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.

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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

they fruited. No significant difference in the number of fruiting bodies or the number of spores formed was found between the two stocks.

The 1-cm² blocks bearing plasmodia of C6 and A20 were placed side-by-side so that the plasmodia fused (Fig. 1*b*). The fused plasmodia were allowed to grow together for periods varying from 15 minutes to 8 hours. At intervals a fused plasmodium was harvested by cutting it (along with the underlying agar) into left, center, and right segments and transferring each segment to nonnutrient agar for fruiting. The left and right segments were cut so that each was approximately 1 cm wide, the width of the original inoculum block; the center segment included the remainder of the fused plasmodium. The resulting spores were grown as clones, and the plaques scored as "A20-type" (those with delayed plasmodium formation) or "C6-type." The results obtained by plating spores from the segment of the fused plasmodia representing the A20 side of the inoculum are shown in Fig. 2. If the contents of the C6 and A20 plasmodia were to mix together without other events occurring, the percentage of "A20-type" plaques should drop to 50 percent and remain at that level. In fact, almost no "A20-type" plaques were recovered, even from the A20 side of the fused plasmodium, when the plasmodia remained fused for 6 hours or more before they were cut into segments. Only rarely was an "A20-type" plaque recovered from the C6 side of plasmodia; less than 10 percent of the plaques derived from the center segment of the fused plasmodia were ever of the "A20-type." Plasmodia of A20 were fused with a second strain isolated from the wild type; the results were comparable.

In another series of experiments the ratio between the amount of C6 and A20 plasmodia fused was varied. Four hours after fusion, a segment 1 cm wide was cut from the most lateral portion of the A20 side and permitted to fruit. The resulting spores gave rise to plaques as shown in Fig. 3. If no more than 50 percent of the fused plasmodium was A20, the results were similar to those of the previous experiment. If most of the fused plasmodium was A20, however, the percentage of spores which gave rise to A20 plaques was at least as great as what would be expected from simple mixing of the plasmodium. If fused plasmodia, which were initially 80 percent A20, were permitted to grow for longer than 8 hours, the percentage of A20-type spores recovered from the A20 side again began to drop. After 8 hours about 50 percent of the spores from the A20 side gave rise to A20-type plaques; after 20 hours 29 percent still gave rise to A20-type plaques.

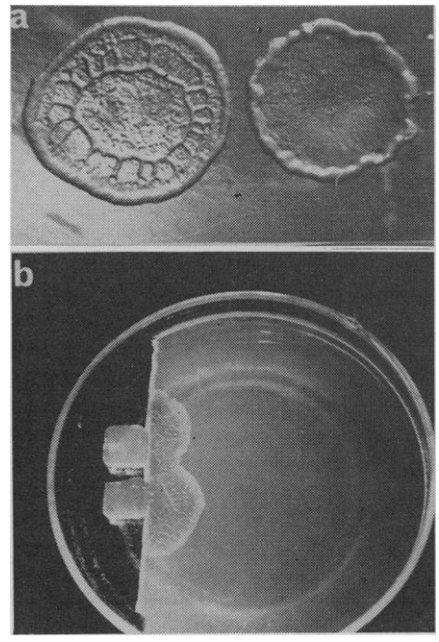

Fig. 1. (*a*) Plaques of C6 (left) and A20 (right) (× 5). (*b*) Fused plasmodium about 5 hours after inoculum blocks were put in place.

During these experiments, strain A20 was scored on the basis of delayed plasmodium formation. Resistance to cycloheximide, a second characteristic of the A20 strain, disappeared at the same rate as did the delayed plasmodium formation. Spores derived from the A20 side of a plasmodium formed by the fusion of C6 and A20 plasmodia which had remained fused for longer than 8 hours were plated at approximately 10^2 spores per plate of GPY/5 and at 10^2 and 10^4 spores per plate on GPY/5 which contained 15 μg of cycloheximide per milliliter. All of the approximately 100 plaques on the GPY/5 plate were of the C6 type. Fewer than 100 plaques appeared on the cycloheximide plate inoculated with 10^4 spores. Thus, resistance to cycloheximide disappeared in parallel with delayed plasmodium formation. The disappearance of the A20 marker continued even after the frequency of the marker was too low to be scored without selection.

