

reveal themselves directly, as visible fluorescent images induced by the ultraviolet illumination, so that the transduction to a video image is really not required. We found the phenomenon to hold consistently for a diversity of pressed flowers known to have ultraviolet patterns in life, including members of the Compositae (*Rudbeckia*, *Heliopsis*, *Helianthus*, *Viguiera*, *Bidens*), Ranunculaceae (*Caltha*), Rosaceae (*Potentilla*), Oleaceae (*Jasminum*), Oxalidaceae (*Oxalis*), Loganiaceae (*Gelsemium*), and Guttiferae (*Hypericum*, *Ascyrum*). Invariably, the fluorescent patterns matched the ultraviolet patterns, in both contour and approximate contrast, leaving no doubt that the former are visible concomitants of the latter. Regions of petals that are ultraviolet-reflecting in life appear reflectant (yellowish-green to blue-green) in the fluorescent image, and ultraviolet-absorbing portions (such as "nectar guides") remain dark in the fluorescent patterns (Fig. 1). In the living flower, the fluorescence is at most only faintly discernible, but it becomes clearly, and sometimes brilliantly, apparent within hours after pressing, as soon as the flowers are dried or nearly dried. Aside from implications relevant to floral chemistry and coloration, the fluorescent phenomenon is of obvious use to botanists, who could, by the simple employ of a conventional ultraviolet "black light" (2), render visible an otherwise invisible floral character of considerable taxonomic value. As a rule, the fluorescence is most intense in relatively fresh herbarium material, but even flowers more than 10 to 30 years old commonly show the pattern. A distinct fluorescent pattern, typical for the species, was clearly apparent in the oldest herbarium flower examined by us, a cultivated specimen of *Rudbeckia hirta* from the collections of John Stuart, third Earl of Bute, who died—following a fall incurred while trying to reach a rare flower on a cliff—in 1792 (3).

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2. Any of a number of commercially available ultraviolet long-wave (about 3660-Å) lamps, such as are commonly used for visualization of fluorescent spots on chromatographic plates, can be employed.

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Pheromone Concentration as a Mechanism for Reproductive Isolation between Two Lepidopterous Species

Abstract. *Pheromone-releasing females of the closely related noctuid moths Trichoplusia ni and Autographa californica attract mainly males of their own species. Sex-pheromone-concentration specificity appears to be an important reproductive isolating mechanism for these two species. Apparently, both species utilize the same pheromone, cis-7-dodecenyl acetate, for mating communication, but T. ni utilizes a higher level than does A. californica. Traps releasing amounts of cis-7-dodecenyl acetate that are highly attractive for males of one of the species catch very few males of the other species.*

Factors such as differing seasonal cycles, geographic distributions, host plant specificities, and times of mating are frequently cited as species-isolating mechanisms (1). *Trichoplusia ni*, the cabbage looper, and *Autographa californica*, the alfalfa looper, frequent many of the same host plants and overlap considerably in their geographic distributions and seasonal cycles (2). Although there is some variation in the timing of their mating rhythms (3), this does not appear sufficient to cause efficient isolation between the species.

Another mechanism for isolation that has recently received considerable attention is sex-pheromone specificity (4). Such a mechanism would appear to be extremely important, because reproductive isolation between closely related species would be most efficiently achieved by mechanisms operating at the sensory and behavioral levels, preventing the two sexes of related species from approaching each other for mating.

The most obvious way in which sex-pheromone specificity between two or more species can be achieved is through each species utilizing a behaviorally and chemically distinct pheromone. Here we report a situation in which two closely related noctuid moth species apparently achieve reproductive isolation by utilizing behaviorally distinct levels of the same sex pheromone in mating communication.

The sex pheromone of *T. ni* is *cis*-7-dodecenyl acetate (5). Based on gas-liquid chromatography, bioassay, and electroantennogram analysis, it appears that *A. californica* also utilizes this

compound as a sex pheromone (6). However, when caged virgin 3- to 4-day-old females of *T. ni* and *A. californica* were used as bait in separate traps (7) in the same field on 30 consecutive nights, they attracted mainly males of their own species (Table 1).

