PARAMUTATION AND SOMATIC MOSAICISM IN MAIZE¹

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PARAMUTATION at the *R* locus in maize is known to occur in vegetative cells (McWHIRTER and BRINK 1963). Evidence to be presented here shows that the course of *R* change is irregular as plant development proceeds, so that an individual tends to become a mosaic in terms of anthocyanin forming potential. No *R* allele has been found, however, the paramutation of which leads to an immediate and readily detectable alteration in shoot phenotype. The somatic variation, therefore, is concealed. Diversity in level of *R* potential within an individual is brought to light by sampling separately the pollen formed in different tassels of a plant or in the several branches of a single tassel. The seed phenotypes resulting from testcrosses on *rr* females of pollen so collected show that in heterozygotes of appropriate kinds readily detectable *R* sectoring is frequent.

Structure of the maize plant and the cryptic nature of the somatic mosaicism (or sectoring) for level of *R* potential restrict what can be deduced directly from the present data. The results gain in meaning, however, when considered in conjunction with corresponding evidence from certain other species and with the data bearing on other aspects of *R* paramutation.

Zonal boundaries would be phenotypically recognizable in a somatic mosaic system well suited to genetic analysis, and all genotypes present would be potentially verifiable by progeny test. These conditions are far from being met in the *R* maize material. Heritability tests can be conveniently made only of sectors that extend into the tassel, and then blindly, in terms of number and distribution of genotypes, because of the hidden zonal boundaries. Only by chance would a given pollen sample be homogeneous under these conditions. Should multiple changes in *R* potential occur in a tassel branch, pollen from even a short segment often would be drawn from more than one *R* zone. Rarely could the character of mixed plant tissue be inferred from the heterogeneous array of kernels resulting from testcrosses on *rr* females of pollen from tassel branches of this kind. Furthermore, restriction of tissue sampling to the tassel, at the apex of the plant, means that the time course of paramutation as development of the individual proceeds can not be measured.

The evidence for *R* mosaicism in maize, although restricted, is adequate to link the case to more patent examples of the phenomenon. A few instances of paramutation in other species have been reported earlier under designations such as "mass mutation" or "somatic conversion," in which mosaicism was both overt

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and genetically verifiable (refs. in BRINK 1960, 1964). When the data from these sources also are considered it becomes apparent that somatic mosaicism probably is a general characteristic of paramutation. Maize has proved especially productive of evidence on certain other aspects of paramutation, the understanding of which becomes clearer when the additional fact of variable genetic change during plant development is taken into account.

MATERIALS AND METHODS

The general procedures used in these studies and the precautions taken to minimize the effect of extraneous genetic variation in aleurone color have been described elsewhere (BRINK 1964).

Aleurone color grade was evaluated by visually matching each kernel in a sample (usually 50) from the middle portion of a testcross ear against a graded set of standard kernels ranging from colorless to fully colored. The mean color grade was then computed and used to represent the sample. Two unlike sets of standard kernels were employed at different times. One set consisted of six kernels, defining seven color classes. The other set represented 11 grades of pigmentation. The accuracy achieved in the respective means probably differed little. Variation in the character of the distributions on which the means were based was better reflected, however, when the finer classification was used.

The usual procedure in testing a plant for mosaicism was to collect pollen separately from a few lateral branches disposed spirally from the base upward in a tassel, and then to apply the pollen samples to the silks of inbred W22 $r^g r^g$ individuals in duplicate. A few testcrosses were made on W23 $r^g r^g$ females. Pollen also was collected in some cases from the central spike at the apex of the tassel. The notation used in identifying the several pollen samples taken from a single plant is exemplified by the expression (52-18)-10-2. The term (52-18) refers to the family concerned, based on a single seed-ear, 10 is the plant number, and 2 is the particular tassel branch in question. In each case, the lowest tassel branch sampled was numbered 1, the next highest was designated 2, and so on. The different pollen samples taken from a given plant were collected in close succession on the same day.

The W22 inbred strain, into which all the *R* alleles used were incorporated, is typically single stalked. Occasionally, however, a basal branch (tiller) is formed which also eventually produces pollen. In the latter case, pollen from the tassels surmounting the two stems may be readily sampled separately for evidence of sectoring within the plant.

EXPERIMENTAL RESULTS

Evidence of mosaicism for level of *R* action was obtained in the first experiment designed to test systematically for occurrence of the phenomenon. W22 plants heterozygous for standard R^r , which is sensitive to paramutation, and R_{117}^{sc} , a mutant from stippled (R^{st}) known from previous tests to be paramutagenic at an average level somewhat below that of the parent allele, were grown under especially favorable soil fertility and moisture conditions in an attempt to induce tillering. Eleven tillered plants were obtained in this way from which pollen samples representing three or more branches each in the respective main shoot and tiller tassel eventually were successfully used in testcrosses on W23 $r^g r^g$ females. Pollen was collected separately in each case from the terminal spike and also from four lateral branches starting at the base and proceeding upward by successive quarter turns.

A parallel experiment was made using plants from a stock culture homozygous for R^r in matings on W23 $r^g r^g$ females. Four $R^r R^r$ individuals, two of which

produced one fertile tiller each, were thus tested for mosaicism. The R^r factor in these R^rR^r plants is not subject, of course, to change in level of action during development in response to the action of a paramutagenic allele like R^{sc}_{117} .

