veying spatial information or as a driving force for the electrophoresis of material to the future branch site.

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3. The female strain, *Achyla bisexualis* T5, was prepared for probe measurements as follows. Vegetative hyphae were sporulated in buffered $CaCl_2$ [D. H. Griffin and C. Breuker, *J. Bacteriol.* **98**, 689 (1969)], and the spores were inoculated onto agar plates containing DMA medium, *p*H 6.5; DMA is the defined medium of D. H. Griffin, W. E. Timberlake, and J. C. Cheney [*J. Gen. Microbiol.* **80**, 381 (1974)], supplemented with the amino acid mixture of D. R. Soll, R. Bromberg, and D. R. Sonneborn [*Dev. Biol.* **20**, 183 (1969)]. After overnight incubation, an agar plug containing one or two mycelia was cut from a plate, attached to a circular cover glass, and covered with liquid DMA. Once hyphae began to grow off the edge of the agar into the liquid, the cover glass was attached to the bottom of a small chamber for vibrating probe measurements. The resistivity of DMA was 1100 ohm-cm.
4. The pattern of current flow at the very tip of the hypha may be complicated by the conical shape of the tip. Current flow into a hyphal tip has also been observed in *Achlya debaryana* by B. Armbruster and M. H. Weisenseel [*Protoplasma* **11**, 65 (1983)].
5. Since the tip grew at 5 μm/min, the probe was moved in order to follow a position 30 μm behind the tip.
6. Branches never emerged closer than 150 μm to the original tip. Therefore, the maximum time from the initiation of branch differentiation to its visible emergence cannot be longer than 30 minutes (the time it takes the old tip to grow 150 μm at 5 μm/min). We can detect inward current at the future branch site at least 20 minutes, and sometimes as early as 40 minutes, before branching; this result suggests that the current acts early in the branching process.
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9. Self-electrophoresis appears dubious a priori for the following reasons. From the total inward current and the hyphal cross-sectional area, we calculate the cytoplasmic current density to be approximately 10 μA/cm^2. If we assume a cytoplasmic resistivity of 200 ohm-cm, the electrical field across the cytoplasm is ~ 2 mV/cm. Let us consider a highly charged protein, such as prealbumin, whose electrophoretic mobility is 0.8 μm per second per volt per centimeter [in *Handbook of Biochemistry*, H. A. Sober, Ed. (CRC Press, Cleveland, ed. 2, 1970), p. C36]. If we ignore back-diffusion, the calculated field could move the protein toward the tip at only 0.1 μm/min, far slower than the tip growth rate. Electrophoresis of membrane proteins would be even slower. Nonetheless, the field strength, and the rate of protein movement, could be significantly larger if the ions that generate the field were tightly bound in the cytoplasm (*7*).
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13. We thank W. J. Betz for computer assistance and R. Kennedy for fabricating the chamber. This research was supported by NSF grant PCM 8009439 and NIH grants AI 03568 and NS 16922.

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Sex Pheromone Biosynthesis in *Trichoplusia ni*: Key Steps Involve Delta-11 Desaturation and Chain-Shortening

Abstract. *In addition to the primary pheromone component (Z)-7-dodecenyl acetate, the sex pheromone gland of* Trichoplusia ni *contains the immediate fatty acyl precursor (Z)-7-dodecenoate and a large quantity of (Z)-11-hexadecenoate. Labeling experiments showed that (Z)-11-hexadecenoate is chain-shortened to (Z)-9-tetradecenoate, and that this in turn is chain-shortened to (Z)-7-dodecenoate. The same mechanism appears to explain the sex pheromone compositions of many other moth species.*

The sex pheromones of more than a hundred species of moths, totaling more than 200 compounds, have been chemically identified (*1*). Most of these compounds are straight-chain acetates, alcohols, or aldehydes, with chain lengths of 10, 12, 14, 16, or 18 carbon atoms, and with one or two double bonds. One of the most puzzling aspects of these studies has been that different double bond positions seem to predominate in different chain lengths. For example, in the Noctuidae, the family of moths for which the greatest number of sex attractants has been characterized, (*Z*)-5-decenyl, (*Z*)-7-dodecenyl, (*Z*)-9-tetradecenyl, and (*Z*)-11-hexadecenyl moieties comprise 80 percent of the known sex pheromone components (*1, 2*). In the course of work with the cabbage looper moth, *Tricho-*

