Chromosomes, Mutations, and Cytoplasm in Maize

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IT seems appropriate here (*1*) to quote the statement made by the late R. A. Emerson at the Sixth International Congress of Genetics in 1932. He said,

> I cannot refrain from noting here a very real advantage experienced by students of maize genetics, which is in no way related to the peculiar characteristics of the maize plant. I am aware of no other group of investigators who have so freely shared with each other not only their materials but even their unpublished data. The present status of maize genetics, whatever of noteworthy significance it presents, is largely to be credited to this somewhat unique, unselfishly cooperative spirit of the considerable group of students of maize genetics.

The Maize Genetics Cooperation was Emerson's creation; its success reflected the high esteem in which he was held. Although maize students no longer are the only group that freely exchanges unpublished information and genetic strains, it was the outstanding success of the Maize Genetics Cooperation that led others to start similar enterprises. It is true that this splendid effort did much to advance the progress of maize genetics, and the spirit that motivated it still prevails today.

Genetic investigations with maize fall into two eras. The first era, which may be said to have ended about 1925, was one in which purely genetic studies dominated; the cytological work was largely concerned with determining the correct chromosome number. Students of maize devoted their efforts to the establishment of a genetic framework upon which the cytogenetical studies of the second era were to be built.

A good deal of experimental work was done with maize prior to the rediscovery of Mendel's laws in 1900. Xenia was described, the vigor of F_1 hybrids of variety crosses was noted, segregation of contrasting characters was reported, and so forth, but no far-reaching conclusions were drawn. Maize genetics truly began in 1900 when De Vries reported the first case of Mendelian inheritance in maize, which involved the sugary locus. This was followed in the next year by Correns' description of the heredity of several endosperm characters, including that of sugary. In the succeeding years, American investigators, notably Collins, Kempton, East, Shull, Emerson, and Hayes, studied the transmission of an ever-increasing number of alternative characteristics, at first chiefly of the more obvious color differences. Emphasis was then rightfully placed upon ascertaining whether heredity in maize conformed to Mendelian principles.

As more and more characters were studied, it was inevitable that linkage would be found. Actually, the first linkage in maize was reported by E. Lewis Sturtevant in 1884 when he described the associated inheritance of the genes for sugary endosperm (*su*) and pod corn (*Tu*), but the true nature of the phenomenon was not recognized. Correns, in 1902, reported aberrant ratios of the starchy-sugary alleles in the F_2 of a cross between a pop and a sugary strain. He mistakenly attributed the deficiency of sugary kernels to selective fertilization. It was later suggested by Emerson that the aberrant ratios were caused by some factor linked with the sugary locus, and in 1926 Mangelsdorf and Jones designated this factor *Ga*. The first unquestionable and clearly recognized linkage in maize was reported in 1911 by Collins and Kempton, who found that the gene for waxy endosperm was linked in its inheritance with a gene for aleurone color, which was shown to be the *C* factor by Bregger in 1918.

One of the most important papers, prior to the advent of cytogenetics, was Emerson's classical analysis of the genetics of plant color in 1921. This paper, possibly more than any other, placed maize genetics on a firm foundation. Other important researches in the second and third decades of this century were the analysis of the complementary interaction of genes in the production of aleurone color by East, Hayes, and Emerson, the demonstration of allelic series at the A_1, *R*, and *P* loci by Emerson and Anderson, the cytological studies by Randolph on the genetically inert supernumerary B chromosomes, the genetic studies of intergeneric *Zea-Euchlaena* hybrids, and the demonstration that pericarp variegation was due to a mutable gene, which was the first proof of such a genetic basis for variegation. Studies of sex expression, of variability in crossing over, of factor interaction, of quantitative inheritance, and of duplicate factor inheritance with its implication of an ancient amphidiploid origin for maize were all fundamental and significant investigations.

