virus from human leukemic cells under unusual culture conditions should perhaps not seem so surprising, despite many previous failures (*2*). Our results raise the possibility that in some patients with leukemia a complete virus particle could be assembled with the potential for horizontal transmission. Against such a mode of transmission, if the acquisition of disease is used as a measurement, is a good deal of epidemiological evidence (*14, 21*). This suggests that if virus can enter from without, interaction with other factors is required to produce myelogenous leukemia in man.

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Polypeptide Composition of Fraction 1 Protein from Parasexual Hybrid Plants in the Genus Nicotiana

Abstract. *Analysis of the subunit polypeptide composition of Fraction 1 proteins gives information on the expression of both nuclear and chloroplast genomes; the large subunits of the protein are coded by chloroplast DNA, whereas the small subunits are coded by nuclear DNA. Fraction 1 protein isolated from the leaves of parasexual hybrid plants derived from the fusion of protoplasts of* Nicotiana glauca *and* N. langsdorffii *contains the small subunit polypeptides of both parent species and the large subunit polypeptides of only* N. glauca. *Fraction 1 protein isolated from the leaves of a hybrid plant obtained after the uptake of chloroplasts of* N. suaveolens *by protoplasts of white tissue of a variegating mutant of* N. tabacum *contains the large subunit polypeptides of both* N. suaveolens *and* N. tabacum, *as well as the small subunit polypeptides of both these species.*

By the fusion of protoplasts derived from mesophyll cells of leaves of *Nicotiana glauca* and *N. langsdorffii*, a parasexual hybrid plant was developed (*1*); it was self-fertile in contrast to conventional, reciprocal F_1 hybrids of *N. glauca* × *N. langsdorffii*, which are not self-fertile. Also, plants of a cytoplasmic hybrid character were derived after the incorporation of isolated chloroplasts of several different *Nicotiana* species into protoplasts obtained from the white tissue of a variegating mutant of *N. tabacum* (*2*).

The expression of both nuclear and chloroplast genomes in these parasexual hybrid plants may be examined by an analysis of the polypeptide composition of a single protein, the Fraction 1 protein. Fraction 1 protein (ribulose diphosphate carboxylase) consists of two kinds of subunits, which differ in size. The large subunit is coded by chloroplast DNA (*3*), whereas the small subunit is coded by nuclear DNA (*4*). By isoelectric focusing of Fraction 1 proteins of *Nicotiana* species in 8*M* urea, it has recently proved possible to resolve, in a single polyacrylamide gel, the large subunit into three polypeptides, and to resolve the small subunit into one or more polypeptides (*5*). The patterns obtained are reproducible and characteristic of each species and thus readily provide phenotypic markers for both chloroplast and nuclear DNA. The three large subunit polypeptides are always inherited and expressed together, with no separation of the inheritance of the individual polypeptides (*6*).

Plants of the parasexual hybrid of *N. glauca* and *N. langsdorffii* were grown from seed obtained from progeny of the original hybrid plant (*1*). The off-

spring all exhibited the characteristic tissue morphology of the parent hybrid plant. Figure 1 shows the polypeptide composition of the large and small subunits of Fraction 1 protein isolated from the leaves of the parasexual hybrid of *N. glauca* and *N. langsdorffii*, in comparison with the two parental species and a series of artificial mixtures of the parental proteins. The results show that the three large subunit polypeptides of the parasexual hybrid are similar to the three large subunit polypeptides of *N. glauca*. There is no indication of the presence of the three large subunit polypeptides of *N. langsdorffii* in the parasexual hybrid. The single small subunit polypeptide of *N. glauca* is present, together with the two polypeptides of *N. langsdorffii*. This indicates that chloroplast DNA coding for the large subunit of Fraction 1 protein of *N. langsdorffii* was not expressed, whereas nuclear genes for the small subunits of both species were expressed in this parasexual hybrid. The electrofocusing method could have detected *N. langsdorffii* large subunit polypeptides at a level of 10 percent of the total polypeptides. Since the progeny of only a single protoplast fusion was available, the production of new parasexual hybrids will be required to ascertain whether the expression of only one of the parental chloroplast genomes is a general phenomenon, and, if so, whether there is an equal chance that the chloroplasts will be of *N. glauca* or the *N. langsdorffii* type.

