plant odor, *Z*7-12:Ac, male (n = 5) odor, and combinations piped into the tunnel. Percentages followed by different letters are significantly different (P = 0.01, χ^2).

Treatment	Attracted (%)	Contact (%)	n
	In flight tunnel		
Cabbage	31a	22a	95
Male *T. ni*	23a	22a	95
Cabbage + male *T. ni*	61b	47b	95
	Piped odor		
Cabbage	16a	6a	50
Male *T. ni*	10a	0a	50
Cabbage + male *T. ni*	44b	14a	50
*Z*7-12:Ac	0.0a	0.0a	30
Male *T. ni*	6.7a	3.3a	30
*Z*7-12:Ac + male *T. ni*	60.0b	47.0b	30

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extracts was a 15-m (0.53-μm film, 1.0-mm inner diameter) Carbowax (Quadrex). Columns were operated at 60°C for 2 min, and then the temperature was programmed at 30°C/min to 160°C. Identification of attractive compounds was based on electron impact and chemical ionization (methane) mass spectroscopy with sample introduction via capillary GC. Fourier transform infrared spectra were obtained on the active compounds. Proton nuclear magnetic resonance spectra (300 MHz) was obtained on natural linalool. The chirality of the *d*-linalool was determined by capillary GC analysis [with a chiral val capillary column (Chrompack, Inc.)] of the diastereomers obtained with (*R*)-phenyl isocyanate, according to the method of E. M. Gaydou and R. P. Randriamihariso [*J. Chromatogr.* **396**, 3781 (1987)]. The *d*-linalool for bioassays was obtained from coriander seeds. The oil obtained was purified by preparative GC and the material (>99% purity) had an enantiomeric purity of 95% dextrorotatory and 5% levorotatory. The *m*- and *p*-cresol (Aldrich) were purified to >99% purity before use.

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The Minimal Number of Class II MHC–Antigen Complexes Needed for T Cell Activation

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Major histocompatibility complex (MHC) molecules are exposed to large quantities of self and nonself antigens. It is not known what fraction of MHC molecules needs to be occupied by antigen to induce a T cell response. A quantitative study of naturally processed antigen indicated that T cells could be activated when only 0.03 percent of the total I-E^d purified from antigen-presenting cells (APCs) was occupied with antigen. B cells and macrophages processed hen egg lysozyme (HEL) with different efficiencies, but similar degrees of occupancy were required for T cell stimulation. Higher occupancy was needed for I-E^d–transfected L cells, possibly reflecting the requirement for other accessory molecules for efficient APC–T cell interaction.

THE HEL PEPTIDE THAT CORRESponds to the I-E^d–restricted determinant, HEL(107–116), can be isolated from naturally processed antigen (*1*). When HEL concentrations of 1 mg/ml are added to APCs, a substantial fraction (10 to 40%) of I-E^d molecules is occupied by HEL-derived antigen fragments (*1*). We have conducted experiments to estimate the minimal number of MHC-antigen complexes necessary to induce T cell activation. Large numbers (~10^9) of the B lymphoma, 2PK3 (*2*), were pulsed with various concentrations of HEL. After 24 hours, cell samples were washed, and their capacity to activate the I-E^d–restricted, HEL(107–116)–specific, T cell hybridoma, IE5, was assessed (*3*). Doses above 1 μg/ml gave an optimal response, and a dose of 0.5 μg/ml or less induced suboptimal interleukin-2 (IL-2) release from IE5 cells (Fig. 1). In parallel to these studies with intact APCs, I-E^d (including I-E^d–HEL peptide complexes) was purified by affinity chromatography (*1*) from each of the cell populations pulsed with the various HEL concentrations, and subsequently these preparations were inserted into planar membranes and used to stimulate IE5 cells (Fig. 2A). For HEL concentrations below 250 μg/ml, the relative potency of the different I-E^d preparations varied as a function of the HEL concentration used in the antigen pulse. To obtain a quantitative estimate of the fraction of I-E^d molecules that were occupied by the HEL(107–116) epitope in the various preparations, we also assessed the relative potency of HEL(107–116)–I-E^d complexes formed in vitro. At the same time, the concentration of antigen–I-E^d complexes formed was determined by Scatchard analysis (*1, 4*). In this way, IL-2 production could be related to the amount of HEL(107–116)–I-E^d complexes used in the T cell hybridoma assay (*1*) (Fig. 2B). Concentrations of 0.7 nM of HEL(107–116)–I-E^d complexes were required to stimulate IL-2 production at 40 U/ml from IE5 cells. About 230 nM of I-E^d purified from cells pulsed at the lowest concentration of HEL that gave stimulatory planar membranes (4 μg/ml) was necessary to achieve similar levels of IL-2 production. It can therefore be estimated that 0.7/230 (0.3%) of the I-E^d molecules purified from 2PK3 pulsed with HEL at 4 μg/ml were occupied by antigenic material. When similar calculations were performed for the other HEL concentrations used to pulse 2PK3 cells, a linear relation resulted (Fig. 2C). If linearity is assumed at low pulsing antigen concentrations, then an estimate of the percentage of

Table 1. Minimal number of I-E^d–antigen complexes required for T cell activation in various APCs. The numbers of I-E molecules per cell was determined by radiolabeling and immunoassay (*5*) with ^{125}I-labeled 14-4-4 monoclonal antibody to detect I-E^d and is stated as the average of two independent experiments. Experiment to experiment variability was contained within 40% of the mean. HEL dose is stated as micrograms per milliliter. Percent occupancy was determined from the capacity of I-E^d isolated from antigen-pulsed cells to stimulate IE5 cells in a planar membrane system, as described in Fig. 2. Average of two independent experiments. Occupancy levels for each cell type varied less than fourfold in replicate experiments.

Measure	APC types			
	2PK3	B cells	BMMϕ	CA36.2.1
Number of I-E molecules per cell	1.8×10^5	0.8×10^5	1.1×10^5	10^5
HEL dose required for suboptimal responses (320 U/ml)	0.3	2	256	32
Percent occupancy	0.03	0.14	0.25	4.6
Number of complexes	60	110	280	4600

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