There is in maize an astonishing array of gene-controlled characteristics. Mutations have been found that affect every conceivable part of the plant. Nearly 500 mutant genes are listed in the recent compilation by Weijer, which is by no means complete, since many induced and spontaneous mutations have not been cataloged. Since 1902 the linkage relationships of

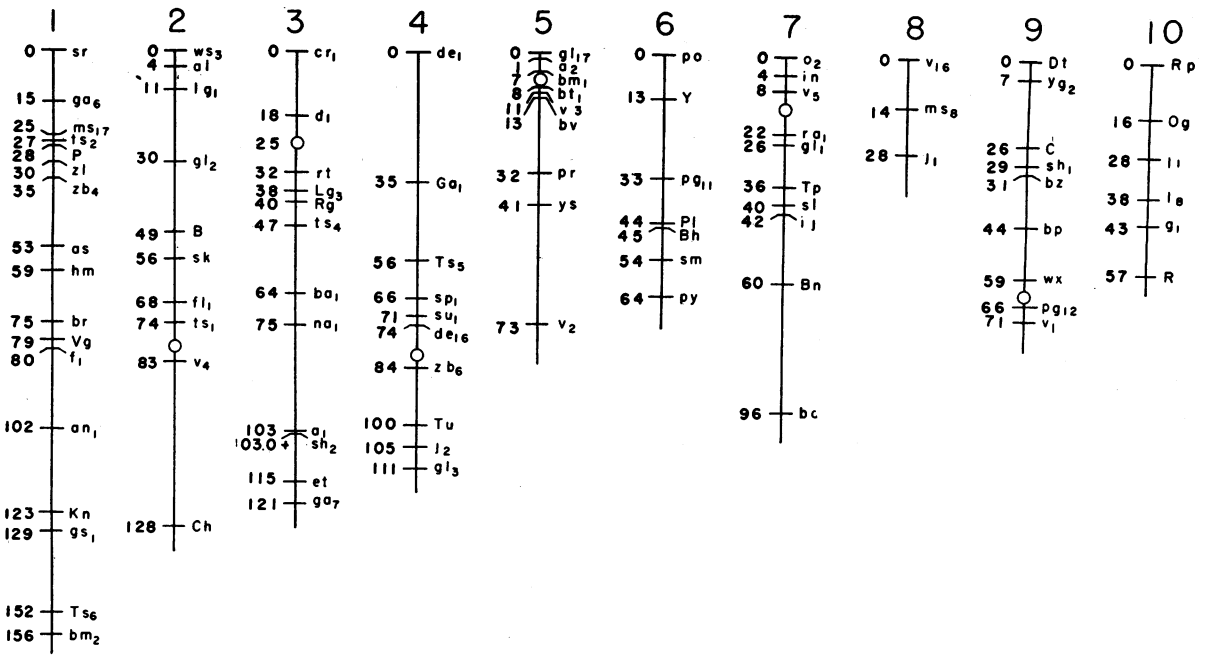

Fig. 1. Linkage maps of maize. The genes in linkage group 1 are carried by chromosome 1, and so forth.

many of these genes have been investigated. These studies culminated in the establishment of ten independent linkage groups, which are shown in Fig. 1. Once this has been done by purely genetic methods, the stage was set for the next great advance, which came with the cytological identification of the chromosomes of the monoploid complement.

In my opinion the second era, marked by the rise of cytogenetics, began with McClintock's study of triploid maize in 1929. The different primary trisomic types derived from this triploid made possible the first correlation of linkage groups with specific chromosomes. Although maize is now considered excellent cytological material, it was not always thus. Prior to the development of the acetocarmine smear technique by Belling in 1921, maize cytologists worked primarily with paraffin sections of root meristems. As can be seen from the polar view of a root tip metaphase (Fig. 2*A*), where little more than chromosome number can be ascertained, maize did not appear to be promising for cytological studies. I recall Emerson relating that Edward Murray East of Harvard, who began his distinguished career as a maize geneticist, decided to forsake corn for *Nicotiana* because maize was in his opinion wholly unsuited for cytological purposes. East's conclusion would be justifiable if nothing better than sections of root tips were available. However, in 1929 McClintock reported that the ten chromosomes could be recognized at the prophase of the first microspore division. The longest member of the haploid complement was designated as chromosome 1, the second longest as chromosome 2, and so forth. The