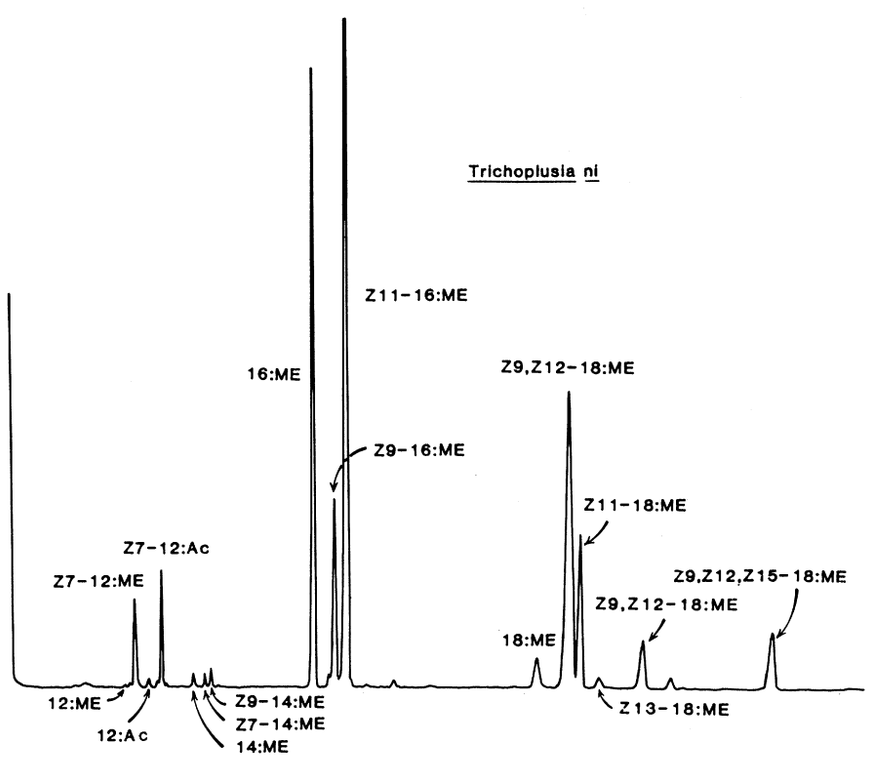

Fig. 1. Capillary GLC trace of sex pheromone components and total complement of fatty acyl moieties in pheromone glands of *Trichoplusia ni*, after methanolysis and acetylation. Abbreviations: *Z7-12:Ac*, (*Z*)-7-dodecenyl acetate; *Z7-12:ME*, (*Z*)-7-dodecenoate; and so on.

plusia ni (Noctuidae), we have found a biosynthetic pathway that appears to explain this widespread feature.

In our interpretation, hexadecanoate and a smaller amount of octadecanoate are produced by fatty acid synthetase in the cytosol in the usual way (*3*). In eukaryotes, these fatty acids can be converted to the unsaturated fatty acids (*Z*)-9-hexadecenoate and (*Z*)-9-octadecenoate by a (*Z*)-9 desaturase in the endoplasmic reticulum (*4*). In addition, an uncommon (*Z*)-11 desaturase is present in the sex pheromone gland of *T. ni*, producing (*Z*)-11-hexadecenoate and a smaller amount of (*Z*)-11-octadecenoate. We have found that (*Z*)-11-hexadecenoate undergoes chain-shortening to generate (*Z*)-9-tetradecenoate and that this in turn is chain-shortened to generate (*Z*)-7-dodecenoate, which is reduced and acetylated to produce the sex pheromone component (*Z*)-7-dodecenyl acetate. Moreover, it is apparent that the (*Z*)-11-hexadecenyl, (*Z*)-9-tetradecenyl, (*Z*)-7-dodecenyl, and (*Z*)-5-decenyl moieties that comprise most of the sex pheromone components known for the noctuid moths can all be produced from (*Z*)-11-hexadecenoate, simply by altering the product specificity of the chain-shortening system. We base these conclusions on the following experiments.

Gland extracts were methanolyzed and treated with acetyl chloride (*5*). Capillary gas-liquid chromatographic (GLC) analysis (*6*) indicated that in addition to the pheromone components (*Z*)-7-dodecenyl acetate and dodecyl acetate (*7*), their fatty acyl precursors (*Z*)-7-dodecenoate and dodecanoate were present in comparable amounts (Fig. 1). The gland contained even larger quantities of two other unusual fatty acids, (*Z*)-11-hexadecenoate and (*Z*)-11-octadecenoate, and also contained small amounts of (*Z*)-9-tetradecenoate, (*Z*)-7-tetradecenoate, tetradecanoate, and (*Z*)-13-octadecenoate. Capillary GLC analysis of fractions from a thin-layer chromatographic separation (*8*) indicated that most of these fatty acids occurred in triacylglycerols, much smaller amounts occurred in choline phosphatides and ethanolamine phosphatides, and very small amounts occurred in diacylglycerols. Common fatty acyl moieties were also present, including hexadecanoate, (*Z*)-9-hexadecenoate, octadecanoate, (*Z*)-9-octadecenoate, (*Z,Z*)-9,12-octadecadienoate, and (*Z,Z,Z*)-9,12,15-octadecatrienoate. These common fatty acids were abundant in diacylglycerols as well as in triacylglycerols and phospholipids.

