

9. Tests to determine whether paramutation is unidirectional or reciprocal in $R^r R^{st}$ heterozygotes.

The R^r allele conditioning self colored aleurone and plant pigment is regularly changed to a weakly pigmenting form in the progeny of heterozygotes possessing the R^{st} allele (Brink, 1956). Two tests were conducted to determine whether the stippled phenotype produced by R^{st} is regularly altered in the $R^r R^{st}$ heterozygote. The first test was made in the following way. A W22 $R^r R^{st}$ heterozygote was selfed. The $R^{st} R^{st}$ and $R^r R^{st}$ progeny from this self were testcrossed on W23 $r^g r^g \phi \phi$. The kernels of $R^{st} r^g r^g$ aleurone phenotype from the testcross ears were scored under a 30x binocular microscope fitted with a 20 x 20 reticule, covering an area of approximately 12 square millimeters. A predetermined area of the abgerminal side of the kernel was brought under the reticule and the number of spots delimited by one-half of the reticule were counted. The results were as follows:

Testcross	No. of kernels scored	Mean no. of spots per kernel
W23 $r^g r^g$ x $R^{st} R^{st}$	360	35.22
W23 $r^g r^g$ x $R^r R^{st}$	240	33.52

A test of significance gave $t = 0.56$, $P > 0.5$.

The second test was made in the following way. A W22 $R^{st} r^r$ heterozygote was selfed and used as the pollen parent on W22 $R^r R^r$. The $R^{st} R^{st}$ and $R^r R^{st}$ progeny were testcrossed on W23 $r^g r^g \phi \phi$. The kernels of $R^{st} r^g r^g$ aleurone phenotype from the testcross ears were scored with a microscope fitted as described above. A predetermined area of the crown was brought under the reticule, and the number of spots delimited by one-twentieth of the reticule area were counted. The results were as follows:

Testcross	No. of kernels scored	Mean no. of spots per kernel
W23 $r^g r^g$ x $R^{st} R^{st}$	240	6.08
W23 $r^g r^g$ x $R^r R^{st}$	240	6.37

A test of significance gave $t = 0.51$, $P > 0.5$.

These facts indicate that paramutation is unidirectional in the $R^r R^{st}$ heterozygote.

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10. The effect of dissociation (Ds) on the stability of the variegated pericarp allele, P^{VV} .

The addition of a transposed Modulator ($tr-Mp$) to a P^{VV}/P^{WR}

heterozygote greatly reduces the frequency of somatic mutations of the variegated pericarp allele (p_{vv}) to self-red (p_{rr}), and thus gives the light variegated phenotype. It has been reported (Genetics 41:901-906) that the addition of an Ac (Activator) element of McClintock's Ac-Ds system has a comparable effect on the stability of the variegated allele. It was of interest, therefore, to determine if Ds (Dissociation) also has an effect on the stability of p_{vv} .

A Ds element, in the standard position on chromosome 9, was introduced into inbred W22, and carried through three generations of backcrossing. Pollen from a plant homozygous for Ds was then placed on silks of p_{vv}/p_{vv} plants of inbred W22 background. The progeny from this cross were grown out, and separated into plants possessing Ds and those lacking Ds, on the basis of losses in the genome of an aleurone marker gene distal to the Ds locus. Seventy-five ears of each group were then scored for the number of somatic mutations of p_{vv} to p_{rr} with the aid of a set of model kernels having mutant areas (red) of graded sizes.

The data obtained are summarized in table 1.

Table 1. Distribution of the number of mutations of p_{vv} to p_{rr} per 1000 kernels in the two genotypes resulting from the cross of $p_{vv}/p_{vv} \times p_{vr}/p_{vr}$, Ds/-.

Genotypes	Estimated Total Kernels	Number Ears Scored	No. of mutations per 1000 kernels distributed according to the size of the mutant area					Total
			1/8-1/4	1/4-1/2	1/2-1	1 kernel	2 or more	
p_{vv}/p_{vv} , <u>Ds</u>	33,355	75	5.40	8.06	3.90	1.20	0.15	18.71
p_{vv}/p_{vv} , no- <u>Ds</u>	32,685	75	8.11	9.15	3.76	1.19	0.34	22.55

The reduction in the frequency of somatic mutations of p_{vv} to p_{rr} in the group possessing Ds as compared to the group lacking Ds is statistically significant at the 5 percent level. However, the reduction in the frequency of somatic mutations due to Ds is not at all comparable to that due to a tr-Mp or an Ac. Wood and Brink (Proc. Nat'l Acad. Sci. 42:514-519) found that the addition of a tr-Mp to a p_{vv}/p_{vr} heterozygote in inbred W23 background reduced the total number of mutations of a size of one-eighth kernel or larger by 69.56 percent and that the relative reduction was quite uniform for mutants in each size class. Comparison of the group totals given in table 1 shows that the total number of p_{vv} to p_{rr} mutations of all sizes exceeding one-eighth of a kernel is only 17.03 percent lower in the group containing Ds than that in the group lacking Ds. Also, the reduction is in the one-eighth to less than one-fourth, and one-fourth to less than one-half kernel classes.

Thus, the effect of Ds in reducing the grade of variegation in $\frac{p^{VV}}{p^{WF}}$ heterozygotes if, indeed, it is real, is much less than that of a tr-Mp or an Ac. Furthermore, the effect of Ds occurs later in ontogeny than does that of tr-Mp, since Ds caused no apparent reduction in the frequency of somatic mutations occurring sufficiently early to give rise to mutant sectors one-half kernel, or larger, in size.

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11. Diffuse.

Diffuse (Df) was previously described (Jour. Hered. 45:47 - 50; M. N. L. 32) as a dominant pattern gene that partially inhibits pericarp pigment produced by the p^{RR} allele. More recent findings indicate that this explanation is inadequate.

$\frac{p^{RR}}{p^{RR}}$, Df/df ears often exhibit colorless or near-colorless sectors of variable size in addition to a fine patchwork of colored, lightly colored, and colorless areas. For the initial tests of heritability of the colorless phase of Df, kernels were selected from both large (100 kernels or more) colorless and dark-diffuse areas on the same ear, and then grown separately so that levels of expression could be compared on a progeny basis. The seven colorless sectors tested all gave offspring indistinguishable from those derived from the dark-diffuse areas. In 1957 a family from a similar colorless sector gave colorless offspring, whereas the plants from the dark-diffuse kernels on the same ear yielded only dark-diffuse. This finding prompted further testing of colorless sectors for heritability of the irregularly expressed colorless phase.

An additional ten colorless sectors were tested in the same manner in 1958, but these sectors were much smaller (less than 30 kernels) than those previously tested. All families from these colorless sectors produced colorless and near-colorless offspring; the dark-diffuse areas gave only dark-diffuse ears. It seems that the plants obtained from kernels in the smaller sectors possess, or at least exhibit, the colorless phase of Df. In contrast, plants obtained from the kernels in the larger colorless sectors, do not show the colorless phase.

In the two cases in which the kernels from entirely colorless Diffuse ears, derived in the previous generation from colorless sectors, were progeny tested some of the resultant ears had the dark-diffuse phenotype, whereas others were very lightly pigmented, but none were colorless, as were the immediate parents.

Df has now been found to be a partial inhibitor of aleurone pigmentation also. When pollen from Df/df plants is placed on silks of A C R plants the resultant ears contain 5 - 10% smoky kernels, with the remainder self-colored. The smoky kernels when grown all prove to be Diffuse, whereas the self-colored kernels give rise to both Diffuse and non-Diffuse plants. The Df expression in the pericarp of plants grown from these