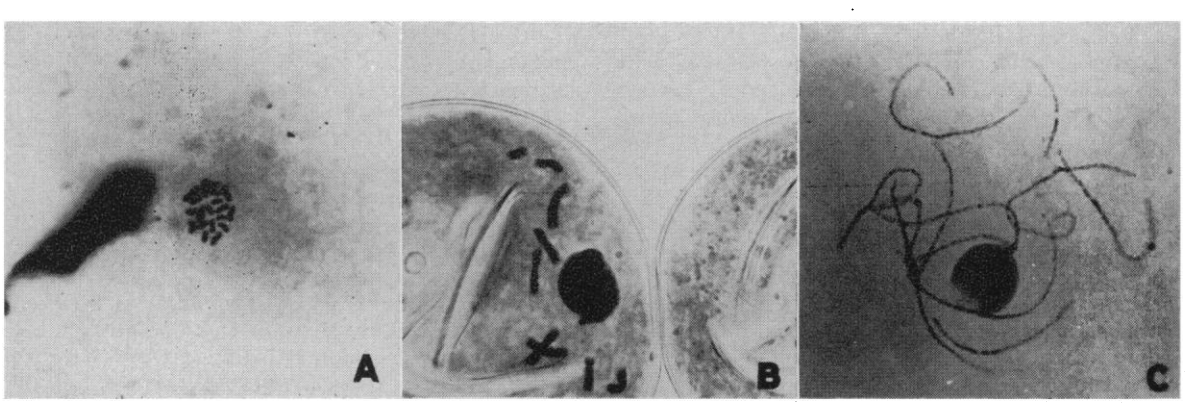

Fig. 2. Chromosome size and morphology in different tissues of the maize plant. (*A*) Polar view of somatic metaphase in root tip. (*B*) Prophase of first microspore division. (*C*) Mid-prophase of first meiotic division [from preparation by D. T. Morgan, Jr.].

microspore chromosomes shown in Fig. 2*B* are more favorable for cytological observations than are those of root tips; but it was from McClintock's later studies of the morphology of the pachytene chromosomes that the great beauty of the maize chromosomes became apparent (Fig. 2*C*). Here the ten chromosomes of the monoploid complement are morphologically distinguishable by (i) their relative lengths, (ii) distinctive chromomere patterns, (iii) deep-staining knobs in characteristic positions, (iv) locations of the centromere (thus determining arm ratios), and (v) the degree of heteropycnosis of the chromomeres adjacent to the centromeres.

The most striking feature of the pachytene chromosomes is the presence of large, deep-staining heteropycnotic knobs. The number of knobs varies greatly in different strains of corn. Knobs have been found in 22 different positions. Some strains have only knobless chromosomes, while in other races a maximum of 16 knobs has been found in a single plant. When a particular knob is present on a certain chromosome, it is a constant feature of the chromosome, and its inheritance is as precise as that of a mutant gene. Knobs are cytological markers in the same sense that mutant genes are genetic markers, and they have been used extensively in cytogenetic experiments. As is diagrammatically illustrated in Fig. 3, one or more knobs may be found on each of the ten chromosomes. Longley, in his extensive study of knob position, found that their locations along the lengths of the chromosomes were not random; there are no knobs in the proximal half of any chromosome arm. Knobs vary in size from those no bigger than prominent chromomeres to ones with a diameter several times that of the pachytene chromosomes. Most knobs are internally located; of the 22 different knob positions, only three are strictly terminal.

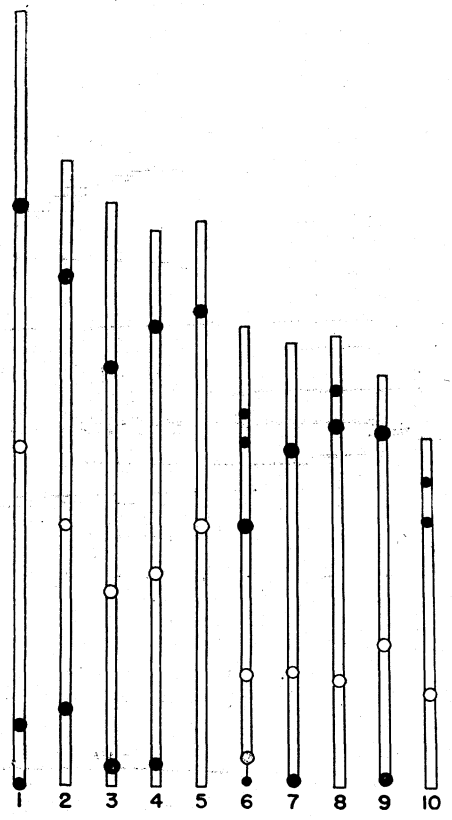

Fig. 3. Diagram of maize chromosomes showing relative lengths, centromeres, and knob positions.

