4. B. Falck, N.-Å. Hillarp, G. Thieme, A. Torp, *J. Histochem. Cytochem.* **10**, 348 (1962); B. Falck, *Acta Physiol. Scand.* **56**, Suppl. 197 (1962); A. Dahlström and K. Fuxe, *ibid.* **62**, Suppl. 232 (1964).
5. K. Fuxe and G. Sedvall, *Acta Physiol. Scand.*, in press; A. Dahlström and K. Fuxe, *Acta Oto-Laryngol.*, in press; K.-A. Norberg and B. Hamberger, *Acta Physiol. Scand.* **63**, Suppl. 238 (1964).
6. U. S. von Euler and F. Lishajko, *Acta Physiol. Scand.* **45**, 122 (1959); U. S. von Euler and F. Lishajko, *ibid.* **51**, 348 (1961).
7. Supported by PHS grant (NB-02854-04) from the National Institute of Neurological Diseases and Blindness and a grant from the Swedish Government Funds for Supporting Medical Research.

10 September 1964

Inheritance of Linoleic and Oleic Acids in Maize

Abstract. *Gas-liquid chromatographic analysis of methyl esters of fatty acids of individual maize seeds of parental and segregating populations suggests that desaturation at the Δ^{12-13} position in oleic acid is under simple Mendelian control. High linoleic acid content is recessive to low.*

Biosynthesis of unsaturated fatty acids in higher plants remains essentially unexplained. According to Erwin and Bloch (*1*) a mechanism distinctly different from two relatively well characterized biosynthetic pathways (anaerobic and oxidative desaturation) appears to exist in higher plants. In the "plant pathway," neither stearate nor palmitate serves as a precursor of oleic acid, but myristate and laurate are transformed to oleate (*2*). Bloch further suggested that oleate is progressively desaturated toward the methyl end of the molecule that is producing linoleic and linolenic acids.

The possibility that the orderly creation of additional double bonds in the oleic acid molecule might be under genetic control presents an attractive problem to a geneticist, particularly one working with maize. Accordingly, an effort was made in the winter of 1963–64 to analyze genetically the distribution of C_{18} fatty acids in the F_1, F_2, and backcross progenies of two strains of maize, R84 and Illinois High Oil (IHO). The two were selected primarily because of their contrasting distributions of fatty acid: R84 was high in linoleic acid and low in oleic acid, whereas IHO was lower in linoleic and higher in oleic.

Gas-liquid chromatographic analyses were made on individual kernels of parental, F_1, and segregating populations. Individual kernels were crushed and the oil was extracted with petroleum ether. The oil was then esterified (*3*). The esters were dissolved in approximately 1 ml of petroleum ether and 3 μl of the solution was injected in the chromatograph for analysis. Some early difficulty was encountered in making satisfactory determinations with so small an amount of oil, but refinement of the extraction methods provided sufficiently large oil samples to make the method successful.

Reliability of the chromatographic analyses was examined by repeated sampling of single kernels. No significant differences were found among repeated individual runs on the oil from individual seeds. The standard error associated with individual-kernel measurements was 1.32 percent for linoleic acid and 0.78 percent for oleic acid.

All analyses were made on an Aerograph Hi-Fi Model 600 gas chromatograph with a 1.5-m, 0.15-cm column packed with diethyleneglycol succinate on acid-washed chromosorb W. The column temperature was maintained at 180°C. Helium flow was adjusted to 18 ml/min. A flame ionization detector was used.

An examination of the frequency distributions in backcross and F_2 populations (Figs. 1 and 2) suggests monohybrid inheritance for oleic and linoleic acid content. The data support the hypothesis that the low linoleic acid content is dominant to high, and the low oleic acid content is recessive to high.

Table 1. Means and ranges of linoleic and oleic acid content (percentage of total oil) of individual maize kernels of parents, F_1, F_2, and backcross generations.

