CONTINUOUS VARIATION IN LEVEL OF PARAMUTATION AT THE R LOCUS IN MAIZE¹

K. S. McWHIRTER² AND R. ALEXANDER BRINK

Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received April 24, 1962

EXPRESSION of the standard R^r allele in maize invariably is altered in the direction of reduced aleurone-pigmenting effect in heterozygotes with either of the aleurone-spotting factors, stippled (R^{st}), or marbled (R^{mb}) (BRINK 1956; BRINK and WEYERS 1957). BRINK (1958) has proposed the term paramutation to describe the genetic process underlying the regularly occurring change in R^r action.

The present report is concerned with paramutation of this R^r gene (colored aleurone, red seedling) in heterozygotes with a series of self-colored (R^{sc}) mutants from stippled. The R^{sc} mutants, which give completely colored aleurone and green seedling, were isolated in this laboratory by ASHMAN (1960). The general result shows that there was wide variation in the level of paramutation of R^r in heterozygotes with the various R^{sc} mutants.

The different levels of paramutation were distinguished by quantitative differences in the mean aleurone-pigmenting effect of the R^r paramutants extracted from the several R^rR^{sc} heterozygotes. The observed levels of paramutation formed a continuous series, paramutant forms of R^r ranging from a near-colorless effect to the darkly mottled phenotype characteristic of unchanged R^r . Thus the R^r gene has the capacity to respond to paramutagenic alleles in a wide variety of ways.

The differential paramutation of the single R^r gene used in this study was a consequence both of the specific property of the gene to undergo differential alteration and of inherent differences among the group of R^{sc} mutants in capacity to promote paramutation of R^r . Characterization of the several R^{sc} mutants on the basis of paramutagenic action shows that the mutation of stippled (R^{st}) to self-colored (R^{sc}) results, in a high proportion of instances, in a simultaneous alteration in the paramutagenic property associated with stippled. Coincident with the mutation of R^{st} to R^{sc} the paramutagenic property may be unchanged, quantitatively reduced, or lost entirely.

MATERIALS AND METHODS

The experimental procedures followed were similar to those used in previous investigations on paramutation at the R locus in maize (BRINK 1960).

¹ Paper from the Department of Genetics, College of Agriculture, University of Wisconsin, Madison, No. 872.

² Present address: C.S.I.R.O., Division of Tropical Pastures, Cunningham Laboratory, Mill Road, St. Lucia, Queensland, Australia.

The various R alleles were incorporated, by backcrossing, in the uniform inbred dent-type strain, W22. A single R^r gene, long maintained in local stocks, and referred to subsequently as *standard* R^r , was used throughout these experiments.

The stippled allele (R^{st}), in W22 background, is characterized by a densely spotted aleurone, green coleoptile and colorless roots and anthers. The scutellum tissue in the embryo is characteristically colorless; or occasionally it is very lightly spotted with pigment. The self-colored mutants give complete pigmentation (self-color) of the aleurone layer of the endosperm in all dosages; slight pigmentation of the coleoptile; colorless roots and anthers. The scutellum tissue in the embryo is conspicuously colored. The mutation of stippled to self-colored thus leads to phenotypic changes in aleurone, scutellum, and coleoptile pigmentation.

No differences in aleurone or plant color could be detected among the groups of R^{sc} mutants. The degree of coleoptile pigmentation of seedlings of $R^{sc}R^{sc}$ or $R^{sc}r^g$ genotype is somewhat variable, but is always substantially less than that characteristic for R^r and r^r (red seedling) alleles. The R^{sc} alleles may be considered as belonging, therefore, to the R^g phenotypic class in EMERSON'S (1921) terminology. They differ from typical R^g alleles in pigmentation of the coleoptile and the scutellum tissue of the embryo, and so the designation R^{sc} is used in this report to distinguish the mutants from R^g alleles from other sources.

All the R^{sc} mutants from stippled were found to be nonparamutable in $R^{sc}R^{st}$ heterozygotes.

The level of paramutation of R^r was determined by evaluating the $R^r r^g r^g$ kernels from testcrosses of the type $r^g r^g \text{♀} \times R^r R^{sc} \text{♂}$ for degree of aleurone pigmentation. The W23 $r^g r^g$ (colorless aleurone, green seedling) inbred strain was used throughout as the pistillate parent in these matings. Individual kernels ($R^r r^g r^g$ endosperm) from the testcross ears were scored for degree of aleurone pigmentation by reference to a set of six standard kernels. The latter kernels were of graded pigmentation level, and defined seven classes ranging from 1 (colorless) to 6 (dark mottled) and 7 (self-colored). A random sample of 100 $R^r r^g r^g$ kernels was scored from each testcross ear, and a mean aleurone color score per kernel for the ear was calculated from the resulting distribution. The testcross ears were coded prior to scoring.