Knobs consist of genetically inert, accessory material. No effect has been associated with them save their role in the formation of neocentromeres at both meiotic anaphases when the abnormal form of chromosome 10 is present (see below). It is improbable, though, that knobs are truly inert; it is more likely that our techniques are too insensitive to detect the part they play in cell metabolism. The largely heteropycnotic supernumerary B chromosomes are also considered to be inert or subinert, and it is significant that Longley found in different races a negative correlation between numbers of knobs and of B chromosomes; that is, strains with many knobbed chromosomes had fewer B chromosomes than did few-knobbed races.

In any consideration of the significance of knobs on maize chromosomes, mention must be made of the hypothesis of Mangelsdorf and Reeves, which holds in part that modern maize originated from an ancient cross between pure knobless maize and a *Tripsacum* species with many terminal knobs. If this is true, all the knobs of modern maize would be of *Tripsacum* origin. Although knobs are genetically inert and would in themselves have no phenotypic effect, it is assumed that segments of genically active chromatin accompanied the knobs when they were transposed to the pure maize chromosomes. It is these remnants of the *Tripsacum* genotype that are held responsible for the tripsacoid features of modern maize. It is, however, somewhat puzzling that we find today no indication of the ancestral rearrangements that were necessary for the transposition of the terminal *Tripsacum* knobs to the interstitial locations they now largely occupy in maize chromosomes.

Although races of maize that have been isolated from one another presumably for hundreds of years exhibit an amazing range in phenotypes owing to genic differences, there is little evidence that gross chromosomal rearrangements have accompanied gene mutations in the evolution of the corn plant. That maize has a stable karyotype is indicated by the failure of both Cooper and Brink and later of Rhoades and Dempsey to find structural changes in their studies of chromosomal homology in exotic races. The only known exception is that of chromosome 10, the shortest member of the complement. There are two kinds of chromosome 10 (Fig. 4). The less frequently found, abnormal kind of chromosome 10 differs from the normal in the chromomere structure of the distal one-sixth of the long arm but more strikingly by having a large, chiefly heteropycnotic segment of chromatin terminally located on the long arm. Inasmuch

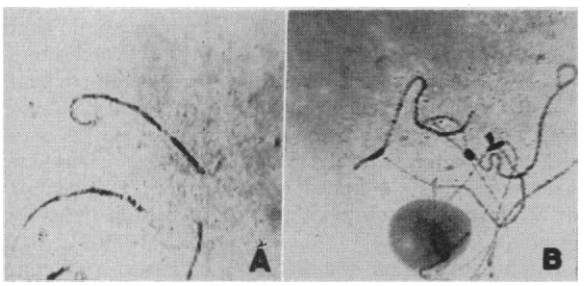

Fig. 4. The two types of chromosome 10 found in races of maize: (*A*) normal type; (*B*) abnormal chromosome 10.

as crossing over is reduced in the distal one-sixth of the long arm in plants heterozygous for abnormal 10, it is probable that this region with dissimilar chromomeres has some kind of structural modification. The lack of homology of the extra segment with regions of other chromosomes is indicated by its failure to synapse with them. Moreover, the exceptional nature of abnormal 10 is demonstrated by its being responsible for preferential segregation and for the formation of neocentromeres.

More surprising than the uniformity of chromosome structure in diverse races of maize is the situation found in hybrids between maize and annual teosinte. Emerson and Beadle found normal crossover values in hybrids between Durango, Chalco, and Florida strains of teosinte and maize for all tested chromosome segments except the short arm of chromosome 9 in the Florida-maize hybrid. The genetic evidence suggests that these species have few structural differences. In agreement with the genetic data is O'Mara's cytological analysis of pachytene pairing, which indicates that maize and Nojoya teosinte have no detectable cytological rearrangements, while Florida teosinte differs from maize by an inversion in the short arm of chromosome 9. This explains why no crossing over was found in this region in Florida-maize hybrids.