Linoleic (%)			Oleic (%)	
Range	Mean		Range	Mean
		R84 ⊗		
57.0–65.5	61.3		21.5–28.8	24.5
		IHO ⊗		
40.5–53.9	48.8		30.5–43.4	35.3
		F_1 (*R84* ♀)		
47.0–55.6	52.2		21.3–35.1	31.3
		F_1 (*IHO* ♀)		
42.0–52.1	47.6		31.3–42.6	36.1
		BC R84		
46.2–61.0	54.3		22.6–38.2	29.1
		BC IHO		
45.5–58.7	51.2		25.3–38.9	32.5
		F_2		
40.9–63.2	51.5		20.8–46.0	32.3

Chi-square analyses of pooled F_2 linoleic acid data indicate a poor fit to a 3:1 ratio. However, analyses of individual F_2 ears of the maize revealed that the ratio in only one ear out of six was quite deviate, and that the remaining five had chi-square probability values of 0.5–0.8. The backcross data were particularly convincing in that pooled data on the backcross of the F_1 to the high linoleic acid parent gave a bimodal distribution. Backcrosses of the F_1 to the low parent gave unimodal distributions.

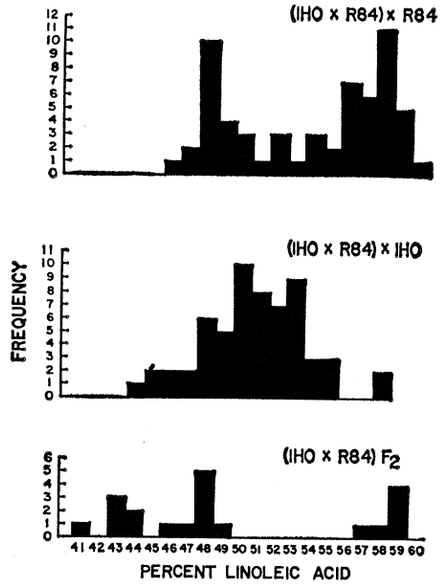

Fig. 1 (left). Frequency distributions of linoleic acid contents of individual maize kernels of backcross and F_2 generations.

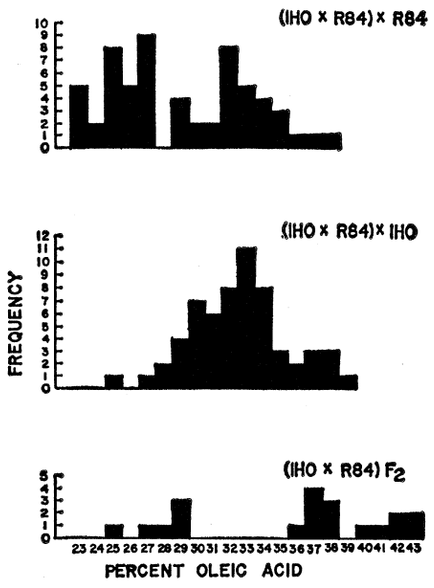

Fig. 2 (right). Frequency distributions of oleic acid contents of individual maize kernels of backcross and F_2 generations.

Oleic acid content, on the other hand, behaved genetically as a virtual reciprocal of linoleic acid content, high oleic acid content being dominant to low. In all analyses, however, the total amount of C_{18} fatty acids—primarily linoleic and oleic—did not vary appreciably (Table 1).

Earlier research on fatty acid composition of plants indicated that linoleic acid and oleic acid content were negatively correlated. Jellum (*4*) showed that a negative correlation of 0.97 existed in maize hybrids he analyzed. Since in individual kernels low linoleic content is always associated with high oleic acid, and vice versa, and the genetic models for control of amount of each acid are "mirror images," it would follow that the two are closely related in the unsaturated fatty-acid biosynthetic pathway.

It has already been suggested that oleic acid is the precursor of linoleic acid in higher plants. Presumably desaturation at the Δ^{9-10} position occurs early, possibly at the C_{12} or C_{14} stage, and subsequent additions of acetate result in the synthesis of oleic acid, although enzyme-bound palmitic and stearic acids may also be desaturated as well. The establishment of a second double bond at the 12–13 position would create linoleic acid.

Since (i) the amount of C_{18} fatty acids was not modified by either the dominant or recessive alleles associated with the linoleic-oleic relationships, and (ii) the amounts of linoleic and oleic acids were reciprocals as a consequence of genotype, and (iii) the genetic model for linoleic acid inheritance is an exact reciprocal for that of oleic acid, it is proposed that the desaturation of the 12–13 position in oleic acid is under simple Mendelian control in maize.