The detailed results of the tests for mosaicism in the $R^rR^{sc}_{117}$ and control R^rR^r plants have been presented by COOPER (1964). In summary, COOPER's findings were as follows: (1) The effect of the paramutagenic R^{sc}_{117} allele in $R^rR^{sc}_{117}$ plants was to lower aleurone pigment producing action of R^r about one class interval on the 1-7 scale. The mean score for the $R^rr^gr^g$ kernels from all r^gr^g female $\times$ R^rR^r male testcrosses was 5.11. The corresponding value for the $R^rr^gr^g$ kernels (R^r = paramutant form of R^r) from all r^gr^g female $\times$ $R^rR^{sc}_{117}$ male matings was 4.05. (2) The R^rR^r plants tested gave no evidence of mosaicism for level of R^r action. Mean color scores for the kernels based on main shoot and tiller pollen samples were alike, within sampling limits, in the two comparisons of this kind that could be made. Furthermore, in each instance, the within tassel variation mean score was statistically insignificant. That is to say, the pollen from different branches within an R^rR^r tassel were indistinguishable in terms of R^r pigment producing potential. (3) All 11 $R^rR^{sc}_{117}$ plants tested, on the other hand, proved to be heterogeneous in terms of level of R^r action. The aleurone color score for the $R^rr^gr^g$ kernels based on tiller pollen significantly exceeded that for kernels resulting from the application to r^gr^g females of main shoot pollen in each instance. Also statistically significant differences in mean aleurone color score were found for different branches within single tassels in seven of the 11 plants examined.

Evidence that the heterogeneity in level of R^r action reflected by the data from $R^rR^{sc}_{117}$ plants rests upon sectoring within the individual is presented in Table 1. The testcross results shown are based on pollen samples taken separately from four lateral branches (1-4) and the terminal spike (5) in the main and also the tiller tassel of plant 41-106-5M.

TABLE 1

Frequency distributions for aleurone color score of $R^r \times r^gr^g$ testcross kernels based on pollen samples from different parts of the main and tiller tassels of $R^rR^{sc}_{117}$ plant 41-106-5M

Sample No.	Aleurone color class							Mean score
	1	2	3	4	5	6	7	
Main tassel								
1	.	.	42	16	22	.	.	3.76
2	.	3	67	8	2	.	.	3.11
3	.	.	56	18	6	.	.	3.38
4	.	4	59	12	5	.	.	3.23
5	.	.	54	19	7	.	.	3.42
Tiller tassel								
1	.	.	27	23	30	.	.	4.04
2	.	.	.	.	36	33	11	5.69
3	.	.	.	.	8	34	38	6.38
4	.	1	51	19	9	.	.	3.45
5	.	.	28	8	38	4	2	4.30

TABLE 2

Mean color scores for the $R^r r^g r^g$ (or $R^g r^g r^g$) testcross kernels representing separate pollen samples from three lateral tassel branches on a series of $R^r R^{st}$ (or $R^g R^{st}$) plants carrying R alleles of diverse geographical origin and also for the $R^r r^g$ (or $R^g r^g$) progeny corresponding to these branches

Allele and family No.		Mean aleurone color score by tassel branch			Significance of difference
		1	2	3	
Argentina R^r	$R^r R^{st}$ parent	5.33	4.10	3.69	*
(52-6)-1	$R^r r^g$ progeny	6.27	5.79	6.49	*
Turkey 13 R^r	$R^r R^{st}$ parent	3.69	7.93	3.99	**
(52-7)-4	$R^r r^g$ progeny	6.68	8.27	6.46	**
Ethiopia 30 R^r	$R^r R^{st}$ parent	5.26	2.93	5.01	**
(52-8)-6	$R^r r^g$ progeny	9.56	5.98	7.49	**
India 6 R^r	$R^r R^{st}$ parent	6.54	3.04	5.96	**
(52-11)-1	$R^r r^g$ progeny	...	4.01	8.73	**
Turkey 18 R^r	$R^r R^{st}$ parent	9.10	5.02	7.04	*
(52-12)-1	$R^r r^g$ progeny	10.55	9.37	10.05	**
Turkey 18 R^r	$R^r R^{st}$ parent	7.81	5.09	2.14	**
(52-12)-8	$R^r r^g$ progeny	9.88	9.30	4.30	**
Standard R^r	$R^r R^{st}$ parent	3.53	3.84	8.62	*
(52-9)-8	$R^r r^g$ progeny	...	6.52	10.50	**
Standard R^r	$R^r R^{st}$ parent	4.15	5.66	2.44	**
(52-9)-11	$R^r r^g$ progeny	6.08	7.06	4.66	**
Turkey 8 R^g	$R^g R^{st}$ parent	4.54	7.87	3.47	**
(52-10)-6	$R^g r^g$ progeny	6.72	9.22	6.03	**

* = .05 > P > .01. ** = P < .01.

Progeny tests for mosaicism with R^r and R^g alleles of diverse geographic origin: Genetic variation during plant development appears to characterize the response of all overtly paramutable R factors to a paramutagenic allele. This conclusion is supported by the results of tests in R^{st} heterozygotes of six R^r and one R^g allele of diverse geographic origin, as listed in Table 2, and known from previous experiments to be sensitive to paramutation.

Pollen from three separately sampled, lateral branches in five to ten R^{st} heterozygotes carrying the respective R^r and R^g factors was used in duplicate testcrosses on W22 $r^g r^g$ females. The resulting $R^r r^g r^g$ (or $R^g r^g r^g$) kernels were then scored for aleurone color on the 1-11 scale. The mean scores, by individual tassel branches, are entered in Table 2 for the particular plants within the total number sampled in which evidence of mosaicism was found and for which the results of a progeny test also are available. Differential induction of paramutation in separate tassel branches of a Turkey 8 $R^g R^{st}$ (52-10)-6 plant is illustrated in Figure 1.