The cytological identification of the individual chromosomes and their correlation with specific linkage groups were the basis for a long series of important cytogenetical studies. Maize cytogenetics was in full flower in the 1930's. Among the many significant investigations were (i) the correlation of cytological and genetical crossing over, (ii) the demonstration of chromatid crossing over by both genetic and cytological methods, (iii) studies of synaptic forces responsible for nonhomologous pairing, (iv) the structure and function of the nucleolar-organizing body on chromosome 6, (v) trisomic and tetraploid inheritance, (vi) cytological and genetic studies of teosinte and *Tripsacum* and of their hybrids with maize, (vii) experimental induction of polyploidy, (viii) analysis of meiotic processes by study of derangements produced by genes such as those for asynapsis, sticky chromosomes, polymitosis, divergent spindle, and so forth, (ix) the relationship between crossing over and chromosome association, and (x) many beautifully executed studies on translocations, deficiencies, inversions, and ring-shaped chromosomes. I realize that it is unfair not to give credit here for the afore-mentioned investigations, but the list would be far too long. Suffice it to say that among those who played a prominent role in these early studies were McClintock above all, Burnham, Stadler, Randolph, Beadle, Longley, Mangelsdorf, Reeves, Brink, Creighton, Cooper, Anderson, and Clark.

It was a fortunate coincidence that Stadler was engaged in his experimental studies on the effects of x-rays in inducing mutations at the same time that McClintock was making her study of the pachytene chromosomes—fortunate for the cytogeneticist, because a great number of chromosomal aberrations were available at the very time mastery of the cytological technique was achieved, and fortunate for the study of induced mutation in that the examination of the pachytene chromosomes permitted a more precise determination of the nature of the induced changes. For example, deficiencies simulating gene mutation, which otherwise would have gone undetected, were now cytologically demonstrable.

Prior to the discovery of the mutagenic effects of short-wave radiations, mutation studies consisted chiefly of work on a highly mutable gene at the *P* locus producing variegated pericarp and of studies on the rate of spontaneous mutation of a few selected loci affecting endosperm characteristics. These loci are especially valuable for mutation studies. Each kernel, as far as the endosperm is concerned, represents an individual, and a single ear comprises a progeny of several hundred. It is possible with relatively little effort to obtain large numbers. Stadler found that the spontaneous frequency of mutation varied widely for the different genes. The *R* gene had a high rate, while the *Wx* gene failed to mutate in his experiments. It was demonstrated that spontaneous mutation rates of different alleles at the same locus vary widely and that it is impossible to characterize a locus as stable or unstable. All that can be done is to compare the mutability of specific alleles. Following the discovery of the mutagenic effects of irradiation with x-rays, the study of spontaneous mutation lagged somewhat while the spectacular effects of this new agent were investigated.

It was at first hoped that a study of x-ray induced mutations would lead ultimately to knowledge of the structure of the gene. However, after a long series of experiments, Stadler concluded that all x-ray induced changes in maize were extragenic in origin and that x-rays had failed to induce mutations similar to those arising spontaneously. Induced mutations having proved ineffective in the attack on the nature of the gene, attention was once more centered on spontaneous mutation. It was concluded that gene mutation could not be studied in experiments where the mutants occur at miscellaneous loci, because of the diverse nature of changes simulating gene mutation. Such extragenic changes may be produced by deficiencies, duplications, small rearrangements, and even by position effects that involve nothing more than a recombination

of genic elements by crossing over. The proper study of gene mutation is concerned with spontaneous changes at specific loci that have favorable characteristics. Two such loci are the A_1 and R loci in maize. In mutation studies, it is essential that the rate of spontaneous changes be sufficiently high to afford adequate numbers of mutations for analysis. The A_1 and R loci have received more than average attention, not only because they meet this requirement, but also because they have alleles with nonlinear effects.

