

Further studies about heritable plastid variations produced by nuclear genes

This letter brings some data and an hypothesis based on molecular genetics that would confirm an already discovered phenomenon (Mazoti, L. B., Cienc. e Invest. 5:387, 1949; Caryologia 6 Suppl.: 1231, 1954). This phenomenon consists in the restoration or normalization of "deficient" extrachromosomal heritable units (proplastids that are incapable of developing and synthesizing chlorophyll) by the action of a simple gene.

The induction of "deficient" heritable variations in the plastids by gene action has been demonstrated in corn (Rhoades, M. M., Proc. Nat. Acad. Sci. Wash. 29:327, 1943; Mazoti, L. B., Rev. Arg. Agr. 12:174, 1945; Stroup, D., J. Hered. 61:139, 1970). Rhoades and Stroup consider that these variations are irreversible. However, the irreversible character of this variation is only true when the R gene is not present, because when these "deficient" proplastids are in the presence of the R-r or the R-g gene they will be normalized (Mazoti, 1954). There follows a summary of the experimental data gathered to the present that would confirm this phenomenon. The material used has been "tester" lines from Cornell University, introduced in Argentina in 1934 and maintained since that date by inbreeding.

1. The line of the ij/ij gl/gl r/r genotype produced in inbreeding 8,624 variegated plants and 408 white seedlings (4.7%).
2. In the cross ij/ij gl/gl r/r x +/+ +/+ r-r/r-r (or r-g/r-g) there were obtained 6,513 normal plants (all green) and 173 lethal white seedlings. This proves heritable plastid variation, because there were "deficient" plastids in the normal nuclear genotype: ij/+.
3. In the cross of ij/ij gl/gl r/r x +/+ +/+ R-r/R-r (or R-g/R-g), using as parents lines that carried R-r or R-g in different "backgrounds," there were obtained progenies that summed up to more than 15,000 individuals. All of them were normal (green chlorophyll), with the exception of chlorophyll variations caused by chromosomal abnormalities, detected by the gl gene used as a marker. The one per thousand of these chlorophyll variations was due to haploid seedlings (Mazoti, L. B., and C. E. Mühlenberg, Rev. Arg. Agr. 25:171, 1958).

In the segregations that were obtained from the cross ij/ij gl/gl r/r x +/+ +/+ R/r, 4,250 colored grains (r/r/R) did not give any white seedlings, and 4,509 individuals of colorless (r/r/r) aleurone gave 40 white seedlings, a result that confirms the specificity of the R gene in the reversion of the "deficient" proplastids.

These studies would confirm that the "deficient" plastids of the mother plant have reverted to normality in the following generation because of a structural change, induced by the R gene, or because of the complementary action produced by a metabolite synthesized by the R gene that would compensate for the plastid structural deficiency induced by the ij gene, with the plastids continuing to be structurally deficient (irreversible condition).

In order to determine which of the alternatives considered above is the valid one, we have to situate the "deficient" plastids in the genotypes in which we know that their deficiency will be shown. To this aim, we cross this F_1 of the gl/+ ij/+ r/R genotype with the +/+ +/+ r/r genotype, and we should expect that 4.7% of the progenies would consist of individuals with mutant plastids (structurally modified) or individuals with plastids structurally reverted to their normal state by the action of the R gene. Specifically 25% of the individuals of those progenies would be of +/ij r/r genotype, in which we know that the "deficient" plastids would be seen if there would not exist a plastid structural reversion to normality.

In order to apply tests of statistical significance, we have to compare the ratio of normal individuals vs. albinos, resulting from the cross ij/ij r/r x +/+ r/r, with the normal progenies vs. segregating progenies resulting from the cross (ij/ij r/r x +/+ R/R) x +/+ r/r (Table 1).

Table 1. Heritable variations of the plastids in relation to genotype.

Cross	Normal Seedling	White Seedling
<u>ij/ij r/r</u> x <u>+/+ r/r</u>	6,513	173
	Normal progenies	Segregating progenies
<u>(ij/ij r/r</u> x <u>+/+ R/R)</u> x <u>+/+ r/r</u>	417	0

$$\chi^2 = 11; P < 0.001$$

Applying to the above results a test of significance by means of a 2 x 2 contingency table we obtain that the heritable variation of the plastids induced by the ij gene appears to be reversible to normality by the action of the R-r or R-g gene, by means of a structural change of the plastids that would normalize them hereditarily.

The interpretation of this phenomenon of the heritable variations of the plastids (mutation and reversion) produced by genes can be the following one if we base our opinion in the knowledge of molecular genetics.

Mazoti (M.N.L. 40:62, 1966; 41:87, 1967; 49:66, 1975; Publ. No. 88 Inst. Fitotec., 1975.) formulated hypotheses based on free replication of DNA to interpret cases of inheritance in corn that deviate from the Mendelian mechanism. The same hypothesis based on free replication of DNA will be useful to us to elaborate our hypothesis on the action of the ij gene over the plastids. Thus, if the ij gene produces its replica, this replica would have the quality of hybridizing with the plastid DNA, in a specific segment; and it will produce heritable variations in the plastids if it acts as an episome. The "mutational" frequency of all the plastids of a cell will depend on the number of free DNA replicas and on the degree of affinity with the plastid DNA.

If we consider that the R gene would also produce its replica, this hypothesis would also interpret the reversion of plastid mutations by the R gene. This replica would be, at least in part, homologous with the ij segment in its nucleotide sequence, and it would produce a segmental trisomy of DNA: R/ij/plastid. Stability of this synaptic state in a small chromosomal segment is rather improbable, because the synapsis of homologous chromosomes in trivalents is partial; and when the pairing of two chromosomes is produced in one segment, the homologous segment of the third one tends to get free. By means of the mechanism mentioned before, plastid reversion to normality would be produced because of the ij replica becoming free from the association.

L. B. Mazoti

IOWA STATE UNIVERSITY
Department of Agronomy, Ames, Iowa

A dominant color allele, A-m(r), responsive to a specific En (Spm)

In the course of investigations of the Fcu controlling element system (Abstracts 1975 International Maize Symposium) two ears segregating an unexpected class of spotted kernels resulting from the cross of a-m(r)/a-dt sh2 plants by an a2 bt/a2 bt tester stock were found. The progeny included 75% solid colored and 25% spotted kernels, leading to the initial interpretation that perhaps a change in state had occurred in the components (r-cu or Fcu or both) responsible for the aleurone color variegation in kernels from the Cuna tribal maize from Colombia (MGNL 48:66-68). Test crosses of the spotted progeny by an r/r tester invalidated the initial