fixed for a different translocation complex. As these isolates expand and integrate, new translocation heterozygotes arise. Further genetic load is produced, which should lead to a second population decline, though of lesser magnitude. In theory this method could be used to replace an insecticide-resistant native strain with a susceptible released strain. Once this was achieved there are alternative courses open for future action. Insecticides could be reintroduced until resistance redevelops and so the cycle repeated.

For a variety of reasons we may prefer to manipulate the population in other directions as well. For example, the introduction of recessive cold-sensitive mutants (*10*) might merit consideration, provided that their pleiotropic effects are found to be suitable. If a multivoltine native insect population could be virtually replaced during a single season by a synthetic strain homozygous for a cold-sensitive mutant, the overwintering stages would succumb and a very high proportion of the population would be eliminated.

If we consider the release of more than one synthetic cold-sensitive strain, the idea becomes feasible for certain dipteran populations. For a species such as the Australian sheep blowfly with six or more generations a year, if we release, early in spring, four synthetic strains each carrying the same recessive lethal which acts only at low temperatures, then we might expect a load in excess of 90 percent over the next five generations while the lethal is being spread through the population. At the onset of winter when the lethal would have its effect, its frequency should exceed 0.999; the resulting genotypic frequencies would be AA, 0.000001; AT, 0.001998; and TT, 0.998; where T is carrying the temperature lethal. Consequently, less than one in a million flies entering winter will both survive the winter and be fertile. This rate of gene substitution is significantly more rapid than any meiotic drive mechanism could achieve.

The release of several translocation strains in which heterozygotes are sterile, once they are available, would appear to be more effective with less effort than the sterile male method of control and may therefore prove economical for some species where cost factors preclude the use of sterile males. For example, a single release of four synthetic strains, in equal frequency to the native population, can be shown to cause a population reduction equivalent to a 20:1 sterile-to-normal-male release repeated for five generations. It provides a further advantage in allowing direct manipulation of the genetic composition of the insect population.

Such a program would seem suited to some higher Diptera, potential candidates including the Australian sheep blowfly, the housefly, and various fruit flies. The presence of crossing-over in male mosquitoes and the availability of only three pairs of chromosomes that would affect the ease with which multiple translocation strains are produced appear to make these important vectors of disease unsuited. However, complete heterozygote sterility is not essential. If the genes to be fixed are included in inversions prior to the induction of translocations it may be possible to prevent their replacement by undesirable alleles from the native strain during the period of displacement, which is the major problem arising from incomplete sterility of the heterozygote. In those instances where there has been leakage of genes through the heterozygote, it may be necessary to make a succession of releases until adequate gene substitution is obtained. Mosquito species have been the subject of a special study (*11*) that indicates it should be possible to substitute genes in less than ten generations while providing a permanent genetic load of 50 percent.

Once a set of suitable strains has been developed and the system has been shown to work over the first cycle of releases, its permanence is ensured since natural selection cannot oppose it; rather, natural selection is an essential ingredient of the program. The prospects of an indefinite life for an insecticide, made possible by genetic manipulation, may provide incentive for the development of better insecticides whose synthesis might otherwise have been economically unattractive.

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Hydrocarbon Sex Pheromone in Tiger Moths (Arctiidae)

Abstract. *2-Methylheptadecane is a sex pheromone compound in many sibling species of the* Holomelina aurantiaca *complex, in* Holomelina laeta, *and in* Pyrrharctia isabella, *which are all arctiids. Habitat preference, temporal distribution, and differing diurnal cycles help effect reproductive isolation among the species, but secondary sex pheromone chemicals are also suggested by the field studies.*

Males of many species in the families Gelechiidae, Noctuidae, Pyralidae, and Tortricidae have been attracted under natural field conditions to specific monounsaturated alcohols or acetates (*1*). Species from many other lepidopterous families have been conspicuously absent in these field screening tests, presumably because monounsaturated fatty alcohols and acetates represent only one of several classes of compounds used as attractants in the Lepidoptera. We have now found that in the family Arctiidae a saturated aliphatic hydrocarbon, 2-methylheptadecane, is a sex pheromone for at least nine species.