The disappearance of the A20 marker when plasmodia bearing it were fused with C6 might be explained in a number of ways. Although it is generally stated that meiosis in the true slime molds occurs during the process of spore formation (*3*), so that spores and amoebas are haploid, there is disagreement as to whether karyogamy occurs at plasmodium formation or sometime after the plasmodia have formed. If the latter were true, the disappearance of the A20 marker might be the result of its being recessive to the C6. This was tested by isolating C6-type plaques resulting from spores formed by the A20 side of a fused A20 plus C6 plasmodium, permitting the plasmodia in these plaques to fruit, and plating the second generation of spores clonally. The procedure was repeated for a third generation. Since A20-type plaques did not reappear, this explanation was rejected.

It is possible that the two characteristics of strain A20, delayed plasmodium formation and cycloheximide resistance, are inherited as a nonchromosomal factor. If, for example, this factor were cytoplasmic, it might be permanently lost once mixed with normal cytoplasm. The disappearance of the A20 marker would then be directly related to the amount of mixing of the contents of the fused plasmodia. Plasmodia have been observed to stream at rates greater than 1 mm/sec. (*4*). To check the rate of mixing of *D. nigripes* plasmodia under the conditions of the preceding experiments, a drop of yeast stained with congo red was deposited just ahead of an advancing plasmodial front. The plasmodium readily ingested the yeast. Within 2 hours, numerous yeast cells could be seen in major veins toward the rear of the plasmodium and several centimeters lateral to the point of ingestion. A few yeast cells were also seen in the advancing plasmodial front more than a centimeter to each side of the point of ingestion. Thus, in less than 2 hours some mixing took place across a plasmodium of the size used. The disappearance of the A20 marker may reflect the degree of mixing of the contents of the two plasmodia.

The usual means of breaking a true slime mold plasmodium into uninucleate pieces is to induce sporulation. An alternate method is to break up the plasmodium mechanically. Portions of plasmodia from the A20 side of C6 plus A20 plasmodia which had been fused for more than 8 hours were suspended in *M*/100 phosphate buffer, *p*H 6.5, and mixed on a Vortex Jr.

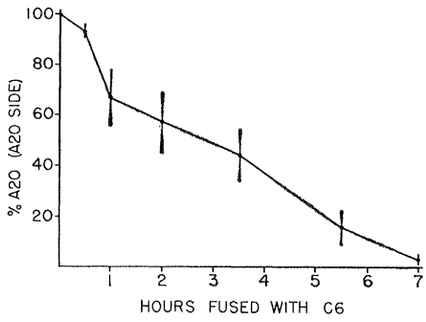

Fig. 2. The percentages of A20-type plaques recovered from the A20 side of a fused plasmodium when equal-sized plasmodia of C6 and A20 were fused. The vertical line equals 2 times the standard error.

mixer until no macroscopic pieces remained. Examination of the mixture with a phase-contrast microscope revealed that, although most of the plasmodia had simply lysed, some microplasmodia containing one or more nuclei remained. The mixtures were plated on agar containing glucose, peptone, and yeast extract with bacteria. Adjacent pieces of fused plasmodia were placed on nonnutrient agar, as usual, for fruiting. After 48 hours the plates bearing the microplasmodia were examined with a dissecting microscope and the smallest plasmodia present were cut out and subcultured to nonnutrient agar spread with bacteria. After these had fruited their spores were plated as usual and scored as C6-type or A20-type. About 20 percent of the microplasmodia yielded exclusively A20-type plaques, whereas less than 2 percent of the plaques from the controls were the A20-type. Microplasmodia isolated from C6 plasmodia in a similar manner gave rise to C6-type plaques only. The A20 characteristic therefore persists in the vegetative plasmodia after being mixed with C6 nuclei and cytoplasm. It disappears during fruiting. Microplasmodia isolated from C6 remained normal; no factor could be eliminated from them.