The $R^r r^g r^g$ kernels from the W23 $r^g r^g \text{♀} \times$ W22 $R^r R^{sc} \text{♂}$ matings usually could be readily distinguished from the $R^{sc} r^g r^g$ kernels on the basis of degree of aleurone pigmentation. If the kernel phenotypes overlapped, the classes were separated definitively by germinating the seeds after scoring. $R^r r^g r^g$ kernels give seedlings with red roots, whereas $R^{sc} r^g r^g$ kernels give seedlings with green roots, with a low frequency of exceptions due to heterofertilization.

EXPERIMENTAL RESULTS

Initial tests for paramutagenic action of R^{sc} mutants: Eighty-six R^{sc} mutants from stippled are listed in ASHMAN'S (1960) Table 5, and 83 of these (pedigree numbers $R^{sc}53$ to $R^{sc}138$) were tested for paramutagenic action by testcrossing

the respective R^rR^{sc} heterozygotes on $r^{\theta}r^{\theta}$ females. Three mutants in this group were not used because seed from the appropriate matings was not available. The R^{sc} mutants tested, therefore, were not selected on the basis of any prior knowledge of their paramutagenic action.

The R^rR^{sc} individuals testcrossed on W23 $r^{\theta}r^{\theta}$ pistillate parent plants were derived, in the case of each mutant, from a mating of the type $R^{sc}r^{\theta} \text{♀} \times R^rr^{\theta} \text{♂}$, since each R^{sc} allele was represented at this initial stage, by a single $R^{sc}r^{\theta}$ plant grown from the original mutant kernel. A single W22 subfamily of the genotype R^rr^{θ} was employed in the preparation of these stocks. Thus it may be assumed that there was no difference in paramutability of the R^r gene used from one R^rR^{sc} heterozygote to another.

The experimental material comprised testcrosses of 533 plants in 87 families representing the 83 R^{sc} mutants and four control families. Two families of F_1 R^rR^{st} heterozygotes were included. The mean aleurone color score for the $R^rr^{\theta}r^{\theta}$ kernels from these testcrosses provided a basis for comparison of the paramutagenic action of each R^{sc} mutant with that of the parent stippled allele. The remaining control families were single ear progenies of the standard R^rR^r stock. These $r^{\theta}r^{\theta} \text{♀} \times R^rR^r \text{♂}$ testcrosses establish the aleurone-pigmenting effect of the standard R^r gene in unchanged form. The aleurone-pigmenting effect of the standard R^r gene also was determined for testcrosses of 20 R^rr^{θ} plants. These R^rr^{θ} male testcrosses were taken at random from among the families containing R^rR^{sc} heterozygotes, since the mating used ($R^{sc}r^{\theta} \text{♀} \times R^rr^{\theta} \text{♂}$) provided both R^rR^{sc} and R^rr^{θ} plants in each case.

The data obtained have been summarized in the form of a diagram (Figure 1), in which the mean aleurone color scores for the $R^rr^{\theta}r^{\theta}$ kernels from the 87 testcross families are arranged in ascending order of magnitude. The family mean score in each case is identified in Figure 1 by the pedigree number of the R^{sc} mutant involved. (The entry for $R^{sc}72$ was inadvertently omitted in preparing Figure 1. The mean score for the $R^rr^{\theta}r^{\theta}$ testcross kernels in this instance was 2.69.)

Since 100 kernels were scored per testcross ear each family mean plotted in Figure 1 is based on $100n$ kernels, n being the number of plants testcrossed from the family in question. The number of R^rR^{sc} plants tested was not uniform from family to family, since the R^rR^{sc} and R^rr^{θ} segregates within these families could not be identified until harvest. Only three $R^rR^{sc}61$ plants were testcrossed, and a further 18 R^{sc} mutants were characterized for paramutagenic action on the basis of testcrosses of four R^rR^{sc} plants in each family. For the remaining families the number of R^rR^{sc} plants testcrossed ranged from 5 to 11.