Extracts of pheromone glands from *T. ni* females evoked a higher response from both *T. ni* and *A. californica* males in laboratory bioassays than equivalent extracts from *A. californica* females (8). When extracts of pheromone glands from females of both species were subjected to gas-liquid chromatography followed by bioassay analysis, quantitative determinations indicated the presence of approximately 0.5 µg and 0.01 µg of pheromone per female for *T. ni* and *A. californica*, respectively. Based on these data, we suggest that females of *A. californica* utilize a smaller quantity of *cis*-7-do-

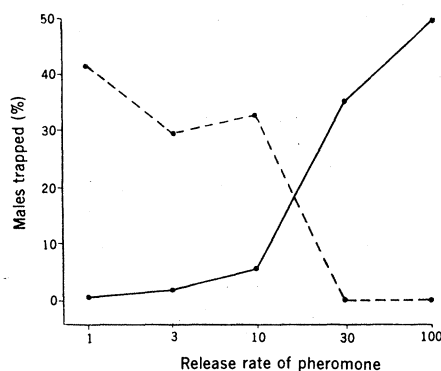


Fig. 1. Percentages of *T. ni* males (solid line; $N = 962$) and *A. californica* males (dashed line; $N = 81$) captured in traps baited with *cis*-7-dodecenyl acetate released at various relative rates.

decenyl acetate in mating communication than females of *T. ni*.

Five traps, each of which was baited with an evaporator (9) that released *cis*-7-dodecenyl acetate (10) at a different rate, were simultaneously placed in a field supporting a mixed population of both species. On each of 30 consecutive mornings, the moths caught in the traps were identified, sexed, and counted, and each trap was randomly moved to a new position. The number of *T. ni* males caught per trap increased with each increase in the rate of evaporation of *cis*-7-dodecenyl acetate (Fig. 1). On the other hand, *A. californica* males were caught only in the traps that released this compound at those low evaporation rates which were largely unattractive to *T. ni* males. These data indicate that the release of the same sex pheromone by females at different rates is a probable mechanism of reproductive isolation between these two species.

Only over a very narrow range of evaporation rates of *cis*-7-dodecenyl acetate will *T. ni* males enter traps baited with this chemical (11). Few males are captured in traps releasing the pheromone at rates tenfold above or below the optimum. We believe that the optimum release rate may be that which causes the pheromone concentration near the entrance of the trap to be similar to the concentration found in the immediate vicinity of a free-living, pheromone-releasing female. We further propose that a male encountering this critical concentration stops its long-distance pheromone-orientation behavior and engages in a short-range "searching" behavior, causing it to enter the trap. If these hypotheses are correct, then *T. ni* males responding to the low quantity of *cis*-7-dodecenyl acetate released from *A. californica* females would not reach their critical concentration and thus would neither terminate their long-distance orientation behavior nor enter traps. On the other hand, *A. californica* males approaching a receptive *T. ni* female would presumably reach this concentration, and thus terminate their long-distance orientation behavior, at some point downwind from the female.

It can be seen that reproductive isolation based on prevention of males orienting to the females of the related species is not complete (Table 1). Essentially no *A. californica* males were captured in traps baited with *T. ni* females. On the other hand, a fairly large number of *T. ni* males was cap-

Table 1. Capture of males in traps baited with ten virgin noctuid females.

Bait species	No. of males captured per trap*	
	<i>T. ni</i>	<i>A. californica</i>
<i>T. ni</i>	1861	1
<i>A. californica</i>	37	79

* Based on 30 trapping nights.

tured in the traps baited with females of *A. californica*. Also, at certain intermediate evaporation rates of *cis*-7-dodecenyl acetate, males of both species were captured. However, previous laboratory research (8) has shown that an additional mechanism can operate at very close range, causing the female to reject copulation attempts made by males of the wrong species.