FIGURE 1.—Ears resulting from matings on $r^g r^g$ females with pollen collected from lower, middle, and top tassel branches of a Turkey 8 $R^g R^{st}$ plant ((52-10)-6). Half the kernels on each ear are of the finely spotted, stippled phenotype. Many $R^g r^g r^g$ kernels on the middle ear are very darkly pigmented, showing that the level of R^g action has been little altered in the corresponding $R^g R^{st}$ tassel branch (average score = 7.87). In contrast, many of the $R^g r^g r^g$ kernels on the right hand ear, representing a top tassel branch, are colorless, or nearly so (average score = 3.47). The $R^g r^g r^g$ testcross kernels on the remaining ear on the left, corresponding to a lower tassel branch, are variable but, on the average, are pigmented at an intermediate level (average score = 4.54).

Progeny testcrosses on W22 $r^g r^g$ females were made using the $R^{st} r^g$ (or $R^g r^g$) offspring of each of the $R^g R^{st}$ (or $R^g R^{st}$) plants in question. Pollen from 8 to 12 $R^{st} r^g$ (or $R^g r^g$) plants each, collected separately but without reference to individual tassel branches, was used in these matings. Mean scores for the progeny are entered in Table 2 just beneath the corresponding parent values.

The data show that the statistically significant differences in mean scores between tassel branches within a plant also tend to characterize the corresponding offspring. The progeny testcross scores regularly are higher, however, than those of the respective parents. The difference is a reflection of the partial reversion in level of R^g action that regularly occurs in a first generation $R^{st} r^g$ plant derived from an $R^g R^{st}$ individual (KERMICLE 1963). Allowing for the general upward shift in magnitude due to partial reversion, it is evident that a low scoring parent tassel branch tends to give low scoring progeny, whereas high scoring branches give relatively high scoring offspring. In eight out of nine progeny tests made the difference between means for branches within tassels is statistically significant at the 1% level, and in the remaining instance, at the 5% level. Thus the data show that the differences in level of R potential arising within tassels are gametically transmissible (Figure 2).

FIGURE 2.—Representative ears from testcrosses on $r^g r^g$ females of plants in two $R^r r^g$ families derived from branches 3 and 2, respectively, of an India 6 R^r/R^{st} individual (52-11)-1). The darkly colored $R^r r^g r^g$ kernels on the ear above show that little change in R^r pigmenting potential occurred in one branch. The many colorless and near-colorless $R^r r^g r^g$ kernels on the ear below demonstrate that the aleurone pigmenting potential of R^r was strongly reduced in another tassel branch on the same $R^r R^{st}$ plant.

The Table 2 data refer only to the $R^r R^{st}$ and $R^g R^{st}$ plants tested that gave evidence of mosaicism and on which a progeny test also was completed. Additional individuals representing each R^r and R^g allele also were tested from which, however, progeny data were not obtained. Addition of these plants to those included in Table 2 gave a total of 186, of which 49 in all showed statistically significant differences between branches within tassels. On this basis, the proportion of obviously sectoried $R^r R^{st}$ and $R^g R^{st}$ plants encountered was about one quarter of those tested.

Additional evidence for mosaicism but without confirming progeny data, was obtained in tests with standard R^r when carried in a K10 chromosome. The latter contains a large heterochromatic knob distal to the R locus the effect of which is to reduce sensitivity of R^r to paramutation (BRINK and WEYERS 1960). Likewise, sectoring was proved to occur in $R^g R^{st}$ plants carrying an R^g allele extracted from the Black Mexican sweet corn variety and known to be highly paramutable.

Diversity in R potential within a single tassel: The diversity of expression encountered in tests for R mosaicism is illustrated in greater detail by the data in Table 3, which relate to testcrosses with a Turkey 13 R^r/R^{st} and an India 6 R^r/R^{st} plant, respectively. Scores for duplicate matings with a given pollen sample agree closely with each other, showing that there is little variation between the pistillate plants used as testers. Scores for different branches within a tassel, on the other hand, may differ widely both in mean level and degree of dispersion.

TABLE 3

Frequency distributions for aleurone score in tests of different tassel branches within two R^{st} heterozygotes carrying paramutable R^r alleles of different geographic origin

Allele	Plant and tassel branch	Aleurone color score											Mean score	
		1	2	3	4	5	6	7	8	9	10	11	Duplicate matings	Average
Turkey 13 R^r	(52-7)-8-1	.	.	2	10	15	18	4	1	.	.	.	5.30	...
	-1	.	1	3	9	17	17	2	1	.	.	.	5.12	5.21
	(52-7)-8-2	.	6	12	15	7	8	2	.	.	.	.	4.10	...
	-2	.	17	8	10	10	4	1	.	.	.	.	3.58	3.84
	(52-7)-8-3	.	32	16	2	.	.	.	.	.	.	.	2.40	...
	-3	.	38	10	2	.	.	.	.	.	.	.	2.28	2.34
India 6 R^r	(52-11)-4-1	.	.	.	.	.	.	.	.	7	43	.	10.86	...
	-1	.	.	.	.	.	.	.	.	3	47	.	10.94	10.91
	(52-11)-4-2	.	3	4	5	3	2	2	4	8	11	8	7.62	...
	-2	.	1	4	7	3	8	3	3	4	10	7	7.26	7.44
	(52-11)-4-3	.	1	.	.	5	14	1	15	8	6	.	7.40	...
	-3	.	.	.	2	8	14	6	8	7	4	1	7.04	7.22

Zones may occur in which the level of R potential is either not reduced at all, or only very slightly, as illustrated by the (52-11)-4-1 entry in Table 3. At the other extreme are zones, such as that represented by entry (52-7)-8-3, in which R potential is uniformly depressed to a low value.