The numerical data upon which Figure 1 is based (on file at GENETICS Editorial Office) were examined by analysis of variance. The differences between family means were highly significant ($F_{87,445} = 36.26$). Duncan's new multiple range test, as modified for unequal family size (STEEL and TORRIE 1960, page 114) was used to determine the significance of differences between individual mean scores.

The data diagrammed in Figure 1 show that the R^{sc} mutants differed markedly

FIGURE 1.—Family mean aleurone color scores for the $R^r r^g$ kernels from $W23\ r^g \text{ } \varnothing \times R^r R^{sc} \text{ } \sigma$ matings, arranged in order of increasing magnitude to show the continuous variation in level of paramutation of the standard R^r gene. Mean R^r scores for the control matings, ($R^r R^{st}$, $R^r R^r$ and $R^r r^g$ plants testcrossed on $W23\ r^g$ females) are indicated by arrows.

in effect on the pigment-producing potential of the R^r gene in $R^r R^{sc}$ heterozygotes. Moreover, the mean scores form a continuous array from near-colorless (mean = 2.06) through a series of intermediate values, up to the high level of aleurone pigmentation characteristic of unchanged standard R^r .

At the upper extreme a group of R^{sc} mutants may be distinguished as non-paramutagenic. This group includes 16 alleles, from $R^{sc}58$ through $R^{sc}113$ in Figure 1. The mean scores obtained for testcrosses of the $R^r R^{sc}$ heterozygotes involving these mutants do not differ significantly from the mean scores obtained for unchanged standard R^r , in testcrosses of $R^r R^r$ and $R^r r^g$ plants. These R^{sc} mutants represent instances in which the mutation of R^{st} to R^{sc} has been accompanied by apparent loss of the paramutagenic property.

Additional data, not included in Figure 1, from testcrosses of sib $R^r r^g$ males enabled a valid distinction to be made between paramutagenic and nonparamutagenic R^{sc} mutants. Testcrosses of five $R^r R^{sc}96$ plants gave a mean aleurone color score of 5.01 ± 0.20 , and this mean was significantly lower than the mean score of 5.77 ± 0.10 obtained for testcrosses of four $R^r r^g$ sib plants. $R^{sc}96$ is, there-

fore, a paramutagenic allele. The next highest mean score was 5.24 ± 0.10 , obtained for testcrosses of five $R^rR^{sc}58$ plants. The latter mean is not significantly different from the mean of 5.26 ± 0.03 obtained for two R^rr^g sib plants. $R^{sc}58$ is classified, therefore, as nonparamutagenic.

Sixty-seven R^{sc} mutants from $R^{sc}126$ through $R^{sc}96$ in Figure 1 are shown by these data to be paramutagenic alleles. The mean aleurone color scores obtained for testcrosses involving them range from 2.06 to 5.01 and, collectively, the data show a continuous series of levels of paramutation of the single R^r gene employed in the tests. An equivalent statement, since a common R^r gene was used throughout, is that the several R^{sc} mutants show continuous variation in level of paramutagenic action.

The 67 paramutagenic R^{sc} mutants may be partitioned, somewhat arbitrarily, into three categories on the basis of the testcross results. These categories are not discontinuous groups but they serve to illustrate the general nature of the differences in paramutagenic action among the R^{sc} mutants.

The first group comprises 36 mutants which are paramutagenic at, or near, the level characteristic of stippled. The upper limit of mean aleurone color scores set for this group is dictated by the significance test, which shows that all means greater than 3.41 are significantly higher than the mean of 2.73 obtained for testcrosses of $F_1 R^rR^{st}$ plants. These mutants are strongly paramutagenic; and the mutation of stippled to self-colored has not resulted, in these instances, in detectable alteration of the paramutagenic property.

Of the 67 paramutagenic R^{sc} mutants recognized, 31 were characterized by mean scores ranging from 3.42 to 5.01. All were paramutagenic, since the pigment-producing action of R^r was significantly reduced in comparison with that of the standard R^r control in each test. Furthermore, the extent of alteration in the pigment-producing action of R^r was quantitatively less than that produced by stippled when similarly tested. Two groups may be recognized among these 31 R^{sc} mutants. Sixteen mutants, characterized by mean scores in the range 4.22 to 5.01, are not significantly different from each other, and comprise a group which may be described as very weakly paramutagenic. Fifteen mutants, characterized by mean scores in the range 3.42 to 4.22, comprise a residual group described as weakly paramutagenic. These two groups are not discontinuous, but the interesting fact is that the 31 R^{sc} mutants represent instances in which the mutation of stippled to self-colored has been accompanied by a significant quantitative alteration of the paramutagenic property in the direction of reduced paramutagenic action.