We wished to know if all these compounds were synthesized de novo from acetate by the gland. Jones and Berger (*9*) demonstrated that the main pheromone component (*Z*)-7-dodecenyl acetate is synthesized de novo from acetate, but they did not analyze other compounds in the gland. We applied sodium [1-^{14}C]acetate topically to sex pheromone glands of female *T. ni* (*10*), and found that ^{14}C was incorporated into the pheromone and into most of the fatty acids in the gland [the greatest proportion appearing in (*Z*)-11-hexadecenoate], but that very little ^{14}C was incorporated into (*Z*)-9-hexadecenoate, (*Z*)-9-octadecenoate, (*Z,Z*)-9,12-octadecadienoate, or (*Z,Z,Z*)-9,12,15-octadecatrienoate.

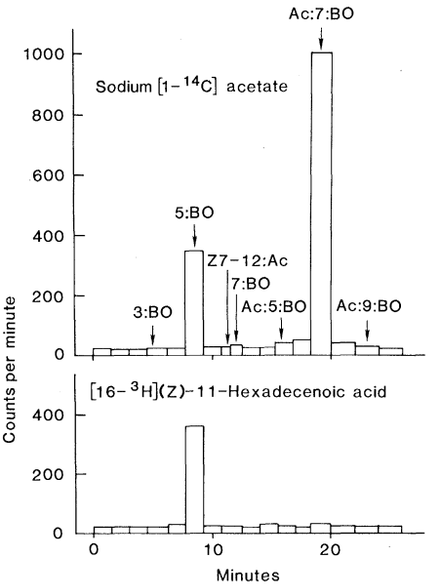

Fig. 2. Benzyloximes of ozonolysis fragments of the main pheromone component (*Z*)-7-dodecenyl acetate after incubation of glands with (A) sodium [1-^{14}C]acetate and (B) [16-^{3}H](*Z*)-11-hexadecenoic acid. Each bar represents a GLC fraction collected from an OV-101 column at the time indicated on the horizontal axis. Abbreviations: *5:BO*, pentylbenzyloxime; *Ac:7:BO*, acetoxyheptylbenzyloxime; and so on.

We then synthesized [16-^{3}H](*Z*)-11-hexadecenoic acid (*11*), applied this topically to the sex pheromone glands of female *T. ni*, and analyzed incorporation into compounds in the gland. The label was incorporated into both (*Z*)-7-dodecenyl acetate and (*Z*)-7-dodecenoate. In addition, we found that although tetradecanoate, (*Z*)-7-tetradecenoate, and (*Z*)-9-tetradecenoate occur in small, almost equal amounts in the gland, and incorporated small, equal amounts of label in the experiment with sodium [1-^{14}C]acetate, only the predicted chain-shortened product, (*Z*)-9-tetradecenoate, incorporated a substantial amount of label in the experiment with [16-^{3}H](*Z*)-11-hexadecenoic acid.

We realized that simply observing incorporation of ^{3}H into (*Z*)-7-dodecenyl acetate after topical application of [16-^{3}H](*Z*)-11-hexadecenoic acid would not necessarily verify our proposed pathway, because if mitochondrial degradation of the [16-^{3}H](*Z*)-11-hexadecenoic acid occurred, labeled acetyl coenzyme A would be produced and might be incorporated into (*Z*)-7-dodecenyl acetate by some other biosynthetic route than chain-shortening. If this occurred, however, the label would occur throughout the (*Z*)-7-dodecenyl acetate molecule, not on the terminal carbon atom alone. This was tested by conducting ozonolysis of the double bond to break (*Z*)-7-dodecenyl acetate into two fragments and comparing the amount of label in each fragment (*12*). When (*Z*)-7-dodecenyl acetate from the sodium [1-^{14}C]-acetate experiment was ozonized and converted to benzyloximes, both fragments were labeled. When (*Z*)-7-dodecenyl acetate from the [16-^{3}H](*Z*)-11-hexadecenoic acid experiment was ozonized and converted to benzyloximes, ^{3}H was found in the pentylbenzyloxime fragment, but not in the acetoxyheptylbenzyloxime fragment (Fig. 2). This demonstrates that chain-shortening of (*Z*)-11-hexadecenoate is responsible for production of the (*Z*)-7-dodecenyl moiety (although the potential occurrence of additional pathways cannot yet be ruled out absolutely).