The extreme diversity in kernel phenotype that may result when pollen from a single branch within a tassel is tested is illustrated by the (52-11)-4-2 arrays in Table 3. The distribution of kernels in this instance extends rather evenly over ten pigmentation grades. The cause of such high local variability is unknown, but a likely explanation is that the pollen involved was produced in two or more nearby, but unlike, zones.

The occurrence of pronounced genetic heterogeneity within the pollen collected from a single tassel branch is further illustrated by the data presented in Table 4. Each of these cases was selected as showing an unusually wide spread in kernel phenotype among distributions most of which were much less variable. The $R'r^g$ testcross kernels were partitioned, rather arbitrarily in certain instances, into two classes, light and dark, with the boundary set between grades 6 and 7. Plants representing the two seed groups were grown out, and ten of each group were then testcrossed on $r^g r^g$ females. It is evident from the respective parent and progeny means scores, shown in Table 4, that the R'' (or R^g) alleles corresponding to kernels in the upper and lower parts of the distribution, on the average, are significantly different from each other in level of action. Other data, not presented here, show that the original plants represented in this test (52-8)-4, (52-10)-5, and (52-11)-1, were mosaics. The present, more detailed analysis demonstrates that zones so recognized may themselves be heterogeneous for level of R potential.

Mosaicism in $R'r^g$ plants: R' alleles in $R'r$ plants derived directly from rr female $\times$ RR^{st} male matings revert partially toward the level of action character-

TABLE 4
*Scores for parent and progeny of R' r^{sp} testcross kernels representing pollen
 from individual tassel branches that appeared to be sectored*

Genotype	Pedigree	Parent kernel group	Aleurone color class (for parents)											Mean score		Statistical significance
			1	2	3	4	5	6	7	8	9	10	11	Parent	Progeny	
Ethiopia 30 R^r/R^{st}	(52-8)-4-2	Light	5	18	55	15	..	..	..	..	..	..	..	3.90	7.11	**
		Dark	..	..	..	..	..	..	37	20	26	20	5	8.41	10.31	
Ethiopia 30 R^r/R^{st}	(52-8)-4-3	Light	21	40	51	38	26	..	..	..	..	..	..	4.05	7.06	**
		Dark	..	..	..	..	..	24	12	11	27	2	..	8.56	8.42	
India 6 R^r/R^{st}	(52-11)-1-1	Light	26	15	12	3	1	..	..	..	..	..	..	2.91	4.96	**
		Dark	..	..	..	..	..	..	11	12	16	32	19	9.40	10.27	
Turkey 8 R^g/R^{st}	(52-10)-5-1	Light	41	11	19	12	11	..	..	..	..	..	..	3.39	5.31	**
		Dark	..	..	..	..	..	..	29	19	19	7	12	8.44	8.92	

** = $P < .01$, based on t-test of difference between progenies of the respective light and dark parent groups.

TABLE 5

Mean scores for testcross kernels from $r^s r^g$ female $\times$ $R' r^s$ male matings in which the testing of pollen samples from three different tassel branches showed that the $R' r^g$ plant in question was sectored for level of R' action

Genotype	Plant No.	Mean aleurone score by tassel branch			Statistical significance
		1	2	3	
Argentina R^r/r^g	R35-1	6.19	5.69	4.31	**
India 6 R^r/r^g	R185-6	8.82	7.40	4.35	**
India 6 R^r/r^g	R186-2	2.93	5.54	4.11	**
Standard R^r/r^g	R66-5	10.21	6.80	10.24	**
Standard R^r/r^g	R109-1	5.70	7.50	6.68	**
Standard R^r/r^g	R127-1	8.65	8.54	5.96	**
Standard R^r/r^g	R138-1	4.95	4.48	6.90	**
Turkey 8 R^r/r^g	R183-1	8.26	10.92	9.72	*

* = .05 > P > .01. ** = P < .01.

istic of R in standard form (KERMICLE 1963). The data in Table 5 show that such partial reversion may proceed to varying levels within a single plant.

In all, 86 $R^r r^g$ plants representing R alleles of four geographic origins were tested for mosaicism by separately sampling the pollen either from branches within a tassel or from the main shoot and tiller. Ten plants were found in this group that clearly were mosaic for level of R action. The data for eight of these individuals in which pollen samples were taken from three lateral tassel branches are summarized in Table 5. In each of these cases differences in mean testcross score, statistically significant at the 1% level, were found between branches within tassels. Corresponding differences were observed in the two remaining plants in which the pollen samples were based on main stalk and tiller tassels (Figure 3).

Effect of different paramutagenic alleles on mosaicism: The data summarized in Table 6 show that mosaicism occurs in heterozygous plants carrying a common R^r factor but varying in the paramutagenic allele present. R_{57}^{sc} , R_{77}^{sc} , and R_{126}^{sc} are strongly paramutagenic mutants from R^{st} , whereas R_{53}^{sc} and R_{84}^{sc} are mutants from the same source that are paramutagenic at a somewhat lower level (McWHIRTER and BRINK 1962).

Seventeen plants carrying standard R^r and one or another of the strongly paramutagenic R^{sc} factors were tested for mosaicism by sampling the pollen in three lateral tassel branches separately. Three definitely sectored individuals were identified in this group. Similarly, among 14 plants heterozygous for standard R^r and either R_{53}^{sc} or R_{84}^{sc} , three mosaic individuals were found. These cases of sectoring were verified by progeny tests the results of which also are assembled in Table 6.

Among 19 plants heterozygous for standard R^r and R_{99}^{sc} , a very weakly paramutagenic mutant from R^{st} , two individuals were identified as mosaic. Progeny tests in these cases, however, were not made.

