R pigment over a ten generation period with R^{st} shows a steady and measurable decrease with each generation--that is, the effects of R^{st} continue to be additive as measured through the phenotype of R pigmentation. It is interesting that the effects of R^{st} on R at this 2-dose level of R has continued to show a linear increase in numbers of kernels which show a mosaic pattern. After 10 generations with R^{st}, one can see in Table 6 that a mosaicism can be detected in over half of the kernels. No other effects of R^{st} have been observed on our inbred lines. It is likely that this reduction in pigmentation can be followed for at least ten more generations since considerable pigment still remains when two paramutated R alleles are present. From the existing data, it appears likely that R can eventually be converted to the completely colorless form and thus it seems possible that a dominant phenotype can be converted to the recessive.

Table 6

Number of generations that R has been heterozygous with R^{st}

	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}
Kernels scored (in thousands)	2.0	6.9	7.8	6.1	6.3	6.1	5.6	4.8	2.6
% mosaic kernels	1.2	10.1	7.7	17.4	23.1	30.1	32.7	42.7	50.4

Progressive conversion of R expression (paramutation by R^{st}) through ten generations. Scores represent the percentage of kernels showing endosperm mosaicism (pigmented and nonpigmented cells) when R is contributed to the endosperm tissue through the pistillate parent. Scored kernels represent the RRr genotype.

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4. High level repression of R expression in the presence of two R^6 alleles.

When R has been kept heterozygous for six generations with R^{st} (by self-pollination), one observes a progressive reduction in the number of aleurone cells with pigment (Vol. 38). When R^6R^6 segregates are self-pollinated so that endosperm cells are $R^6R^6R^6$, one can note some 20-25 kernels whose aleurone pigmentation is considerably reduced--near the colorless level. The presence of such kernels with highly repressed pigment furnishes a striking contrast to the otherwise heavily pigmented kernels on the same ear. If pigment is repressed in certain kernels, as such lightly pigmented kernels suggest, then it may be possible to place into cells of such kernels a "normal" R and detect repression in the resulting endosperm on a standard (unparamutated) R introduced through the pollen. The

genotypic constitution of such cells will be $\underline{R}^{6}\underline{R}^{6}\underline{R}$; with $\underline{R}$ introduced through the pollen, a dark kernel would be expected. At the same time it was also of interest to ask what degree of repression might be observed if the same standard $\underline{R}$ allele were introduced into aleurone cells against the background of highly paramutagenic alleles such as $\underline{R}^{st}$-- endosperm cells with $\underline{R}^{st}\underline{R}^{st}\underline{R}$.

The 25 lightest kernels were removed from each of six ears from the crosses $\underline{R}^{6}\underline{R}^{6}$ x $\underline{RR}$ and $\underline{R}^{st}$ x $\underline{RR}$. These selected kernels were scored by matching them against a set of standard kernels as described above. In Table 7 it can be seen that the weakest $\underline{R}$ pigment expressions for standard $\underline{R}$ are found on $\underline{R}^{6}\underline{R}^{6}$plants and not on the highly paramutagenic $\underline{R}^{st}\underline{R}^{st}$ plants where $\underline{R}$ pigment scores were uniformly dark. Table 7 confirms the results of the Wisconsin Laboratory which reported that no difference in $\underline{R}$ expression was found when standard $\underline{R}$ pollen was placed on plants which carried a highly paramutagenic colorless allele (compared to $\underline{R}$ on nonparamutagenic controls). It is highly interesting, therefore, that such a marked reduction in pigmentation can take place in the presence of two $\underline{R}^{6}$ alleles in the endosperm, especially when the paramutagenic alleles appear to be ineffective in this respect.

It would be premature to call this reduction of $\underline{R}$ pigmentation paramutation, if by paramutation we mean a transmissible change in the mosaic pattern of $\underline{R}$ pigmentation. There is no way of testing for transmissibility of the altered $\underline{R}$ expression in the endosperm. Whether this change represents paramutation or is simply a repression of the normal $\underline{R}$ in the presence of two highly paramutated $\underline{R}$ genes remains to be determined.

Table 7

Endosperm genotype $\underline{R^{st}R^{st}R}$	Endosperm genotype $\underline{R^{6}R^{6}R^{6}}$
19.96	17.00
19.92	17.32
20.16	17.00
20.04	15.96
19.52	15.48
20.12	16.60
	16.88
Pooled $\overline{X}$ 19.95	16.61

Comparison of R repression in the endosperm in the presence of two R^{st} and two R^{6} alleles. Each of the above figures represents ear mean scores based on 25-kernel samples. The lightest 25 kernels were selected from each of the ears for the above comparisons.

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5. Heritability of light and dark phenotypes from RR and Rr tassel mosaics.

In MGCNL 40 we reported that tassel mosaics could be found from RR and Rr combinations and that such mosaics parallel variations to be found for R from RR^{st} heterozygotes. Such differences in R expression when R is removed from the RR and Rr combinations may involve the same mechanisms responsible for differences in R from RR^{st} combinations. In Vol. 40 we reported that the differences in R expression from RR^{st} tassel sectors were heritable. Table 8 below shows that heritable differences, though very much smaller than from RR^{st}, can be found for R from RR and Rr backgrounds. Dark kernels, resulting from pollen collections made on the first day pollen was shed, were planted for comparison with the lightest kernels resulting from a pollen sample taken six days later from the same tassel. Scores of testcrossed plants from these light and dark seed selections show their means separated by two standard deviation units. Likewise, selected light and dark phenotypes from testcrosses from Rr plants show heritability. Seeds in this last case were provided by pollination from a single pollen sample from an Rr plant in 1965. Plants resulting from selected seeds were tested in 1966; the light and dark selections produced scores whose means were separated by two standard deviation units. In another test, in Table 8, it can be seen that where tassel samples were separated by six days in 1965 and where testcross scores were nearly alike from these pollen samples, again, the scored seeds produced plants whose testcrosses, in 1966, show no significant difference for pigment scores. This variation in R expression from the RR and Rr combinations must be considered to originate in somatic sectors arising during the course of tassel differentiation. These somatic sectors, in turn, result in pollen transmissible levels of mosaic expression (different states of R) visible in the aleurone layer of the endosperm.

Heritability of different states of R (light and dark pigment mosaics) from RR and Rr backgrounds. Seeds were selected from 1965 testcross ears reported in MGCNL 40. Selected Rr seeds were grown and resulting plants were testcrossed in 1966 for the scores given on the next page.