C. G. Poneleit
D. E. Alexander
Department of Agronomy,
University of Illinois, Urbana

References and Notes

1. J. Erwin and K. Bloch, *Science* **143**, 1006 (1964); P. K. Stumpf, *Nature* **194**, 1158 (1962).
2. A. T. James, *Biochim. Biophys. Acta* **70**, 9 (1963).
3. E. J. Gauglitz and L. W. Lehman, *J. Am. Oil Chem. Soc.* **40**, 197 (1963).
4. M. D. Jellum, personal communication.
5. The research described here was supported in part by a grant from the Corn Industries Research Foundation, Washington, D.C. Part of a thesis submitted by C.G.P. in partial fulfilment of the requirements for the M.S. degree, University of Illinois.

4 February 1965

Disappearance of a Genetic Marker from a Cytoplasmic Hybrid Plasmodium of a True Slime Mold

Abstract. *Two strains of the true slime mold* Didymium nigripes *form distinctive plaques when their spores are plated clonally. Strain C6 gives rise to plaques surrounded by large plasmodia. Plaques of strain A20 at first contain only amoebas, the formation of plasmodia being delayed; and strain A20 is resistant to cycloheximide. Plasmodia of C6 and A20 were fused, and at intervals the fused plasmodia were cut into segments and each segment was permitted to fruit. The resulting spores were plated clonally and the plaques scored as C6-type or A20-type. When equal-sized C6 and A20 plasmodia were fused, the A20 characteristic of delayed plasmodium formation disappeared with time so that after 8 hours few A20 plaques could be recovered even from the A20 side of the fused plasmodium. When the ratio between sizes of C6 and A20 plasmodia to be fused was varied, similar results were obtained. The A20 characteristic did not disappear until fruiting.*

The plasmodial stage in the life cycle of the Eumycetozoan, *Didymium nigripes*, consists of a macroscopic fan-shaped network of rhythmically streaming multinucleate protoplasm. When placed on an agar substrate which has been spread with *Aerobacter aerogenes*, it feeds on the bacterial layer while growing and migrating across the agar. If deprived of a bacterial food supply, the plasmodium differentiates into spore-containing fruiting bodies within 48 hours (*1*). The resulting spores germinate, liberating myxamoebas. Each spore or myxamoeba is capable, if plated clonally, of giving rise to a plaque which at first contains amoebas, and later, plasmodia. In this report I describe the results of fusing plasmodia of strains which differ in their plaque morphology.

A strain, designated C6, was isolated by selection from the wild type, 101. Strain C6 forms plaques containing a single, entire plasmodium with a prominent secondary vein (Fig. 1*a*). A second strain, called A20, was originally isolated because of its resistance to 15 μg of the antibiotic cycloheximide per milliliter (*2*). A number of cycloheximide-resistant strains were isolated; most were permanently without plasmodia. The A20 strain was selected from one of these. It forms plaques which at first contain only myxamoebas (Fig. 1*a*); within a day or two small plasmodia appear. Strain A20 has retained its cycloheximide resistance. Large plasmodia of C6 and A20 cannot be distinguished from one another.

Culture techniques described by Kerr and Sussman (*1*) were used. Plasmodia were maintained on agar containing glucose, peptone, and yeast extract (*1*) poured into petri dishes (100 × 15 mm) in 25-ml portions so as to assure a constant thickness. After the agar had solidified, 0.3 ml of *A. aerogenes*, grown for 48 hours in broth containing glucose, peptone, and yeast extract, was spread evenly over the surface of each plate. The plates were incubated for at least 16 hours before use so as to permit the surface to dry and the bacteria to multiply. When a piece of plasmodium along with the underlying agar was inverted onto such a plate near one edge, the subcultured plasmodium grew and migrated over the surface. It reached the far side after 36 to 72 hours, depending on the size of the inoculum. For experimental purposes, plasmodia which had migrated more than half way across the plate but which had not reached the far side were used.

Blocks of agar, 1 cm^2 and bearing the plasmodia to be fused, were cut from the advancing plasmodial fronts with a flamed nickel cutter and were placed side-by-side against a cut edge of a previously prepared plate (Fig. 1*b*). The size of each plasmodium could be varied by using different numbers and sizes of blocks. After the plasmodia had crawled off the inoculum blocks they were watched under a stereoscopic microscope until they fused. The presence of a continuous stream of protoplasm between the plasmodia was used as the criterion of fusion. (If incompatible strains were examined, the plasmodia expanded laterally until they touched, but remained distinct even after migrating across the plate.)

The variation in the amount of plasmodium on a 1-cm^2 block was determined by plating such blocks bearing plasmodia on nonnutrient agar where