FIGURE 3.—Differential reversion in an Argentina R^r/r^g plant. The ears shown resulted from testcrosses on $r^g r^g$ females using pollen collected from the main shoot (left) and tiller (right) of a single $R^r r^g$ individual derived from an $r^g r^g$ female $\times$ Argentina R^r/R^{st} male mating. The unlike levels of R^r action in the $R^r r^g r^g$ kernels on the two ears shows that the paramutant R^r allele in this $R^r r^g$ individual underwent different amounts of reversion in main shoot and tiller.

If a comparison is made by inspection between mean scores for parent zones and progenies (Tables 2 and 6) a general correspondence in ranking will be evident. Correlation and regression analyses were carried out to confirm this correspondence and to express the relationship quantitatively. A highly significant correlation (0.80) between scores for the parental zones and the progenies was found. The confirmatory results of the regression analysis are presented in Figure 4. Both the correlation ($-.18$) and regression values ($-.64 \pm .48$) for the differences between duplicate pollinations and the differences between their progenies, on the other hand, are nonsignificant, showing that the small initial differences observed between the testcross ears obtained by pollinating different female parents ($r^g r^g$) with the same sample of pollen are not heritable.

Distribution of zones within the tassel: Three lateral branches taken in ascending order around the central axis of the tassel were sampled in collecting pollen from most of the plants tested for mosaicism. If paramutation occurs progressively during plant development, it is conceivable that the most distal tassel branch in the set of three would give a lower aleurone color score, on the average, than the proximal one. A tabulation of the data on this basis, however, disclosed no regular relation between aleurone testcross score and branch order.

This result should not be construed as evidence that excludes the progressive occurrence of paramutation during plant development. The sampling method employed probably was much too coarse, relative to the small differences in stage of development represented by three such tassel parts, to provide critical evidence on the point.

Variation in level of R action and sensitivity to paramutation: The question

TABLE 6

Mean scores for the R^r r^{sg} testcross kernels representing separate pollen samples from three lateral tassel branches from each of a series of R^r heterozygotes carrying different paramutagenic R^{sc} mutants from R^{st} , and also the $R^r r^{sg}$ progeny corresponding to these plants

Pedigree No.	R^{sc} mutant	Plants tested	Mean aleurone color score by tassel branch			Significance of difference
			1	2	3	
(52-14)-2	R^{sc}_{57}	$R^r R^{sc}_{57}$ parent	2.95	4.85	3.87	**
		$R^r r^{sg}$ progeny	5.95	7.08	6.52	*
(52-15)-6	R^{sc}_{77}	$R^r R^{sc}_{77}$ parent	5.66	2.81	4.96	**
		$R^r r^{sg}$ progeny	8.51	6.04	8.57	**
(Mc 772)-1	R^{sc}_{126}	$R^r R^{sc}_{126}$ parent	6.11	3.23	5.63	**
		$R^r r^{sg}$ progeny	8.89	5.65	7.22	**
(52-18)-5	R^{sc}_{53}	$R^r R^{sc}_{53}$ parent	6.65	10.91	4.94	**
		$R^r r^{sg}$ progeny	9.98	10.97	8.27	**
(52-19)-4	R^{sc}_{84}	$R^r R^{sc}_{84}$ parent	5.64	8.75	5.68	**
		$R^r r^{sg}$ progeny	7.76	9.35	...	**
(52-19)-8	R^{sc}_{84}	$R^r R^{sc}_{84}$ parent	3.32	2.51	4.52	**
		$R^r r^{sg}$ progeny	6.60	5.09	7.62	**

* = .05 > P > .01. ** = P < .01.

may be asked whether the observed variation in $R^r r^{sg}$ aleurone color following testcrosses with pollen from different tassel branches of a mosaic individual reflect changes in sensitivity of R^r to paramutation during plant development or are the result merely of different amounts of R^r change without alteration in inherent sensitivity.

An earlier study by BROWN (1963) disclosed no evidence that the R^r allele in gametes from $R^r R^{st}$ plants that gave rise to unusually dark kernel phenotypes in the initial testcross on an $r^{sg} r^{sg}$ female had acquired insensitivity to paramutation. The R^r factors carried by such darkly pigmented seed when retested in $R^r R^{st}$ heterozygotes displayed the usual sensitivity to paramutation. Additional evidence pointing to the same conclusion was obtained in the present study.

Frequency distributions for kernel color score following duplicate testcrosses on $r^{sg} r^{sg}$ females using pollen collected separately from three lateral tassel branches on plant (52-18)-5, heterozygous for standard R^r and the paramutagenic allele R^{sc}_{53} are shown in Table 7. It is evident that this plant was mosaic for level of R^r action. The mean score of 10.9 for branch 2 is near the top of the aleurone

FIGURE 4.—Regression of *R'r^g* progeny mean score (Y) on *R'r^g* parent sector score (X).

TABLE 7

Frequency distributions for aleurone color score following duplicate r^{gr} female × R^rR^{sc}₅₃ male testcrosses in which the pollen was collected separately from three lateral tassel branches in plant (52-18)-5

Pollen sample	Aleurone color class											Mean score	
	1	2	3	4	5	6	7	8	9	10	11	Single	Average
52-18-5-1	.	.	.	.	5	22	11	5	4	3	.	6.80	...
-1	.	.	.	.	5	25	15	2	1	2	.	6.50	6.65
52-18-5-2	.	.	.	.	.	.	.	.	.	6	44	10.88	...
-2	.	.	.	.	.	.	.	.	.	3	47	10.94	10.91
52-18-5-3	.	3	8	5	9	13	7	1	4	.	.	5.32	...
-3	.	4	10	12	8	10	6	.	.	.	.	4.56	4.94

color scale, and is much higher than those for branches 1 and 3, namely, 6.65 and 4.94. Little or no change in level of R^r action has occurred in branch 2, since 90% of the testcross kernels fall in the highest pigmentation class. Has the R^r allele in this case become insensitive to paramutation? An additional test provided the answer to this question.