Concomitantly with the mutation of stippled to self-colored, therefore, the paramutagenic property may be unchanged, quantitatively reduced, or lost. Fifty-seven percent of the group of 83 R^{sc} mutants studied differed detectably from the parent allele, R^{st} , in paramutagenic action.

Photographs of testcross ears illustrating four general levels of paramutation of R^r are shown in Figure 2. Single testcross ears representative of the strong, weak, very weak, and nonparamutagenic categories recognized are arranged in

FIGURE 2.—Ears produced by testcrosses on W23 $r^g r^g$ females of four different $R^r R^{sc}$ plants, showing different levels of paramutation of R^r . From left to right, the levels of paramutation are:
 ear 1 strong paramutation ($R^r R^{sc126} \delta$)
 ear 2 weak paramutation ($R^r R^{sc69} \delta$)
 ear 3 very weak paramutation ($R^r R^{st99} \delta$)
 ear 4 no paramutation ($R^r R^{sc83} \delta$).

The $R^{sc} r^g r^g$ kernels are fully pigmented, and the $R^r r^g r^g$ kernels range from very lightly pigmented (ear 1), to dark mottled (ear 4).

order of decreasing level of paramutation (and hence in order of increasing level of aleurone pigmentation of the $R^r r^g r^g$ kernels) from left to right.

Coincidence of alteration in paramutagenicity with mutation of stippled to self-colored: Certain of the self-colored mutants provide evidence suggesting that, in the mutation of R^{st} , the alterations in aleurone phenotype and paramutagenicity are coincident changes consequent upon a single mutational event. ASHMAN (1960) showed that the rate of mutation of stippled to self-colored is relatively high (19.9×10^{-4}). It was not surprising to find, therefore, that in the present group of 83 R^{sc} mutants there were 15 instances in which a single parent stippled ear bore two or more independent self-colored mutants. Thirteen parental ears each bore two independent mutations, and two parental ears each bore three independent mutations. Comparison of the R^{sc} mutants derived from a common parental ear showed that in 12 of the 15 comparisons available there were differences in paramutagenic action.

This result is illustrated by the data obtained for the R^{sc} mutants belonging to four of the 15 groups of R^{sc} mutants with this origin (Table 1). A particularly clear example is provided by Group 3, comprising R^{sc} mutants 125, 126 and 127. R^{sc125} was nonparamutagenic, R^{sc126} was strongly paramutagenic, and R^{sc127} was very weakly paramutagenic. In this case three mutants independently derived from a single parental ear differed from each other in paramutagenic action.

This result precludes the possibility that differences in level of paramutagenic

TABLE 1

Mean aleurone color scores of R^rR^{sc} kernels from the $W23\ r^gr^g \times R^rR^{sc}$ matings involving R^{sc} mutants established from a common parental ear

Group	Pedigree number of R^{sc} mutant	Number of R^rR^{sc} plants tested	Mean aleurone color score	Level of paramutagenic action
1	64	8	2.69 ± 0.12	Strong
	65	7	5.61 ± 0.08	Nonparamutagenic
2	89	9	3.79 ± 0.21	Weak
	90	7	4.90 ± 0.19	Very weak
3	125	4	5.82 ± 0.04	Nonparamutagenic
	126	7	2.06 ± 0.05	Strong
	127	8	4.52 ± 0.12	Very weak
4	130	4	3.61 ± 0.26	Weak
	131	5	3.04 ± 0.28	Strong
	132	11	2.33 ± 0.08	Strong

action among the R^{sc} mutants reflect prior differences between parental stippled plants in paramutagenic action. If the latter condition obtained then all R^{sc} mutants from a single parental ear would be expected to exhibit the same level of paramutagenic action. The alteration in level of paramutagenic action, or loss of paramutagenicity, observed for a majority of the R^{sc} mutants studied must have occurred coincidentally with the mutation of stippled to self-colored. It appears, therefore, that alteration in aleurone phenotype and paramutagenicity are consequences of a single mutational event.