The pheromone was obtained by extracting the abdominal tips of 50 *Homomelina nigricans* (*2*) females

Table 1. The presence and field attractancy of 2-methylheptadecane in various Arctiidae.

Species	Origin of laboratory stock	Trapping localities	Field attractancy	Presence in female
Holomelina aurantiaca complex				
H. aurantiaca	Connecticut	Connecticut	*	Yes
H. immaculata	New York	New York, Pennsylvania	*	Yes
H. rubicundaria		Florida	*	
H. lamae	Maine	Maine, Michigan	†	Yes
Undescribed Black Hills species	Wyoming	Wyoming	†	Yes
H. nigricans	New Jersey	Pennsylvania	‡	Yes
H. fragilis	Colorado			Yes
H. ferruginosa	Connecticut	Connecticut, Pennsylvania	§	Yes
Holomelina laeta		Florida, New York	‖	
Pyrrharctia isabella	New York	Connecticut, Florida, New York	‖	Yes

* Efficient male attraction (> 10 to 20 males per trap daily). † Males attracted to the vicinity of trap (1.0 to 0.1 m) but few in traps (one or two). ‡ Very few males trapped; no apparent long-range attraction. § No males attracted. ‖ Trapped in low numbers; population levels unknown.

with methylene chloride. Intense male responses to the crude extracts in laboratory bioassays were still elicited after various chemical treatments of the extract, such as saponification, acetylation, and bromination. Elution of the active component from a silica gel column with petroleum ether indicated that the attractant was very nonpolar. Gas chromatographic retention times, obtained by bioassaying 1-minute collections after injection of crude extract, show that the attractant behaved like octadecane on both polar and nonpolar columns. The attractant and octadecane have retention times of 4.9 and 5.6 minutes, respectively, on 3 percent cyclohexanedimethanol succinate at 156°C, and 17.7 and 21.3 minutes, respectively, on 3 percent OV-1 at 148°C. A mass spectrum of the attractant shows the following prominent peaks at mass-to-charge ratios (*m/e*) 254 (1.5 percent), 239 (3.3 percent), 211 (13.3 percent), 210 (3.8 percent), 197 (1 percent), 183 (1.5 percent), 169 (2.7 percent), 155 (4.1 percent), 141 (6.4 percent), 127 (7.5 percent), 113 (11.3 percent), 99 (16.7 percent), 85 (45 percent), 83 (10 percent), 71 (74 percent), 70 (12 percent), 69 (14 percent), 57 (98 percent), 56 (55 percent), 55 (72 percent), 43 (100 percent). Fragmentation at M-15 and M-43 is quite characteristic of 2-methyl alkanes (*3*). Since the molecular ion at *m/e* 254 indicates a C_{18} compound, only 2-methylheptadecane and 2,15-dimethylhexadecane are possible structures for the attractant. Although these two compounds (*4*) give identical fragmentation patterns with mass spectrometry, they are easily separated on polar and nonpolar gas chromatographic columns since the dimethyl isomer elutes faster from both. The attractant has retention times identical to those of 2-methylheptadecane.

Holomelina nigricans is one of at least nine sibling species in the *aurantiaca* complex (*5*), of which at least eight have 2-methylheptadecane as an attractant (Table 1). Further, *Holomelina laeta*, a species not closely allied to the *aurantiaca* complex, and *Pyrrharctia isabella* also are lured to the same compound. The presence of 2-methylheptadecane in the female extract of other arctiid species (Table 1) was confirmed by gas chromatography and mass spectrometry. Cases in which related species utilize the same sex attractant compound are not uncommon in the Lepidoptera, a salient example being the use of *cis*-7-dodecenyl acetate by many noctuid species in the subfamily Plusiinae (*1*). In such cases it is interesting to determine what factors are operating to effect reproductive isolation among the species. In the case of the *aurantiaca* complex, differences in habitat preference and temporal distribution isolate some species that occur together. For example, in southern Connecticut *H. aurantiaca* and *H. ferruginosa* occupy distinct but continguous habitats. Separation is effected by differing seasonal flight periods: *H. aurantiaca* is bivoltine with adults emerging from mid-May to mid-June and again in August, whereas *H. ferruginosa* is univoltine with the only flight in July. Full sympatry (temporal and spatial), however, is found for species in some geographical areas: for example, *aurantiaca, ferruginosa,* and *lamae* in Nova Scotia (*6*); *aurantiaca, immaculata,* and *nigricans* in southeastern Pennsylvania; and *fragilis* and an undescribed species in the Black Hills of South Dakota.