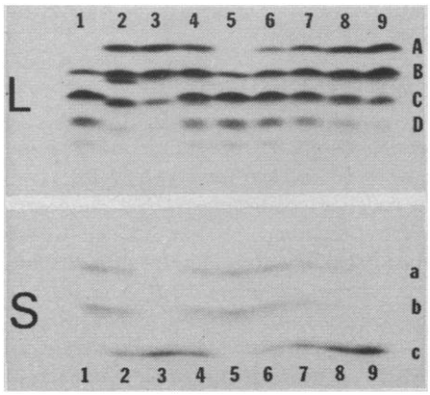

Fig. 1. Polypeptide composition of Fraction 1 protein isolated from the parasexual hybrid of *N. glauca* and *N. langsdorffii*. Polypeptides of carboxymethylated Fraction 1 proteins were separated by isoelectric focusing in 4.5 percent polyacrylamide gel slabs containing 1 percent Ampholine, *p*H 5 to 7, and 8*M* urea. Polypeptide bands were stained with bromophenol blue. Minor bands, below the three large subunit polypeptides, are artifacts arising by carbamylation of the protein by the cyanate present in urea solutions (*5*). All samples applied contained 20 μg of protein. Samples from left to right: No. 1, *N. langsdorffii*; No. 2, parasexual hybrid of *N. langsdorffii* and *N. glauca* (split in band B may be an artifact as it did not occur in repetitions); No. 3, *N. glauca*; No. 4, 1 : 1 mixture of *N. langsdorffii* and *N. glauca* proteins recrystallized as mixture before carboxymethylation; No. 5, *N. langsdorffii*; No. 6, a 3 : 1 mixture of carboxymethylated proteins of *N. langsdorffii* and *N. glauca*; No. 7, a 1 : 1 mixture of carboxymethylated proteins of *N. langsdorffii* and *N. glauca*; No. 8, a 1 : 3 mixture of carboxymethylated proteins of *N. langsdorffii* and *N. glauca*; No. 9, *N. glauca*. *L*, large subunit polypeptides, *p*H 6.5; *S*, small subunit polypeptides, *p*H 6.0.

A further hybrid plant was derived after the incorporation of chloroplasts of *N. suaveolens* into protoplasts from the white tissue of a maternally inherited variegating albino mutant of *N. tabacum* cv. Xanthi NC (*7*). Protoplasts were isolated from albino leaves in which all tissue layers lacked green chloroplasts and were cultured by the methods of Nagata and Takebe (*8*). Chloroplasts were isolated from green leaves of *N. suaveolens* (*9*) and were mixed immediately after preparation with protoplasts in a ratio of 10^4 : 1 in liquid regeneration medium containing 1 μg of poly-L-ornithine per milliliter (molecular weight, 120,000). The mixture was centrifuged at 50*g* for 1 hour, and the pellet was incubated undisturbed for 3 hours. After the 3-hour period, the pellet was resuspended, and the centrifugation and incubation processes were repeated. After the second incubation period the pellet was gently resuspended and plated in fresh regeneration medium. Green calluses were observed to arise from the regenerating protoplasts at an approximate rate of 2.0×10^{-4}, a rate greater than that observed for reversion, which was approximately 10^{-8}. Attempts to regenerate whole plants from the green isolates were uniformly unsuccessful, with the exception of a single individual. This individual is a variegating albino of abnormal morphology and is sterile. An average chromosome number of 64 (range 61 to 66) was determined by counting eight mitotic figures in the cells of young leaves (*10*). The somatic chromosome numbers of the *N. suaveolens* and *N. tabacum* used as the source of chloroplasts and protoplasts were 32 and 48, respectively.

Leaves of the hybrid plant were sent by air mail from New York to Los Angeles, and Fraction 1 protein was isolated by crystallization (*11*). Transporting the leaves of other *Nicotiana* species and hybrids by this method does not result in any changes in the patterns obtained on isoelectric focusing of the protein.

Figure 2 shows the polypeptide composition of Fraction 1 protein from the hybrid plant compared to the protein isolated from *N. suaveolens* and from the white tissue of the variegating *N. tabacum*. It is evident that the large subunit polypeptides of Fraction 1 protein of *N. suaveolens* are present together with the polypeptides characteristic of the protein from *N. tabacum*. This indicates that chloroplast DNA from both *N. suaveolens* and *N. tabacum* is present in hybrid plant. Examination of the composition of the small subunit of Fraction 1 protein from the hybrid plant reveals the presence of six polypeptides—four characteristic of the protein from *N. suaveolens* and two from *N. tabacum*. This indicates that nuclear DNA from *N. suaveolens* is present in the hybrid plant in addition to the nuclear DNA from *N. tabacum*. Chloro-

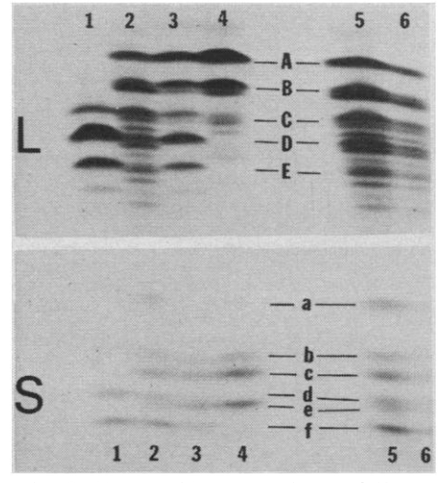

Fig. 2. Polypeptide composition of Fraction 1 protein isolated from a plant derived from *N. tabacum* protoplasts that had taken up *N. suaveolens* chloroplasts. Conditions are described in the legend to Fig. 1. Band splitting is due to the reaction of polyphenols with the protein during isolation (*12*). Samples from left to right: (1) 20 μg of protein from wild-type *N. tabacum* cv. Turkish Samsun (pattern identical to protein obtained from white tissue of the variegating mutant that was used as a source of protoplasts); (2) 20 μg of protein from plant derived from *N. tabacum* protoplasts and *N. suaveolens* chloroplasts; (3) 20 μg of protein of 1 : 1 mixture of proteins from *N. suaveolens* and *N. tabacum*; (4) 20 μg of protein from *N. suaveolens*; (5) repeat of (2) but with 30 μg of protein; (6) repeat of (2) but with 10 μg of protein.

