situated in the short arm. The ten chromosomes of Elyonurus tripsacoides from both the collections are found to be more similar to the first ten out of 18 chromosomes of Tripsacum dactyloides in their relative lengths than they are to those of maize.

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1. Mutational analysis of R^{ch}.

The R stocks and the treatments employed. One of the various expressions of the R phenotype, such as determined by R^{ch}, is the production of anthocyanin in the aleurone, silks, pericarp, and other plant tissues.

The experimental evidence so far obtained suggests that R^{ch} is a complex separable into four components, symbolized P, S, Si, and Ch, controlling pigment synthesis in the plant, seed, silk, and pericarp tissues respectively. This evidence was provided by analysis (Sastry, 1970) of a series of mutants with colorless endosperm that appeared on ears of testcrosses involving R^{ch}/R^{st} plants. Three independent collections of R^{ch} were employed in this study. Two of them presumably carried a heterochromatic knob (K10) situated distally on chromosome 10, because they segregated preferentially for R. The majority of these mutants had lost pigment both in aleurone and in the sporophytic tissues. Because of their heterozygous R^{ch}/R^{st} parentage, either one of their parents is accountable for their origin. However, since 21 out of 25 carried the distal marker K10 it is likely that they were R^{ch} mutants. A minority of mutants had lost the aleurone pigmenting capacity but retained some of the R^{ch} features thus suggesting that R^{ch} is a complex of genes.

To find further information on this small chromosomal region, we designed additional tests. If R^{ch} is separable into various components, it should be possible to induce deletions involving two or more genes of the complex, and from a study of their pattern of loss, information

should be obtained on the number of the R components and on their sequence.

Accordingly R^{ch}/R^{ch} plants were treated with the alkylating agent EMS, effective in inducing mutation and chromosome breakage (Malling et al., 1968), and the treated individuals were crossed reciprocally with a $g\ R^{st}$ / $g\ R^{st}$ tester. The stem of plants of about the same developmental stage was injected with a freshly prepared buffered solution of this agent (2.37 or 4.75×10^{-3}M).

Cytological analysis of the male inflorescence indicated that the majority of the germ cells reached the stage of microspores while a minority was still in meiosis at the time of the treatment.

The R^{ch} form used in this study originally was obtained from the collection of Stadler, while the $g\ R^{st}$ tester (without the stippled modifier M^{st}) came from Dr. Brink's laboratory. A series of spontaneous mutants from standard R^{r} were also isolated and analyzed in order to compare the mutational spectrum of the two R forms. To test whether the EMS solution injected into the stem is taken up by the plant tissues, M-2 seeds, obtained from crosses of treated $R^{ch}R^{ch}$ plants with the $g\ R^{st}$/ $g\ R^{st}$ tester, were germinated in sandbenches and studied, using appropriate controls.

A significant proportion of seedlings with abnormal growth was found only in the treated population (Table 1). These seedlings, when transferred to soil, died at the stage of 2-4 leaves. A similar phenotype associated with lethality is expected as a result of chromosomal deletions or other aberrations leading to an impairment in plant metabolism. These results suggest that EMS was incorporated into the nuclei of the germ line.

Results with R^{ch}. The exceptional kernels isolated as presumed mutants from the reciprocal crosses of R^{ch}/R^{ch} plants with the $g\ R^{st}$ / $g\ R^{st}$ tester have either colorless or pale aleurone (Table 2). Either one of the R parents could contribute to their production.

However, their origin from the R^{st} parent can be excluded since overlying both phenotypes is a fine stippling pattern determined by R^{st}. The EMS treatment did not alter their frequency significantly.

Table 1

Frequency of lethal seedlings with an abnormal morphology obtained from the reciprocal crosses of G R^{ch} / G R^{ch} plants with a g R^{st} / g R^{st} tester

R^{ch} contributed by	Treatment	No. seedlings scored	Abnormal seedlings	Fr x 10^{-3}
Pistillate parent	Nil(1)	4,000	0	0.00
"	EMS-1(2)	4,000	12	3.00
"	EMS-2(2)	4,000	30	7.50
Staminate parent	Nil(1)	4,000	0	0.00
"	EMS-1(2)	3,500	46	13.14
"	EMS-2(2)	4,000	40	10.00

(1) 2.37 x 10^{-3}M; (2) 4.75 x 10^{-3}M

Table 2

Number and phenotype of exceptional kernels observed on ears obtained by the reciprocal crosses of a GR^{ch} / GR^{ch} WxWx line with a g R^{st} / g R^{st} wx / wx tester

R^{ch} contributed by	Treatment	Number of kernels	No. and phenotype of exceptions	
			Light st.	Light st. on pale backgr.
Pistillate parent	Nil	6,000	6	1
"	EMS	22,310	22	3
Staminate parent	Nil	5,250	8	0
"	EMS	8,830	9	4
Total		42,390	45	8

Almost all (16/17) the exceptional kernels isolated from ears that derived $\underline{R}^{ch}$ from the pistillate parent were germinally transmissible while about one half (6/13) of those obtained from the reciprocal cross failed to repeat that altered phenotype in the offspring (Table 3). Upon selfing they segregated about 3/4 stippled and about 1/4 homozygous colorless or pale mutants. Since they all segregated for wx and g, we ruled out contamination with alien pollen as their possible origin. The mutant progeny has been grown for a further generation to analyze pigment distribution in the sporophytic tissues, namely in the roots, coleoptile, primary internodes, anthers, silks, and pericarp by removing the husks and exposing the ears to the sunlight from 5 to 15 days after pollination.