$R^{st}R^{st}$ and $R_{126}^{sc}R_{126}^{sc}$ individuals were pollinated by the plants grown from $R^{r'}r^g$ kernels resulting from the testcrosses of branches 1 and 2. The respective R^rR^{st} and $R^rR_{126}^{sc}$ heterozygotes were then grown out and testcrossed on rr females. Five groups of R^rR^{st} plants representing branches 1 and 2 gave average testcross scores of 2.48 and 4.57, respectively. The corresponding values for the $R^rR_{126}^{sc}$ testcrosses, based on three parent plants each, were 1.82 and 1.98. Thus both retests of R^r derived from branch 2 resulted in reduced scores. The latter, in fact, were below the levels shown by branches 1 and 3 in the first r^gr^g female $\times$ R^rR^{st} male matings. These results show that failure to undergo reduction in level of action in this case is not attributable to an inherent incapability of the R factor in question to undergo such change.

DISCUSSION

The foregoing evidence on mosaicism was obtained under the experimental disadvantage that change in level of R action in maize is not reflected at once in plant phenotype. This limitation does not apply to certain earlier described examples of paramutation in other species. Progressive morphological changes in these cases appeared along a growing axis. The changes could be shown to be gametically transmissible if flowers were present. Genotypic and phenotypic alterations in somatic tissues went hand in hand. Plants developed that were overtly mosaic for the character in question. Three examples will be mentioned here of paramutation expressed in this way. They are significant for the light thrown on progressive change during plant development, an aspect of somatic mosaicism on which the maize data are relatively uninformative.

In considering the examples from other species now to be cited of progressive paramutation during ontogeny, the fact should be borne in mind that, whereas parallel evidence has not been obtained from maize, it has been demonstrated that change in level of action at the R locus in the latter species is progressive in successive sexual generations (cf. BRINK 1964).

The rabbit ear rogue, which appears occasionally as a mutant in various garden pea varieties, involves a pronounced narrowing of stipules and leaflets. BATESON and PELLEW (1916, 1920) observed that rogues selfed gave only rogues, and that when rogues and normal plants were crossed, and the hybrids were allowed to self-pollinate, most of the offspring, and sometimes all of them, were rogues. F_1 plants, which were intermediate in phenotype as seedlings, became progressively more rogue-like as the stem elongated, so that the uppermost nodes were typically rogue in phenotype. The further important fact was established that with the progressive change in form of leaflets and stipules along the plant axis there was a corresponding increase in the proportion of rogue offspring from seeds

borne at successively higher nodes. BATESON (1926) later reported that as the stem of a heterozygous plant was ascended the proportion of gametes transmitting the rogue characteristic increased more rapidly for sperm than for eggs.

Several points are noteworthy when the observations on rogues in peas are considered in relation to *R* mosaicism in maize. In F_1 rogue-type pea plants, the genetic change that occurs is highly directed, as it is in $F_1 R'R^{st}$ maize. The shift in the pea is from type toward rogue, and in $R'R^{st}$ maize it is regularly from a higher to a lower level of *R* action. Secondly, at maturity in both kinds of plants most, or all, gametes carrying the allele subject to change have been changed, in fact, in one degree or another. There is visual evidence in the pea, confirmed by progeny tests, that paramutation is progressive as plant development advances. Sectoring for level of *R* action in maize shows that the somatic tissues become heterogeneous in this species also, but the data obtained do not reveal the relation between amount of change and growth stage.

It is significant that the anisogamy which BATESON (1926) reported in rogue pea heterozygotes has a counterpart in maize also. KERMICLE (1963) has proved that the paramutant R'' allele derived from $R'R^{st}$ plants regularly is transmitted at a potentially lower level of pigmenting action through male than through female gametophytes. The basis of this extraordinary manifestation of mosaicism is quite unknown, but the parallel between pea and maize in this respect is remarkable.

LILIENFELD (1929) has described an example of paramutation in *Malva parviflora* in which concomitant alterations in genotype and phenotype within plants also occurred. A mutant with deeply incised leaves, termed *laciniata*, was found to breed true when homozygous. *Laciniata*, however, was unstable, and gave rise to several mutants, one of which, called normal-2, conditioned non-incised leaves, like the wild-type allele. F_1 hybrids between *laciniata* and normal-2 were intermediate between the two parental forms as seedlings, but changed progressively during growth in the direction of normal-2. Progeny tests, using seed from separate branches on such plants, showed that the progressive phenotypic changes reflected gametically transmissible differences.

The last example to be mentioned here of visible changes in form within a developing plant that resulted directly from local genetic changes is the *cruciata* character in *Oenothera*. RENNER (1937, 1959) found that the *cruciata* locus is highly unstable in certain heterozygotes. Normal plants selfed or crossed with each other yielded only *cruciata* progeny, but of varying grades depending on the parents. Heterozygotes between normal and *cruciata* individuals of whatever source, however, invariably were inconstant. Such plants frequently were obviously mosaic. RENNER demonstrated, as he notes DE VRIES had done previously, that the distribution of offspring of a mosaic plant was correlated with the form of the flower that produced the seed, namely, narrowly cross-shaped (*cruciata*), intermediate, or normal.