plast preparations obtained by the method of Honda *et al.* (*9*) contain intact nuclei, and it seems probable that an isolated nucleus was taken up by the *N. tabacum* protoplast together with the chloroplasts. However, further analyses of individual plants derived from the uptake of chloroplasts into protoplasts will be required for assurance that the phenomenon can be reproduced. In any event, the results from both experiments confirm that in vitro technology can be utilized to alter the genetic makeup of higher plants.

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Sex Pheromone of the Oak Leaf Roller: A Complex Chemical Messenger System Identified by Mass Fragmentography

Abstract. *The sex pheromone of the oak leaf roller,* Archips semiferanus *Walker, is composed of a complex mixture of chemical signals. The attractant component of the pheromone contains a series of tetradecenyl acetates having double bonds in positions 2 to 12. Mass fragmentography of the ozonolysis products of the attractant component was used to locate the double bonds in the various isomers.*

Recent reports on insect phermones have revealed that few insect communication systems involve single chemical messengers. Moreover, sexual signals in some species consist of exact ratios of positional isomers (*1*) as well as geometrical isomers (*2*). The purity of some optical isomers, identified as pheromones, has also been viewed as important in certain insects, as shown by Riley *et al.* (*3*).

The sexual message in some species is composed of different chemicals which elicit separate behavioral responses from the insect. This segmentation of insect behavior in response to different chemical stimuli has been reported in the sex pheromones of Coleoptera (*4*) and in a host-searching kairomone in the Hymenoptera (*5*). In Lepidoptera, a sexual excitant and a sexual attractant have been distinctly isolated from the pheromone extracts of the oak leaf roller moth, *Archips semiferanus* Walker (Lepidoptera: Tortricidae) (*6*). We report here the identification of compounds found in the attractant portion of the oak leaf roller pheromone.

The oak leaf roller moth, a forest defoliator, has recently infested large portions of timberlands in the northeastern United States. In Pennsylvania, tree mortality due to this pest has climbed as high as 90 percent in more than 1 million acres of forest; approximately $70 million has been lost in oak stumpage values alone in this area (*7*). In the process of searching for a control method for the oak leaf roller, a sex pheromone was discovered in the adult female (*8*). Laboratory and field biological assays (*9*) revealed that two chromatographically isolable fractions of the pheromone extracts were involved in the female's sexual message, that is, an excitant and an attractant (*6*). (*Z*)-10-Tetradecenyl acetate was isolated, synthesized, and shown to serve as the major attractant signal (*10*); however, compounds having similar chromatographic properties were unidentified. These compounds represented generally a much smaller portion of the active attractant fraction, and consequently presented a formidable analytical task to identify them.

The analysis of these additional components was conducted as follows. Fifty virgin female oak leaf rollers were anesthetized with carbon dioxide; the last two abdominal segments were excised and ground in a tissue grinder with methylene chloride. The resulting suspension was filtered, and the solvent was evaporated under a stream of nitrogen. The extract was subjected to thin-layer chromatography on silica gel (Brinkmann Silplate F-22) and was subsequently purified by gas chromatography (GC) on nonpolar (SE30) and polar (DEGS) columns, as previously described (*6*). The active portion (*11*) that was known to attract males upwind in our laboratory flight chamber and to trap males in field tests was collected, and half of the product ($\sim$ 50 ng) was subjected to computerized gas chromatography–mass spectrometry (GC-MS) (*12*). A full mass spectrum was recorded every second. The result was an unresolved band of peaks which had identical qualitative mass spectra. Key ions included *m/e* (mass to charge) 254 (M^+, the parent peak), 194 (M^+–HOOC–CH_3), 166, and 61 (H_2OOC–CH_3^+). The spectra were indicative of a mixture of 14-carbon monounsaturated acetates. (No interfering compounds of higher or lower molecular weight were detected by computer search.)

To locate the double bond positions in the acetates, the remaining GC product collected was subjected to microozonolysis (*13*) in highly purified carbon disulfide and analyzed by GC-MS. The ozonolysis products, which were aliphatic aldehydes, were below the limit of detection by mass spectroscopy (< 1 ng). To detect the aldehyde products, the GC-MS was programmed to scan only for a few appropriate ions which were intense in standard aldehyde spectra (*14*). This novel technique, mass fragmentography (also called multiple ion detection), has been utilized in many areas of chemistry (*15*), but this is the first time, to our knowledge, that it has been applied to an insect pheromone study. Since mass fragmentography requires that the GC-MS scan for a few ions rather than over an entire mass range, the sensitivity increases dramat-