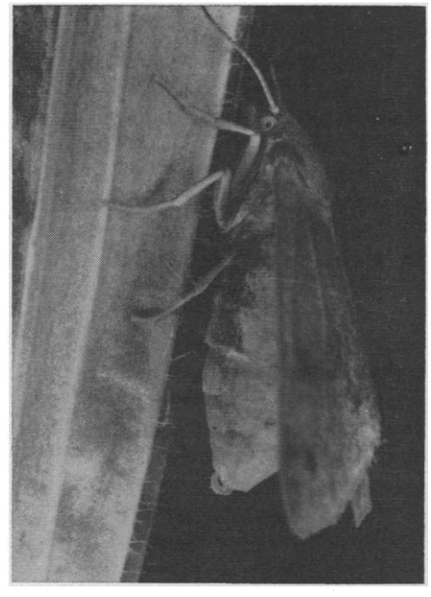

Fig. 1. *Holomelina nigricans* female in calling stance.

Another important partitioning mechanism is differing diel periodicity for mating. For instance, females of the saturniid (silkworm) moths *Callosamia promethea* and *Samia walkeri* (= *cynthia* of recent authors) apparently use the same sex attractant but are isolated by exclusive diurnal cycles (*7*). Similar factors play a role in preventing cross attraction among some species of the *aurantiaca* complex. *Holomelina lamae, H. nigricans,* and the undescribed Black Hills species appear to be day mating since the males are attracted to 2-methylheptadecane in the field only during the day, and the *nigricans* females call (*8*) (Fig. 1) and mate in the laboratory from hours 9 to 16 in a 16-hour photoperiod regime.

Other species, such as *aurantiaca, immaculata, rubicundaria, fragilis,* and *ferruginosa,* appear to have broadly overlapping nocturnal cycles in that laboratory females call and mate only at night. In the field, *immaculata* males are attracted to 2-methylheptadecane from 2 hours before until 2 hours after sunset. We have also observed six successful courtships within one-half hour of sunset. The exact diurnal cycle of *aurantiaca* is anomalous, as males

are lured to 2-methylheptadecane from 10 a.m. to 10 p.m. (sunset 8 p.m.), while a laboratory population from the same material mates exclusively at night.

Sex pheromones can also play an important role in effecting reproductive isolation in the *aurantiaca* complex even though 2-methylheptadecane is common to them all. Specificity could be effected by the use of secondary chemicals (*9*) or by differences in the release of the primary attractant, or both. Field responses (Table 1) (*10*) of males in the *aurantiaca* complex to 2-methylheptadecane suggest that other chemicals may be involved. Males of *aurantiaca, immaculata,* and *rubicundaria* are easily attracted into the traps, whereas *lamae* and males of the undescribed Black Hills species approach the traps upwind but rarely enter. Upon reaching the vicinity of the trap, males walk or hover a few centimeters over the herbage and get to about 0.1 to 1 m from the trap before abruptly flying 10 m or more away. Lower concentrations of attractant did not change this behavior. *Nigricans* never oriented toward the traps, and exceedingly few of the feral population were actually caught—although males in laboratory bioassays are attracted to filter paper disks containing 2-methylheptadecane and often attempt copulatory movements. *Ferruginosa* males were never trapped in the field. No orientation flight was noted in daytime observations, but such behavior could have occurred at night during the normal period of mating. Varying the rate of release of 2-methylheptadecane and modifying possible visual clues with the use of black traps or of dead females as calling models did not seem to change the field responses of males of these various species. It was interesting to find that 2-methylpentadecane, 2-methylhexadecane, 2-methyloctadecane, 2-methylnonadecane, 4-methylheptadecane (*4*), and *n*-octadecane were totally unattractive to tiger moth males. 2,15-Dimethylhexadecane did attract some *immaculata* males, but it was about one-tenth as attractive as 2-methylheptadecane.