The evidence from *Pisum*, *Malva*, and *Oenothera* suggests that progressive, genetic change during development of the individual is a common attribute of paramutation. Confirming evidence is not obtainable from maize because pollen

sampling is limited to the tassel at the apex of the plant and, in any case, *R* mosaicism is not directly expressed in vegetative tissues of the sporophyte in the W22 strain used. The latter difference, however, is superficial, and depends merely on the character with which the particular locus is associated. It might be supposed that at least a rough test for progressive change in level of *R* action in maize during plant development could be made by comparing the pigment producing potential of *R* gametes from pollen (tassel) and ovule (ear shoot). This procedure, however, would not yield uncomplicated evidence on the question. As pointed out earlier, there is regularly a difference in paramutant maize in the potential of *R* from these two sources (anisogamy) that seemingly has no relation to ordinary vegetative growth of the plant.

Mosaicism for level of *R* action in maize was observed in one or more individuals in each group of plants tested that were heterozygous for a paramutable and a paramutagenic allele. It was not found when R^rR^r plants from W22 stock cultures were tested. One might conclude from this difference that mosaicism with reference to the action of a paramutable *R* allele is not autonomous but occurs only when such an *R* factor is subject to paramutation by virtue of the presence in the homologous chromosome of a paramutagenic allele. Such an inference, however, would be mistaken. Positive evidence for mosaicism was obtained for $R''r^g$ plants also, accompanying the partial reversion toward the level of action of standard R^r which R'' , as a primary paramutant, undergoes in such individuals. KERMICLE (1963) demonstrated that partial reversion of an R'' allele occurs even in hemizygotes (R''/R -deficient), that is, in the absence of any *R* partner. This independent reversion reflects a metastable condition of R'' . Evidently mosaicism for level of *R* action can arise from the same cause.

These facts are understandable if it is assumed that the primary consideration in *R* paramutation is an inherent instability of *R* (STYLES 1965) and its paramutant derivatives. The effect on *R* of a paramutagenic partner in the homologous chromosome in a heterozygote on this view is to increase the likelihood that *R* will change rather than to direct that such change shall occur in a given instance. Whether change occurs at a particular somatic mitosis and the amount of change are both indeterminate. Mosaicism is a consequence of this indeterminacy; it rests primarily upon the metastability of R^r and R'' , in R^rR^{st} or $R''r^g$ plants.

The generality of the relationship between *R* paramutation and somatic mosaicism is attested by the fact that each of the paramutable and paramutagenic alleles tested gave a positive result for heterogeneity in level of action. Confirmation of mosaicism was obtained in progeny tests of seven of the former, representing maize races of diverse geographic origin. Two others should be added to this group from which offspring were not grown. Likewise, nine different paramutagenic R^{sc} mutants from R^{st} gave rise to mosaicism in heterozygotes with standard R^r . Confirmatory progeny data were obtained in six of these cases.

Little can be deduced from the available data on the frequency of *R* mosaicism. The examples discussed probably represent the most extreme differences and only a small fraction of all the sectors that occurred in the plants examined.

Testing the pollen from different tassel branches of an R^r heterozygote discloses, of course, only the terminal states of R^r . Information is not forthcoming by this procedure concerning either the number or the distribution during plant development of the intermediate states through which a given R^r as tested in the pollen will have passed in somatic cells. McWHIRTER and BRINK (1962) showed that the pigment producing potential of standard R^r could be reduced to any one of a wide range of levels by passing the allele through appropriate R^{sc} heterozygotes. The possible courses of R^r change during plant development, therefore, are very large. Probably every plant in which R paramutation occurs is a mosaic of greater or lesser complexity. Reduction of standard R^r in an R^rR^{st} individual to a relatively low level of pigmenting potential in a given cell lineage could involve a succession of small changes, a few widely separated large ones, or various combinations between these extremes.

It also appears likely that considerable variability in level of R action in R^rR^{st} plants, for example, is extinguished during the course of development. Such net reduction would result if R^r were reduced ultimately to a given low level in all of several cell lineages within an individual, but at a different average rate in each lineage. The relative uniformity in level of paramutant R' action often observed in testcross ears from certain classes of matings could be accounted for in this way.

BRINK (1964) postulated that paramutation involves a heterochromatic segment at or near the R locus consisting of varying numbers of repeating units, termed P_A , the effect of which is to repress R gene action. Degree of repression was assumed to be proportional to the multiplicity of P_A units making up the heterochromatic segment. It was further postulated that the essential feature of paramutation was change in number of P_A units. An R gene accompanied by many P_A units was only weakly expressed, whereas few P_A units permitted strong gene action.

Somatic mosaicism may be explained in terms of a model of this kind. The main postulates may be outlined as follows: (1) Genetic diversity originates at a mitosis in which change occurs in number of P_A units in the heterochromatic segment adjacent to the R gene. (For convenience the heterochromatic segment in question will be termed "Repressor," and a P_A unit will be called a "metamere.") (2) Change in number of metameres results from misreplication of Repressor during a somatic mitosis. Copying of given metameres more than once in a mitotic cycle leads to an increase, and failure to copy some of them results in a decrease, in total metamere number. (3) One of the conceivable ways in which an overtly paramutagenic allele, like R^{st} in an R^rR^{st} heterozygote, could function would be to prolong the period within a mitotic cycle during which Repressor replicates, and so favor excess copying of metameres associated with the paramutable R^r allele in the homologous chromosome. A corresponding reduction in level of R^r action would ensue. Recessive r in an R^rr , or $R^r'r$, plant could have the opposite effect on replication time, thus causing undercopying of Repressor and a rise in level of R^r (or R^r') action. (4) Since, as KERMICLE (1963) observed, partial reversion of R'' toward the pigmenting level of R^r in standard

form occurs in R''/R -deficient plants it must be postulated also that in a highly repressed R'' the number of metameres may decline autonomously, that is, in the absence of any allele in the homologous chromosome.