Table 3

Germinal transmission of the characters of the exceptional kernels

$\underline{R}^{ch}$ contributed by	Treatment	Exceptional kernels			Fr. x 10^{-3}
		Tested	Mutant	Non-mut	
	A. Colorless Aleurone				
Pistillate parent	Nil	3	3	0	1.0
" "	EMS	11	11	0	.98
Staminate parent	Nil	3	2	1	1.01
" "	EMS	6	3	3	.50
	B. Pale Aleurone				
Pistillate parent	Nil	1	1	0	.16
" "	EMS	2	1	1	.06
Staminate parent	Nil	0	0	0	.00
" "	EMS	4	2	2	.22

From each original mutant 5 to 10 plants were studied. Surprisingly, none of the mutants tested (23) produced visible amounts of pigment in the sporophytic tissues. The loss of aleurone pigmentation is consistently associated with loss of pigment in all the tissues of the

plant. These results are unexpected on the basis of observations of $\underline{R}^r$ mutants.

Results with $\underline{R}^r$. Homozygous $\underline{R}^r$ plants were pollinated with a $\underline{r}^g$ tester carrying wx as pollen marker. Out of 24,000 kernels produced, 24 were classified as colorless seed mutants and all the 18 progeny tested transmitted the trait germinally. All of them produced, however, pigment in the roots and in the anthers.

These results are consistent with the model first proposed by Stadler (1951), envisaging R as a complex of two genes, P and S, controlling pigment synthesis in the plant and seed tissues, respectively. The results of the mutation experiments with $\underline{R}^{ch}$ do not support such a simple model. Though the possibility of exploring the structure of the locus by means of small induced R deletions did not seem practical, as we first hoped, yet our study allows us to propose the following working hypotheses concerning the organization of the R region:

1. $\underline{R}^{ch}$ is a single gene. Its mutation leads to loss of pigment production in the whole plant. Accordingly, pale mutants could be either the result of the mutation of a closely linked color modifier or they are leaky mutants.
2. $\underline{R}^{ch}$ is a gene complex:
   a. Individuals with colorless or pale aleurone result from mutation of a regulatory component of the R complex that affects the expression of the other genes of the complex.
   b. The R complex carries duplicate segments, delimiting the R region, that favour pairing of the two homologues at meiosis. Being duplicate segments they can either pair equally or unequally (see fig. a and b).

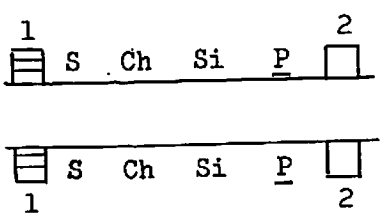

fig. a

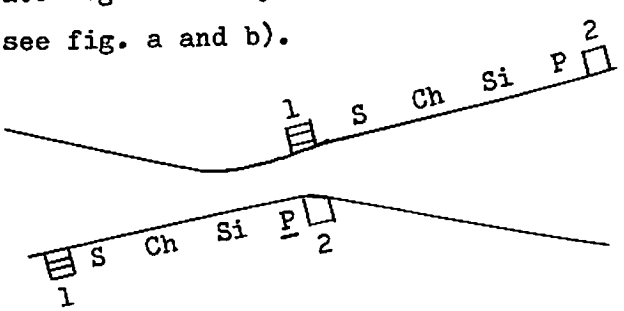

fig. b

In the first case, crossing over has no genetically detectable effect (fig. a); in the second case crossing over is limited to a region of effective pairing (fig. b). Any exchange occurring in this region gives rise to a crossover strand missing the entire R locus and to another carrying it in duplicate. Assumption (b) does not account for the pale mutants. Rather, their low frequency of production suggests that they are associated with a mutational event of a regulatory component (see 2a). Even though the data do not allow us to make a definite choice among these plausible assumptions, the working hypotheses proposed are easily amenable to experimental tests that should resolve the problems of the structural organization of R^{ch}.

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2. Effects of the "opaque-2" gene in maize: quantitative variations.

The quality of protein in the corn kernel can be genetically modified by the action of the gene opaque-2 (o_2) when homozygous. The main effect of this gene is an increase of lysine and tryptophan content (Mertz, Bates and Nelson, 1964).

In addition to this variation, some negative effects are correlated with the same genotype; the most important characters involved are kernel weight and kernel texture (Alexander et al. 1969; Salamini et al. 1969).

Some experimental results obtained during the last few years have shown that most of the effects of the o_2 gene can vary in intensity in relation to the genetical background. Therefore, it has been suggested that it should be possible to select genetical backgrounds in which the negative effects of o_2 would be minimized. Following the same reasoning, it should also be possible to select for high Tryptophan/Protein and Lysine/Protein ratios. The experiment described in this report was designed to evaluate all these possibilities, studying the genetical variability of those characters in a segregating population to which the selection could be applied.

An F_2 population segregating for o_2 was the material used for this research. The parent o_2/o_2 was an inbred line selected by Prof.