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6. In Nova Scotia, *aurantiaca, ferruginosa,* and *lamae* often fly within sight of each other, but *lamae* is unique in its bog habitat requirement, while the other two species are widely distributed, typically on blueberry barrens [D. C. Ferguson, *Proc. Nova Scotian Inst. Sci.* **23**, 212 (1954)].
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8. The gland which produces the pheromone is situated between the eighth and ninth abdominal segments. In calling, the female raises its wings slightly from the rooflike resting position and protracts and retracts the ovipositor and gland at short (< 2-second) intervals. Night observations were made with a low intensity light filtered with Kodak Wratten filters 29 and 92 (> 6100 Å).
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10. 3M Co., white Sectar traps were deployed a few centimeters above the herbage and baited with 20 μl of synthetic compound inside a polyethylene cap [see (*1*)] or with rubber septa containing 10, 30, 100, or 1000 μg of attractant, or with a saturated rubber septum.
11. We thank Dr. D. Habeck for conducting the Florida field studies, J. Muller and Dr. A. Shapiro for help in locating *H. nigricans* colonies, D. Angell for invaluable assistance with the mass spectrometry, R. W. Mackey, Jr., for chemical synthesis, and Dr. J. G. Franclemont for helpful discussions.

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Sea Urchin Embryos Are Permeable to Actinomycin

Abstract. *When eggs and cleaving embryos of the sea urchin are exposed to [^{3}H]actinomycin D, they become radioactive, and autoradiograms show that the radioactivity is inside the cells. At midcleavage, nuclei are more radioactive than cytoplasm. Extraction and chromatography of the intracellular labeled compounds identify them as actinomycin D and a water-soluable derivative. Conversion does not take place outside the cells. Treatment of embryos for 90 minutes with actinomycin D inhibits synthesis of RNA by more than 90 percent, leaving unaffected turnover of the pCpCpA terminals in transfer RNA. These data justify earlier interpretations of actinomycin experiments with embryos and justify use of the drug as a tool in the analysis of gene expression.*

Actinomycin has been used as an inhibitor of RNA synthesis in studies on gene expression during sea urchin development (*1*). Recent claims have been made to the effect that actinomycin fails to enter the embryos before hatching (*2*), and on this ground doubts have been expressed as to the validity of conclusions drawn from such experiments. Concepts evolved from actinomycin experiments are of some importance in developmental biochemistry (*3*); it is therefore important to be certain of the permeability of early stages to the drug. Results of a critical examination prove that the drug does penetrate eggs and cleavage stages.

Standard culture techniques were

Table 1. Radioactivity in nucleic acids from control and actinomycin-treated embryos labeled with ^{32}P. Percentages of distributions of total radioactivity (count/min) were obtained by summation in appropriate parts of the gradients shown in Fig. 4. Nucleic acid preparations were treated with deoxyribonuclease prior to gradient sedimentation and the radioactivity (count/min) in the DNA was all in oligodeoxynucleotides sedimenting at less than 4*S*. The number of counts per minute in the DNA was determined by resistance to alkaline hydrolysis. Total incorporation of ^{32}P in the presence of actinomycin was 0.3 of that in controls, hence 0.3 times the values in column 2 gives the radioactivity in each RNA and DNA fraction, labeled in actinomycin, expressed as percentage of total incorporation in controls. Values in column 3 can be expressed as percentages of corresponding fractions in column 1, and subtracting such percentages from 100 gives the percentage *inhibition* of incorporation into each fraction, attributable to presence of the antibiotic. Since 4*S* RNA labeling is unaffected, actinomycin treatment did not alter the uptake of precursors. The percentage of inhibitions (column 4) is an explicit measure of decrease in synthesis. Actinomycin D, Act. D.

Fraction	Radioactivity (% total count/min)		Act. D incorporation (% control total count/min)	Inhibition (% by fractions)
	Control	Act. D		
RNA > 4*S*	65.4*	15.5*	4.7	**92.8**
"RNA ≤ 4*S*"	34.6*	84.5*		
DNA	22.2	41.0	12.3	**44.6**
4*S* RNA	12.4†	43.5†	13.0	**—5.0**

* These are distributions of all labeled nucleic acids and sum to 100 percent. † The radioactivity in the 4*S* RNA was determined by difference.