Replication of Repressor, according to this point of view, is subject to regulation in definite ways. Certain conditions at the R locus, either in the same or in the homologous, chromosome in a heterozygote, or both, favor overcopying, whereas other local conditions promote undercopying of metameres. These conditions underlie the directed nature of paramutation.

The evidence on somatic mosaicism, however, shows that an important additional variable is involved in paramutation. This variable underlies the amount of change in level of R action at a mitosis as distinguished from direction of change. The direction of change in a given kind of heterozygote is determinate. The amount of change, on the other hand, is indeterminate. It is this indeterminacy that leads to R mosaicism during plant development.

It was pointed out earlier that only the terminal states of a paramutable R in the cell lineages extending into the tassel are measurable by the procedures followed in the present study. The terminal state reflects the sum of the changes that have occurred in this cell lineage between fertilization and maturity of the plant but tells nothing about the size and distribution of the single items entering into the total. If, within a given tassel, however, more than one terminal state of R is found this is evidence, *ipso facto*, for occurrence within the plant of at least one mitosis at which R paramutation has occurred and from which a different R cell lineage was derived.

The chance factor in paramutation is clearly exemplified by the occasional occurrence of tassel zones in plants heterozygous for a paramutable and paramutagenic R allele in which no change in the paramutable member of the pair is demonstrable in testcrosses. Illustrative cases are the distributions in Tables 1, 3, and 7 in which all the entries fall in the upper classes. No change in level of R action has occurred at any mitosis in the long succession which these lineages represent. Tests show that the unaltered R alleles remain sensitive to paramutagenic action. Failure to change in these rare cases is attributable to chance escape from paramutation at each successive somatic mitosis within the lineage throughout plant development. The commonly observed effect of paramutation within plants of a given genotype is a variety of levels of potential R action. The shift is in a common direction but to varying degrees. Seemingly numerous factors operating at random control the amount of a change that is otherwise determinate.

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SUMMARY

Testcrosses on colorless aleurone (r^0r^0) maize plants using pollen collected separately from different branches within the tassel of an individual in which paramutation was occurring often resulted in kernel populations differing sig-

nificantly in mean aleurone pigmentation. Such mosaicism was found to characterize plants carrying paramutable R^r or R^g alleles of various geographic origins and one or another paramutagenic allele. It was observed that somatic mosaicism also occurred in $R''r^g$ plants, in which the R'' factor undergoes partial reversion toward the standard R^r level of pigmenting action. The R system in maize is unsuitable for determining in detail the nature of the somatic mosaicism here encountered. Others have shown, however, that mosaicism associated with paramutation also occurs in *Pisum*, *Malva*, and *Oenothera*. The evidence from all sources suggests that progressive genetic change during development is a common attribute of paramutation and that the amount of change frequently varies in different cell lineages within the individual. An hypothesis is offered to account for mosaicism in maize that postulates misreplication during somatic mitosis within an assumed heterochromatic segment near the R locus. It is suggested that this segment consists of repeating elements, and that R action is repressed according to multiplicity of these elements.

LITERATURE CITED

- BATESON, W., 1926 Segregation. *J. Genet.* **16**: 201–235.
- BATESON, W., and C. PELLEW, 1916 Note on the orderly dissimilarity in inheritance from different parts of a plant. *Proc. Roy. Soc. London B* **89**: 174–175. — 1920 The genetics of “rogues” among culinary peas (*Pisum sativum*). *Proc. Roy. Soc. London B* **91**: 186–195.
- BRINK, R. A., 1960 Paramutation and chromosome organization. *Quart. Rev. Biol.* **35**: 120–137. — 1964 Genetic repression of R action in maize. *Symp. Soc. Study Develop. Growth* **23**: 183–230.
- BRINK, R. A., and W. H. WEYERS, 1960 Effect of an abnormal knob-carrying chromosome 10 on paramutation of R^r in maize. *Genetics* **45**: 1445–1455.
- BROWN, D. F., 1963 Paramutability of R^g and r^r mutants from the standard R^r allele in maize. Ph.D. thesis, University of Wisconsin Library, Madison, and unpublished material.
- COOPER, H. B., 1964 Paramutation and reversion of the R^r allele in maize. Ph.D. thesis, University of Wisconsin Library, Madison.
- KERMICLE, J. L., 1963 Metastability of paramutant forms of the R gene in maize. Ph.D. thesis, University of Wisconsin Library, Madison, Wisconsin.
- LILIENFELD, F. A., 1929 Vererbungsversuche mit schlitzblättrigen Sippen von *Malva parviflora*. I. Die laciniata Sippe. *Bibliotheca Genet.* **13**: 1–214.
- MCWHIRTER, K. S., and R. A. BRINK, 1962 Continuous variation in level of paramutation at the R locus in maize. *Genetics* **47**: 1053–1074. — 1963 Paramutation in maize during endosperm development. *Genetics* **48**: 189–203.
- RENNER, G., 1937 Über *Oenothera atrovirens* Sh. et Bartl. und über somatische Konversion im Erbgang des *cruciata*—Merkmals der *Oenotheren*. *Z. Ind. Abst. Vererb.* **74**: 91–124. — 1959 Somatic conversion in the heredity of the *cruciata* character in *Oenothera*. *Heredity* **13**: 283–288.
- STYLES, D., 1965 The metastable nature of paramutable R alleles. *Maize Genet. Coop. News Letter* **39**: 171–172.