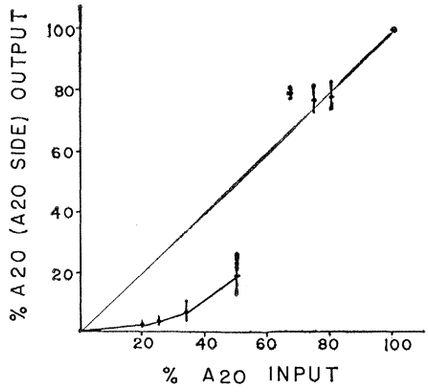

Fig. 3. The percentages of A20-type plaques recovered from the A20 side of a fused plasmodium when the plasmodia remained fused for 4 hours. Various ratios of C6 and A20 plasmodia were fused. The results expected on the basis of a simple mixing are represented by the fine line.

The disappearance of the characteristic of delayed plasmodium formation might be due to nuclear selection. Since the characteristic can be recovered from microplasmodia, the selection cannot take place in the vegetative plasmodium. Furthermore, when C6 and A20 plasmodia were fused on nonnutrient agar, where little growth would occur, and were left together until the fused plasmodium fruited, few A20 plaques were recovered. It is possible, however, that nuclear selection might occur during the formation of fruiting bodies. Cadman (*5*) reported that many nuclei in the developing fruit became pycnotic and did not undergo meiosis or become incorporated into spores. The possibility that C6 nuclei might be preferentially incorporated into spores was tested by means of radioactive tracers. Plasmodia were labeled by growing them on GPY/5 plates spread with bacteria, 250 μc of H^3-thymidine having been added to the plates as a narrow band near the inoculum. Twenty-four hours after the plasmodia had crossed the labeled band, they were fused with unlabeled plasmodia. Twelve hours later the fused plasmodia were transferred to nonnutrient agar for fruiting. Amoebas that germinated from the resulting spores were fixed with buffered osmium, air-dried on clean slides, and washed extensively with distilled water. Autoradiography was done according to the technique described by Prescott (*6*) with Kodak NTB-2 liquid emulsion. After 4 weeks of exposure, amoebas were examined by phase-contrast microscopy and scored as labeled or unlabeled. Comparisons were made between the percentages of labeled amoebas recovered from fused labeled A20 plus unlabeled A20 plasmodia and labeled A20 plus unlabeled C6 plasmodia, and from fused labeled C6 plus unlabeled C6 plasmodia and labeled C6 plus unlabeled A20 plasmodia. No significant differences were found. In every case 40 to 60 percent of the amoebas were labeled. Silver grains were seen above pieces of capillitium, indicating that there had been breakdown of nuclear material. The results obtained by autoradiography suggest that the phenomenon of the disappearance of the two characteristics, delayed plasmodium formation and resistance to cycloheximides, cannot be explained by nuclear selection.

The observed phenomenon could be explained either by assuming that some nonchromosomal factor responsible for the two characteristics of strain A20 is selected against during the fruiting process, or by assuming that such a factor derived from the C6 parent is somehow incorporated into the A20 nuclei during the fruiting process.

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Erythropoiesis in the Chick Embryo: The Role of Endoderm

Abstract. *The endoderm can be separated from the ectomesoderm of the area vasculosa of the early chick embryo. The development of the separated germ layers in organ cultures shows that the endoderm is necessary for the normal formation of blood islands by mesodermal cells. In the absence of endoderm the mesodermal cells undergo erythropoiesis in dispersed groups of cells but do not form endothelium.*

An early developmental event leading to the initiation of erythrocyte formation in the chick embryo is the condensation of newly formed mesoderm of the area vasculosa, which results in the appearance of blood islands. Thus, at the head process stage, blood islands composed of a few thousand cells are observed in the splanchnic mesoderm (*1*). At about the seven-somite stage all the central cells of the blood islands begin to make hemoglobin and produce erythrocytes (*2*); the peripheral cells of the blood islands flatten and form the endothelium of the extraembryonic blood vessels (*3*). Organogenesis elsewhere in the embryo often involves similar compact groups of