Table 8

Source of R	Ear $\bar{X}$	Stan. Dev.	Ear $\bar{X}$	Stan. Dev.	Pollen Collection Detail 1965
RR	20.73 n=9	.29	20.13 n=9	.35	Pollen from same tassel; collections separated by 6 days; darkest and lightest seeds selected from darkest and lightest ears, respectively.
Rr	21.35 n=6	.23	20.42 n=5	.41	Single pollen sample; darkest and lightest seeds selected from single ear.
Rr	20.82 n=6	.58	20.81 n=7	.37	Pollen samples separated by six days; randomly selected seeds from two testcross ears whose pigment scores were alike.

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6. Ranges of R expression (paramutation) from RR, Rr, RRst and R^{l}R^l plants.

Brink and his students have reported that different levels of R expression from RR^{st} heterozygotes can be attributed to the somatic sectors which occur during tassel formation. Our data in MGCNL 39 and 40 confirm the Wisconsin reports for tassel mosaics--our methods of sampling pollen from tassels differed from those used by the Wisconsin group. By sampling RR^{st} tassels daily during the time of pollen shed, usually a period of seven days, we have found that earliest pollen collections produce the lightest R^l expression; the darkest R^l expressions come from the last pollen samples from the tassels. It would appear, therefore, that paramutation can be defined as somatic mosaicism which is manifested as heritable changes in R expression. The level of R expression--the state of R--depends, in turn, on the position in the tassel from which the gamete carrying R emerges. It is hypothesized that different ranges of mosaicism result from different allelic combinations; evidence for this hypothesis is presented below.

Samples of pollen from single plants representing the different allelic combinations, RR, Rr, RR^{st} and RR, were taken over a period of seven days and applied to colorless inbreds. Such testcrosses were scored by the method of matching testcross kernels against a set of standard kernels, detailed in earlier reports above. Each test plant was represented, on the average, by four testcrosses (four pollen samples made on different

days over a seven day period) from which standard deviations for tassel samples were computed for each plant. Standard deviations for each of the four genotypes are averaged for comparison purposes. It can be seen in Table 9 that single plants of the $\underline{Rr}$ and $\underline{RR}$ combinations show only a third of the range of variation for $\underline{R}$ expression when compared to the standard deviations for $\underline{R}^l$ from $\underline{RR}^{st}$ combinations. It is appropriate to suggest, therefore, that the four allelic combinations represent variation from a continuum of possible states of $\underline{R}$; any particular state of $\underline{R}$ is dependent on its previous history of association with its alleles (as well as its position of origin in the somatic mosaic of the tassel, discussed below).

Such differences in $\underline{R}$ scores reflected in the standard deviations, suggest that somatic sectors from different positions in the tassels determine the particular state of $\underline{R}$. The Wisconsin Laboratory has not been able to demonstrate somatic sectors for either the $\underline{RR}$ or $\underline{Rr}$ combinations, though they have reported heritable differences in $\underline{R}$ expression from $\underline{RR}$ and $\underline{Rr}$ combinations. Our evidence for tassel mosaicism (Vol. 40) and its heritability (Table 8) together with evidence for a continuum of variation in $\underline{R}$ expression (Table 9) depending on allelic combinations, requires that we consider $\underline{RR}$, $\underline{Rr}$, $\underline{RR}^{st}$ and $\underline{R}^l\underline{R}^l$ as paramutagenic combinations.

Samples of pollen from a single plant, made over a seven day period, show a "polarity" which is reflected in an orderly change in $\underline{R}$ expression during development. We have reported, for instance, that pollen collections from tillers produce $\underline{R}$ expressions which score darker than those of the main tassel; this confirms a report of Brink and his students. From the $\underline{RR}^{st}$ combinations, we find almost invariably, over the past three years, that the earliest collections show $\underline{R}^l$ expressions to be lighter from the upper part of the tassel than pollen collections made lower (later) on the tassel. On the other hand, in the case of $\underline{R}^l\underline{R}^l$ segregates, from the $\underline{RR}^{st}$ sibs, we find the opposite "polarity"; the earliest pollen collections from $\underline{R}^l\underline{R}^l$ plants produce darkest testcrosses. Table 10 shows somatic sectors can result in opposed gradients with respect to the state of $\underline{R}^l$--from $\underline{RR}^{st}$, the earliest pollen yields the lightest testcrosses, later pollen samples become progressively darker; from $\underline{R}^l\underline{R}^l$ segregates of $\underline{RR}^{st}$ the darkest testcrosses came from the earliest pollen samples, while the later pollen samples produced progressively lighter kernels. It is quite possible that the environment may make some contribution to the differences in these tassel mosaics. In 1965 we reported for $\underline{RR}$ and $\underline{Rr}$ that the earliest pollen collections gave the darkest testcrosses and the latest collections showed the lightest testcrosses. In 1966 testcrosses of $\underline{RR}$ (Table 10) and $\underline{Rr}$, lightest testcrosses are found from among the first pollen samples, the reverse of the data of 1965.