Let us first consider mutation at the A_1 locus, which is concerned with aleurone, plant, and pericarp color. Of the considerable number of alleles at this locus, there are two that merit brief consideration here. The A^b allele derived from an Ecuadorean race is similar to the standard A allele typical of North American races in its aleurone and plant color effects but differs by producing a dominant brown pericarp color. Stadler found that the A^b gene mutated to a new allele, A-dilute, which has the dormant brown pericarp effect but whose pale aleurone and red-brown plant colors are recessive to those produced by standard A. Laughnan in 1949 demonstrated that the A^b locus was compound, its two component parts, alpha and beta, being separable by crossing over. He showed that, in 92 percent of the mutations of A^b to A-dilute, there was a separation of the alpha and beta components by crossing over. The A-dilute mutants have only the alpha component. The 8 percent of exceptions where mutation of A^b to A-dilute was not accompanied by detectable crossing over has been taken to indicate that mutation of A^b to A-dilute can occur without crossing over. Evidence for this conclusion is that mutation to A-dilute occurs in heterozygotes where A^b is opposite a deficiency. However, the recent demonstration by Schwartz of sister-strand crossing over gives some support to Laughnan's suggestion that some of these exceptional cases could be due to unequal sister-strand crossing over. It is unlikely, though, that mutation of A^b is invariably associated with crossing over. This is assuredly not so at the R locus.

The R locus is similar to the A_1 locus in that it affects both aleurone and plant color. Mutations occur in either the aleurone or plant color component, but they are independent events, since never, or rarely, does the same mutational event involve both. Moreover, mutations of one component have no influence on the expression of the nonmutated component. The two components also respond differently to modifying genes. All these facts suggest that the R gene is a compound locus—that is, composed of two independent but closely linked genes. However, the finding that the mutation rate of the aleurone color component was substantially reduced following plant color mutation indicated that aleurone and plant color effect were not wholly independent. The question of whether or not the R effect was produced by two closely linked genes or by one with dual effects was resolved by the recent report of Stadler and Nuffer that the two components were separable by crossing over. In heterozygotes for certain R alleles, crossing over between the two adjacent genes yielded recombination types identical to those produced by gene mutation. It is no doubt significant that both the A and R loci, which have alleles with nonlinear effects, are compound loci.

That mutation in maize is subject to genic control was demonstrated by the finding that the recessive a allele is made highly mutable by the Dt gene, although it is stable in the absence of this controller of mutability (Fig. 5). The Dt locus is in, or is adjacent to, the heteropycnotic knob terminating the short arm of chromosome 9, while the a locus is in chromosome 3. Chromosomal rearrangements are not associated with Dt-induced mutations of recessive a. Increasing the dosage of Dt causes an exponential increase in the number of mutations but does not affect the time at which they occur. Similar in certain respects to the a-Dt situation are the results obtained by McClintock in her remarkable analysis of mutable loci involving the Activator-Dissociation complex. Her results can be only briefly mentioned. Both Ac and Ds are believed to consist of heterochromatin. The nature or state of the Ds locus determines the kind of event that will happen at this locus, while the state of Ac controls both the time and frequency of these events. Two kinds of events occur at Ds loci if Ac is present in the nucleus. The chromosome breaks or becomes dissociated into two fragments at the Ds locus. Following breakage at the position occupied by Ds, fusion of broken ends of sister chromatids may produce a dicentric chromatid and an acentric fragment, or the Ds locus may simply drop out, thus producing a normal chromosome deficient for this locus. Which of these two events takes place is determined by the state of Ds, while Ac is the controller of mutability, since in its absence breaks do not occur at Ds. McClintock has found that when Ds was transposed next to a dominent allele—and transpositions of both Ds and Ac are frequent—the normal action of this allele is suppressed and it behaves as a recessive. However, with the loss of Ds by means of the breakage mechanism induced by Ac, the dominant allele recovers its normal activity. This restoration of normal function simulates a mutation from recessive to dominant, but nothing more is involved than the removal of inhibiting heterochromatin. Not only are McClintock's results of the greatest importance to the problem of mutation, but they also have wide im-

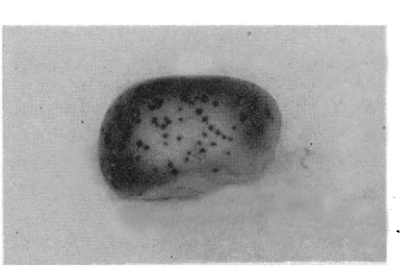

Fig. 5. Maize kernel homozygous for the recessive a gene which is made mutable by the Dt gene. Each black dot represents a mutation of a to a dominant A allele.

plications regarding the nature of gene action and the genetic control of differentiation.