Our proposed pathway for chain-shortening offers an explanation for several features of the ultrastructure of the pheromone gland cell whose functional significance has so far remained unclear. Percy (*13*) demonstrated that large lipid spheres are abundant in the pheromone gland cells of *T. ni*. The triacylglycerols, which contain the bulk of the (*Z*)-11-hexadecenoate, are expected to occur in lipid spheres (*13*). Percy observed that each lipid sphere is completely surrounded by a "halo" of microperoxisomes. Peroxisomes in mammalian cells are able to carry out β-oxidation of fatty acids, a process previously thought to take place exclusively in the mitochondria (*14*). Unlike mitochondrial β-oxidation, peroxisomal β-oxidation is incomplete. At most, five β-oxidation cycles are involved, and products of the first two or three β-oxidation cycles predominate (*15*). It seems likely that the principal function of the microperoxisomes in the sex pheromone gland of *T. ni* is to carry out two cycles of β-oxidation (that is, chain-shortening) of (*Z*)-11-hexadecenoate to produce (*Z*)-7-dodecenoate, which is then reduced and acetylated to produce the main sex pheromone component (*Z*)-7-dodecenyl acetate.

(*Z*)-11-Octadecenoate was also abundant in the sex pheromone gland of *T. ni*, and chain-shortened products of this can account for an additional 10 percent of the sex pheromone components known for the Noctuidae (*1*). In a preliminary experiment with *Xestia dolosa*, in which (*Z*)-7-tetradecenyl acetate is the principal sex pheromone component, we have observed that large quantities of the expected precursors (*Z*)-11-octadecenoate and (*Z*)-9-hexadecenoate are present [but (*Z*)-11-hexadecenoate is absent]. In *Argyrotaenia velutinana* (Tortricidae), in which the principal sex pheromone components are (*Z*)- and (*E*)-11-tetradecenyl acetates, we found earlier that hexadecanoate is chain-shortened to tetradecanoate and that this is desaturated to produce (*Z*)- and (*E*)-11-tetradecenoates (*16*). The (*Z*)- and (*E*)-9-dodecenyl moieties are also common as sex pheromone components in many other species in the Tortricidae (*1*), and these may arise as chain-shortened products of (*Z*)- and (*E*)-11-tetradecenoates. The small proportion of (*Z*)-13-octadecenoate found in the gland of *T. ni* may represent a chain-elongated product of (*Z*)-11-hexadecenoate. Combinations of (*Z*)-11-hexadecenyl and (*Z*)-13-octadecenyl moieties are common in sex pheromone blends of many species in the Pyralidae (*1*). The sex pheromone compositions of all these families, together with our present results, indicate that chain-shortening or chain-elongation systems, in conjunction with delta-11 desaturases that react with 18-, 16-, or 14-carbon chain fatty acyl groups, may be involved in the biosynthesis of many of the known lepidopteran sex pheromone components.