Since pigment mosaics are characteristic of the $\underline{R}$ gene for all allelic combinations with $\underline{R}$, it may be well to give some consideration to terminology to be applied to the conditions affecting mosaics controlled by the $\underline{R}$ locus. At the present time these phenomena are being described by such terms as: reversion, mosaicism, induction, paramutation, enhancement, conversion, variegation, sectoring, states of $\underline{R}$,

mottling and still others. Operationally, the result observed, is either an increase or decrease in the numbers of cells in the aleurone layer with pigment. The simplicity of the observations may be obscured by the multiplicity of terms for the observations. One may also be left with the impression that the paramutation alleles are a class apart from "normal". As the data above suggest, this implication cannot be defended. The increase or decrease in numbers of cells--the state of $\underline{R}$-- is a function of the allelic combinations and the position of origin in the tassel of the gamete carrying $\underline{R}$. There are no allelic combinations (even the hemizygote) where mosaicism is absent in the testcrosses of the $\underline{R}$ allele, therefore paramutation (mosaicism) is universal for $\underline{R}$.

The genetic apparatus reflected by the mosaic expression of the $\underline{R}$ alleles above, represents a system of biological "memory", a "summing device", whose current phenotypic range of mosaicism represents an expressive summation of its genetic history. The expressivity of this system responds to both allelic and environmental regulation in its memory-storage capabilities. The availability of the many states of $\underline{R}$ and the ranges of expression which show heritability provide a mechanism of extreme genetic versatility of the individual plant. In so far as a single gene is concerned, the choices of $\underline{R}$ expression available to the next generation from a single plant or from a few plants would be very great; still further opportunity for increase in the range of expression for a particular $\underline{R}$ would take place with each generation. Should this system examined for $\underline{R}$ prove to be at all applicable to other genetic systems, the problem of accounting for sources of variation for evolutionary purposes would cease to exist and the behavior of the $\underline{R}$ locus will have made possible the opening of a Pandora's box the biological consequences of which stagger the imagination with all its implications.

Table 9

Standard deviations of testcrosses for pollen samples of single plants

$\underline{Rr}$	$\underline{RR}$	$\underline{R}^1\underline{R}^1$	$\underline{RR}^{st}$
.37	.33	1.01	.90
.23	.39	2.11	1.42
.35	.50	1.13	1.84
.33	.24	.67	1.32
.30	.55	.93	1.61
.60	.47	1.55	1.37
Pooled $\overline{X}$ .36	.41	1.23	1.41

Comparison of standard deviations for $\underline{R}$-expression from four different genotypic backgrounds. Each standard deviation figure above represents, on the average, four or more pollen samples (50 scored kernels/sample) from the same plant taken over a period of from four to seven days.

Table 10

	Day	#1	#2	#3	#4	#5	#6
				Plant Number			
rr x R¹R¹	1	19.00	13.82	18.26	19.94	19.88	20.18
	2						
	3	17.96	13.52	15.40	13.26	17.56	19.78
	4	17.20	11.82	14.78	18.36	18.02	19.26
	5	16.00	11.46	14.74	16.58		17.74
	6					16.82	
	7		11.62	12.38			
rr x RRst	1	11.76	9.92	11.28	13.96	13.82	15.56
	2	12.74		10.10	14.36	17.06	14.22
	3	14.84				15.42	17.42
	4		6.78		13.74		18.14
	5	14.00	11.60	14.22	15.16	14.28	17.18
	6			13.18	17.76	16.60	
	7	15.68	10.88		15.82		
rr x RR	1	20.70	20.74	20.70	20.34	20.44	19.94
	2			21.74	21.44		
	3		21.90	20.86			
	4	20.46	21.00	21.88	20.46	20.06	20.10
	5	20.96	21.00		21.22	20.26	20.64
	6	21.24	21.20			21.06	20.82
	7	21.36				21.10	20.98

Selected examples of tassel gradients for R expression from three different allelic backgrounds, R¹R¹, RRst and RR. Pollen samples were made over a seven day period and put on rr for scoring purposes.

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