Recently, Brink and Nilan, in a reinvestigation of varigated pericarp, reported data which suggest that mutation at the *P* locus involves a mechanism similar to that of *Ac-Ds,* and Peterson from his analysis of a mutable pale green locus concluded that an *Ac*-like factor was operating.

As was true in the study of genes, information on the nature of the cytoplasm and its particulate components can come only when heritable modifications occur whose characteristics can be compared with the normal condition. The chloroplast is a particulate cytoplasmic component that has a high degree of autonomy. Although morphological evidence for chloroplast continuity may be inconclusive, there is good evidence for genetic continuity from breeding experiments.

Plastid mutations not only occur spontaneously but can be induced by the action of specific nuclear genes, as has been demonstrated in two cases in maize. A second type of cytoplasmic change is that responsible for pollen abortion or male sterility. This kind of cytoplasmic mutation has occurred a number of times and is currently of great interest to the hybrid-seed producer, since its utilization promises to make unnecessary the costly and tedious task of detasseling. It is likely that the cytoplasmic condition responsible for male sterility arises from the mutation of a particulate cytoplasmic factor, but this has not yet been fully demonstrated. That interaction of genic and cytoplasmic factors is involved in the expression of the male sterile phenotype became apparent when it was found that certain races carried specific genes that were able to suppress the male sterility factors in the cytoplasm, while other strains had genes that were quite ineffective in this respect. Jones found that some lines had a single dominant gene that would restore pollen fertility. The dissimilar effect of specific fertility-restoring genes on different sources of sterile cytoplasm suggests that these cytoplasmic mutations are not all identical. A complex situation was encountered in the male sterile condition analyzed by Schwartz, where pollen abortion was produced only when a specific kind of cytoplasm was combined with a dominent gene for male sterility and with the recessive allele of a suppressor locus.

Although the amount of work on cytoplasmic inheritance has been negligible compared with that on genes and chromosomes, it may be confidently expected that significant advances will be forthcoming in the future.

Note

1. Presented in the symposium "Species that feed mankind," AAAS meeting Boston, 27 Dec. 1953.

Nuclear Function and Mitosis

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WHEN a cell goes into mitotic division, the state of the nucleus is altered in every visible respect from that which is observed in the cell when it is not dividing (interphase). In interphase, the nucleus is seen as a distinct body, encased in a membrane. The chromosomes exist in a diffuse condition and are seldom resolvable as distinct threads. Often one or more nucleoli are present. In the preparatory stages of mitosis, the nuclear membrane and the nucleoli disappear, and the chromosomes condense into compact bodies of high density. The relationship between these states of the nuclear apparatus and the functions of the nucleus is a question of some importance. The nucleus performs two classes of functions. The reproductive functions include the processes that lead to the production of a new set of chromosomes identical with the original set, guaranteeing that the daughter cells of a division will obtain a complete representation of the genetic material present in the mother cell. It now seems to be established with fair certainty that the syntheses involved in chromosome reproduction take place during interphase and not during the mitotic process itself (*1*).

The class of nuclear functions that may be termed "physiological" includes all nuclear activities that are necessary for the long-term survival of the cell when it is not reproducing. It is reasonably certain that the interphase nucleus performs such functions (*2*).

The question to be considered is whether the physiological activities of the nucleus depend on its being in interphase condition, or whether they can be carried out during division when the chromosomes are condensed and are not separated from the cytoplasm by a membrane.

An experimental attack on this question poses two requirements: (i) an adequate supply of interphase cells and dividing cells for comparison; (ii) a criterion for evaluating the physiological activity of the nucleus. Both of these requirements may be met by choosing the familiar ameba, *Amoeba proteus,* as experimental material.

It has recently been found by T. W. James (*3*), in this laboratory, that members of clones of *A. proteus* will divide quite synchronously if the temperature is cycled. In these experiments, a daily cycle of 12 hr at 18°C and 12 hr at 26°C was employed. At appropriate times (shortly after the beginning of the high-