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5. Glands were dissected from the ovipositors of females (3 to 4 days old) and extracted with 1 ml of a 2:1 mixture of chloroform and methanol [J. Folch, *J. Biol. Chem.* **226**, 497 (1957)]. All fatty acyl moieties were converted to methyl esters by acid methanolysis, and subsequent treatment with acetyl chloride converted fatty alcohols to acetates.
6. Capillary GLC was conducted with a 45-m Carbowax 20-m column used with splitless injection and programmed from 80° to 200°C at 10° per minute after an initial delay of 2 minutes. Packed GLC columns were 3 percent OV-101 (methyl silicone) on 100- to 120-mesh Gas-Chrom Q, 2-m glass column (inside diameter, 4 mm) and 10 percent XF-1150 (50 percent cyanoethyl, methyl silicone) on 100- to 120-mesh Chromosorb W-AW-DMCS, 2-m glass column (inside diameter, 2 mm).
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8. Precoated TLC plates (Whatman K5, 0.25 mm, 20 by 20 cm) were used to separate gland extracts. Plates were developed for 2 cm with a mixture of chloroform, methanol, and water [62:34:4 (by volume)] to separate ethanolamine phosphatides and choline phosphatides (neutral lipids moved with the solvent front), then developed for 7.5 cm with a mixture of Skelly B, diethyl ether, and acetic acid (80:20:2) to separate diacylglycerols, triacylglycerols, and acetates. These lipid classes were scraped from the plate, methanolyzed, and acetylated for GLC analysis.
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10. A 5-μl solution of 1 μCi of sodium [1-^{14}C]acetate (Amersham) in a 1:1 mixture of water and dimethyl sulfoxide was applied to the pheromone glands of five female *T. ni* (1 to 2 days old). The glands were held everted by clamping the abdomens with smooth-jawed microalligator clips. The droplets completely absorbed into the gland in about 1 hour, and the clips were removed. After 24 hours, the glands were extracted, and the extract was methanolyzed and treated with acetyl chloride (*5*). The reaction products were separated on XF-1150 at 140°C (*6*). Fractions were collected in 30-cm glass capillary tubes. Compounds were recovered by washing through each tube with scintillation fluid (0.5 percent PPO in toluene) into a scintillation vial. Overall incorporation of ^{14}C into sex pheromone components and fatty acyl moieties was 2.2 percent.
11. Methyl [16-^{3}H](*Z*)-11-hexadecenoate was prepared by adding a dimethyl sulfoxide solution of NaB^3H_4 (Amersham) to methyl [16-tosyl](*Z*)-11-hexadecenoate, and base hydrolysis produced [16-^{3}H](*Z*)-11-hexadecenoic acid. A 5-μl solution of 1 μCi of the acid in dimethyl sulfoxide was applied to the pheromone glands of 19 female *T. ni* (1 to 2 days old). Overall incorporation of ^{3}H into sex pheromone components and fatty acyl moieties [other than the starting material (*Z*)-11-hexadecenoate] was 2.0 percent.
12. Unsaturated compounds were dissolved in Skelly B and added to a solution of ozone in Skelly B in a dry ice–acetone bath. This (0.5 ml) was flushed with N_2 to dispel excess ozone and added to a solution of benzylhydroxylamine hydrochloride (0.5 mg) in pyridine (0.5 ml) in a 4-ml vial with a Teflon-lined lid. The tightly capped vial was heated at 100°C for 16 hours. The products were separated on OV-101 (*6*), programmed from 100° to 225°C at 6° per minute with a 1-minute delay.
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Suppressor T Cell Action Inhibits the Expression of an Excluded Immunoglobulin Gene

Abstract. *Cells of the murine plasmacytoid line MOPC-315 synthesize two distinct immunoglobulin light chains: a normal λ_{II} protein, which is incorporated into secretory and surface-bound immunoglobulin, and a truncated, nonfunctional λ_I protein found only in the cytoplasm. Idiotype-specific suppressor T lymphocytes selectively inhibit the expression of both λ_{II}- and λ_I-specific messenger RNA by MOPC-315 cells. This finding demonstrates that phenotypically excluded light chain genes can be subject to immunoregulatory control and suggests that the expression of divergent λ isotypes may be coordinately regulated in immunoglobulin-secreting cells.*

Immunoglobulin light chain proteins of the mouse occur in four isotypic varieties, designated κ, λ_I, λ_{II}, and λ_{III}, each characterized by a specific amino acid sequence of the constant (C)-region domain (*1*). Each of the four C-region isotypes is encoded by a corresponding C-region gene, present as a pair of alleles in the normal diploid genome. Individual lymphoid cells, however, selectively express only one of these eight available C genes as functional protein; the light chain secreted or displayed as surface-bound immunoglobulin by a given clone is of a single isotype and is exclusively derived from either the maternal or the paternal C-region allele. The remaining allelic and isotypic C genes are said to be excluded.

Molecular analysis of the structure and ontogeny of immunoglobulin genes has begun to clarify the mechanisms by which lymphoid cells select a single C-region gene for expression (*2*). The formation of an active light chain gene requires specific DNA rearrangements that occur at an early stage in commitment to the B lymphocyte lineage (*3*). These rearrangements bring together separate genetic elements that encode the variable and C-region domains and align these elements in a precise manner for transcription and subsequent translation. While rearrangements of this type provide the major source of sequence diversity among immunoglobulin proteins, they are not without genetic risk. Aberrant rearrangements are common (*4*) and can result either in deletion of the affected locus or in the formation of a structurally anomalous gene. Such aberrantly rearranged genes may be transcriptionally silent or may be capable of rudimentary expression, giving rise to inactive transcripts or to transcripts that specify a nonfunctional light chain. One currently held view (*5, 6*) is that the differentiating lymphocyte rearranges its