2. Enhancement of R expression in plants hemizygous for the R locus.

In the 1965 News Letter it was stated that paramutable R alleles are metastable, i.e., they have a capacity to vary heritably in plants not carrying an overtly paramutagenic allele. One evidence of such metastability is that alleles conditioning a mottled phenotype in single dose are enhanced in level of action toward self-color when maintained through successive generations heterozygous with a recessive r allele. R_2^g (a plant color mutant from standard R^r, comparable with standard R^r in paragenetic properties) has been maintained for three successive generations in stocks heterozygous with r^r, and also in otherwise comparable stocks hemizygous for the R locus. Enhancement has occurred in parallel fashion in both cases. The mating scheme consisted of an initial pollination of $R_2^g R_2^g$ on silks of $r^r r-x_1$ plants ($r-x_1$ = deficiency in chromosome 10 spanning the R locus), followed by recurrent pollinations of $R_2^g r^r$ and $R_2^g r-x_1$ sibs on $r^r r-x_1$ females. Mean single dose aleurone scores for the parental $R_2^g R_2^g$ stocks ranged from 5.38 to 5.72 (seven class scale; 1 = colorless, 7 = self-colored). Mean scores from two $R_2^g r^r$ lines after three generations of heterozygosity were 6.46 and 6.50. Mean scores from two comparable $R_2^g r-x_1$ lines after three generations of hemizygosity were 6.61 and 6.24. It appears, therefore, that enhancement may occur autonomously; i.e., it is not of necessity directed by the partner allele as with paramutation of R to R' in R R^{st} heterozygotes.

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3. Complete reversion of R'.

$R_2^g R^{st}$ plants from $R_2^g R_2^g$ X $R^{st} R^{st}$ matings were used as pollen parents in crosses with r^r $r-x_1$ plants ($r-x_1$ = deficiency in chromosome 10 spanning the R locus). R_2^g r^r and R_2^g $r-x_1$ sibs were then used to establish R' lines which were further subdivided at each generation by recurrently mating with $r^r r-x_1$ females and separating again into $R' r^r$ and $R' r-x_1$ sublines. A number of R' lineages were obtained in this manner, some of which were successively heterozygous ($R' r^r$), and some successively hemizygous ($R' r-x_1$). As there was no consistent difference in reversion pattern between heterozygous and hemizygous lineages, the separation into $R' r^r$ and $R' r-x_1$ classes at each generation served only as a basis for establishing new sublines. Change in R' aleurone expression was followed by testcrossing representatives of each lineage at each generation on W23 $r^g r^g$ females.

Testcross scores of homozygous $R_2^g R_2^g$ plants average ca. 5.50 (seven class scale; 1 = colorless, 7 = self-colored). The R' class on testcross ears from the three $R_2^g R^{st}$ plants used to start this experiment scored 2.10, 2.04, and 2.72.

Reversion toward the original $\underline{R}$ expression occurred at each generation and was consistent <u>on the average</u>, but the amount of reversion from one generation to the next within any one subline was irregular and unpredictable. The overall mean scores from all sublines in the first, second and third generations were 3.86, 4.28, and 4.72 respectively. Forty-eight sublines had been established by the third generation, and although expressions within sublines were relatively uniform, their mean scores ranged from 2.22 to 6.39. Thus in some lineages there had been essentially no reversion in three generations. In other lineages, however, reversion had progressed to the point that the reverted $\underline{R}'$ had a mean score higher than that of the parental $\underline{R^r_g R^r_g}$ stock. Further "reversion of $\underline{R}'$" in this case could be described equally well as "enhancement of $\underline{R}$". The phenomenon of $\underline{R}'$ reversion further reflects the innate metastability of paramutable alleles.

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4. **Paramutation of standard $\underline{R}^r$ in $\underline{a_1 a_1};\underline{R^r R^{st}}$ and $\underline{c_1 c_1};\underline{R^r R^{st}}$ plants.**

Evidence so far indicates that paramutation of standard $\underline{R}^r$ in $\underline{R^r R^{st}}$ heterozygotes occurs in somatic tissues, and that there is no direct correlation between the pigmenting action of paramutagenic alleles and their paramutagenicity. A small scale test has been conducted to determine whether the actions of other genes concerned with pigment production have any effect on the process of paramutation. Matings were of two types:

Mating	Class of interest	No. of Plants	Aleurone color score when testcrossed on W22 $\underline{ACr^g r^g}$ ♀♀
$\underline{A_1 a_1};\underline{R^{st} r^r}$	$\underline{A/-};\underline{R^r R^{st}}$	7	3.21
X $\underline{A_1 a_1 R^r r^g}$	$\underline{a\ a};\underline{R^r R^{st}}$	6	3.00
$\underline{C_1 c_1};\underline{R^{st} r^r}$	$\underline{C/-};\underline{R^r R^{st}}$	15	3.20
X $\underline{C_1 c_1};\underline{R^r r^g}$	$\underline{c\ c};\underline{R^r R^{st}}$	8	3.28

There is no indication that overt function of the $\underline{A_1}$ or the $\underline{C_1}$ gene is a requirement for paramutation of standard $\underline{R}^r$ in $\underline{R^r R^{st}}$ heterozygotes.

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