Innate variability of paramutation in R^rR^{sc} heterozygotes: Most R^{sc} mutants proved to be relatively uniform in paramutagenic action from plant to plant. Five of the 83 mutants tested, however, regularly exhibited high variability among sibs in the progenies derived from matings which precluded undetected cross contamination with other R^{sc} mutants. These mutants were $R^{sc}53$, $R^{sc}84$, $R^{sc}111$, $R^{sc}117$, and $R^{sc}120$.

Data showing the variable paramutagenic action of R^{sc} mutants 53, 111, and 117 are entered in Table 2; and the high variability characteristic of $R^{sc}111$ is illustrated in Figure 3 by representative testcross ears, whose mean aleurone color scores were 3.84, 4.13 and 5.12, obtained by testcrossing three sib $R^rR^{sc}111$ plants on $W23\ r^gr^g$ females.

The high variability within families was not a general characteristic, however, of very weakly, and weakly paramutagenic R^{sc} mutants. As shown in Table 2, other families within these classes, 820 ($R^rR^{sc}99$) and 994 ($R^rR^{sc}69$), for example, gave relatively uniform scores from plant to plant.

The $R^rr^gr^g$ kernels from testcrosses on r^gr^g females of either standard R^rR^r or $F_1\ R^rR^{sc}$ control plants, as the data in Table 3 show, form relatively homogeneous populations at the two respective levels of aleurone pigmentation. The strongly paramutagenic mutant, $R^{sc}126$ (family 831) in Table 2, similarly gave a relatively homogeneous population of near-colorless or very weakly pigmented $R^rr^gr^g$ kernels in $W23\ r^gr^g \times R^rR^{sc}126$ matings. Certain R^{sc} mutants, on the other

TABLE 2

Frequency distributions and mean aleurone color scores for the $R^r r^{sc}$ kernels from testcrosses on W23 $r^{sc} r^{sc}$ females of W22 $R^r R^{sc}$ plants

Staminate plant no.	Parent genotype	Aleurone color classes							Mean score	Type of R^{sc} mutant
		1	2	3	4	5	6	7		
831-1	$R^r R^{sc} 126$	1	91	7	1	..	..	..	2.08	Strongly paramutagenic
-4		..	76	24	..	..	..	..	2.24	
-7		..	81	18	1	..	..	..	2.20	
-13		..	90	10	..	..	..	..	2.10	
-17		..	90	10	..	..	..	..	2.10	
994-12	$R^r R^{sc} 69$	3	7	61	23	6	..	..	3.22	Weakly paramutagenic
-28		..	2	31	59	8	..	..	3.73	
-30		..	2	35	50	13	..	..	3.74	
-42		..	4	37	46	13	..	..	3.68	
-48		1	1	42	41	15	..	..	3.68	
820-3	$R^r R^{sc} 99$	..	..	3	23	70	4	..	4.75	Very weakly paramutagenic
-4		..	..	..	21	77	2	..	4.81	
-6		..	..	6	30	54	10	..	4.68	
-8		..	3	41	44	12	..	..	3.65	
-13		..	..	..	26	68	6	..	4.80	
813-4	$R^r R^{sc} 83$	..	..	..	..	19	81	..	5.81	Non-paramutagenic
-5		..	..	..	..	14	86	..	5.86	
-12		..	..	..	..	28	70	2	5.74	
-13		..	..	..	..	19	81	..	5.81	
-16		..	..	..	1	66	33	..	5.32	
637-4	$R^r R^{sc} 53$	1	18	43	21	17	..	..	3.35	Weak and variable in paramutagenic action
-5		..	46	48	6	..	..	..	2.60	
-6		..	2	30	44	22	2	..	3.92	
-11		..	3	21	55	20	1	..	3.95	
-17		..	..	..	9	73	18	..	5.09	
793-2	$R^r R^{sc} 111$	..	..	4	9	58	29	..	5.12	Weak and variable in paramutagenic action
-3		1	39	52	8	..	..	..	2.67	
-9		..	1	19	67	13	..	..	3.92	
-10		..	25	54	21	..	..	..	2.96	
-12		..	5	33	36	25	1	..	3.84	
698-1	$R^r R^{sc} 117$	..	6	41	37	16	..	..	3.63	Weak and variable in paramutagenic action
-5		..	1	9	17	51	22	..	4.84	
-8		2	16	33	28	17	4	..	3.54	
-11		..	..	10	40	48	2	..	4.42	
-16		1	19	60	16	4	..	..	3.03	

hand, characteristically gave a wide spectrum of $R^r r^{sc}$ phenotypes on each test-cross ear even though the mean scores differed little from ear to ear. Examples of such R^{sc} mutants are $R^{sc} 69$ and $R^{sc} 99$, as the data for families 994 and 820 in Table 2 show. An extreme case is the distribution from plant 698-8 ($R^r R^{sc} 117$) in Table 2 in which six classes, in terms of aleurone pigmentation, are represented on a single testcross ear.