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6. Near identical retention times for the biologically active compound in extracts from female pheromone glands of *T. ni* versus *A. californica* were recorded with a flame ionization detector after gas-liquid chromatographic separation on each of three different columns (Carbowax 20 M, neopentylglycol adipate, and SF 96). Based on the previous identified structure of *T. ni* pheromone, these data limit the structure of *A. californica* pheromone to several closely related straight chain 12-carbon monounsaturated acetates. T. L. Payne, H. H. Shorey, L. K. Gaston (*Ann. Entomol. Soc. Amer.*, in press) obtained electroantennograms (EAG) from *A. californica* and *T. ni* in response to *cis*-7-dodecenyl acetate and three closely related compounds (*cis*- Δ -dodecenyl acetate, $x=6, 8$, and 9). Significant EAG responses were obtained with both *T. ni* and *A. californica* males for all compounds. However, from 100- to 10,000-fold more (compared to *cis*-7-dodecenyl acetate) of each compound was required to obtain the response. In addition, *trans*-7-dodecenyl acetate does not attract males of either species at any of several release rates that we have tested in the field.
7. Double-cone traps [R. K. Sharma, H. H. Shorey, L. K. Gaston, *J. Econ. Entomol.* 64, 361 (1971)] were baited with ten virgin females of *T. ni* or *A. californica*. Based on earlier experimentation with *T. ni* [R. S. Kaae and H. H. Shorey, *Ann. Entomol. Soc. Amer.* 65, 436 (1972)], we estimate that no more than two or three of these females were releasing pheromone at any one time.
8. H. H. Shorey, L. K. Gaston, J. S. Roberts, *Ann. Entomol. Soc. Amer.* 58, 600 (1965). The pheromone extracted from females of each species was bioassayed in a dilution series based on female equivalents.
9. The construction and calibration of the evaporators is described by H. H. Shorey, R. S. Kaae, L. K. Gaston, J. R. McLaughlin, *Environ. Entomol.* 1, 641 (1972). Five evaporators were designed to release *cis*-7-dodecenyl acetate at 1, 3, 10, 30, and 100 ng/min under constant laboratory conditions of 18°C and 0.4 m/sec airflow. Temperatures and air velocities in the field were lower than these values during the conduct of the experiment, and actual release rates probably varied between one-tenth and one-half of the above-stated rates. Therefore, release rates in Fig. 1 are given as relative, rather than absolute, values.
10. The *cis*-7-dodecenyl acetate used gave only one peak on gas-liquid chromatography (lower limit of detection of other compounds was 0.5 percent), indicating that the material was at least 99.5 percent 7-dodecenyl acetate. An upper limit of 10 percent for the *trans* isomer was estimated by nuclear magnetic resonance (δ and J are slightly different for the *cis* and *trans* olefinic protons).
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12. This is paper No. 36 of a series entitled "Sex pheromones of Lepidoptera." We thank B. Wilk and S. U. McFarland for their assistance. This research was supported in part by a grant from the Rockefeller Foundation.

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Leaching: Use of a Thermophilic and Chemoautotrophic Microbe

Abstract. *A chemoautotrophic, thermophilic, and acidophilic microorganism capable of oxidizing reduced sulfur and iron compounds and leaching concentrates of molybdenite and chalcopyrite at 60°C has been characterized by transmission and scanning electron microscopy. This constitutes the first direct observations of microorganisms on ore fines.*

Field studies indicate that temperatures within low-grade copper ore dumps may reach 80°C (1). These elevated temperatures undoubtedly inhibit the leaching activity of strains of sulfur- and iron-oxidizing bacteria whose optimum temperature range is

25° to 45°C (2, 3). Thermophilic organisms have not previously been considered in leaching activities. We therefore report the characterization of a chemoautotrophic, thermophilic, and acidophilic microorganism, possibly related to *Sulfolobus* (4), with the capa-