Reproducibility of levels of paramutation given by R^{sc} mutants: The level of paramutation of R^r given by a particular $R^r R^{sc}$ heterozygote is highly reproducible, as demonstrated in two different experiments.

FIGURE 3.—Ears produced by testcrosses on W23 $r^g r^g$ females of $R^r R^{sc111}$ sib plants (family Mc793), illustrating the weak and variable paramutagenic action of the R^{sc111} mutant. The $R^{sc} r^g$ kernels are fully pigmented, and the $R^r r^g$ kernels range from light to dark mottled. The phenotype of the $R^r r^g$ kernels reflects differences between the three sib plants in level of paramutation.

TABLE 3

Frequency distributions and mean aleurone color scores for the $R^r r^g r^g$ kernels from testcrosses on W23 $r^g r^g$ females of $R^r R^{st}$ and $R^r R^r$ plants. Control matings for comparison with the data in Table 2

Staminate plant no.	Parent genotype	Aleurone color							Mean score
		1	2	3	4	5	6	7	
783-1	$R^r R^{st}$	..	90	10	..	..	..	..	2.10
-2		..	15	63	20	2	..	..	3.09
-3		..	76	24	..	..	..	..	2.24
-4		..	68	32	..	..	..	..	2.32
-5		..	83	17	..	..	..	..	2.17
803-2	$R^r R^r$	..	..	..	..	14	86	..	5.86
-3		..	..	..	..	9	91	..	5.91
-5		..	..	..	..	8	92	..	5.92
-7		..	..	..	..	9	91	..	5.91
-10		..	..	..	..	28	72	..	5.72

In the first experiment R^{sc} mutants distributed over all the levels of paramutagenic action disclosed by the initial test were again made heterozygous with standard R^r , and then were retested for paramutagenic action. The $R^r R^{sc}$ progenies involved in the second test were derived from either the mating $R^{sc} r^g \varnothing \times R^r R^r \delta$ or $R^{sc} r^r \varnothing \times R^r R^r \delta$. The $R^{sc} r^g$ and $R^{sc} r^r$ pistillate parent plants used were from backcross sublines of the R^{sc} mutants, developed from the original $R^{sc} r^g \varnothing \times R^r r^g \delta$ matings. In each pedigree R^{sc} had not previously been heterozygous with R^r . The second test for paramutagenic action was independent, therefore, of the initial test.

The results of the two series of tests are compared with each other in Figure 4. In interpreting the data allowance should be made for seasonal effects and level of incorporation in W22 inbred background, as well as possible changes in paramutagenic action of the R^{sc} mutant tested.

The correlation diagram (Figure 4) shows, however, that there was close correspondence between the results of the two independent tests. Seemingly the extraneous factors mentioned had little influence on level of paramutation. There are minor differences, however, between the results of the two tests, the most significant being with respect to $R^{sc}73$. This mutant was initially classified as nonparamutagenic, but in the second test it yielded a mean score for five $R^rR^{sc}73$ plants of 4.83 ± 0.06 , which was significantly lower than the mean of 5.38 ± 0.10

FIGURE 4.—Correlation diagram relating the mean aleurone color scores obtained for the $R^r r^g r^g$ kernels from $W23\ r^g r^g\ ♀ \times R^r R^{sc}\ ♂$ matings in two independent tests of the paramutagenic action of R^{sc} mutants. The numbers at each of the experimental points identify the R^{sc} mutants tested.

obtained for testcrosses of five $R^r r^r$ sib plants. $R^{sc}73$, therefore, may be very weakly paramutagenic, rather than nonparamutagenic as first concluded. Minor differences are apparent also with certain other R^{sc} mutants (e.g., $R^{sc}126$ and $R^{sc}69$) but, in general, results of the second test do not significantly alter the classification initially arrived at.

It is noteworthy that the difference in aleurone color score between the weakly paramutagenic and very weakly paramutagenic groups of R^{sc} mutants was observed in both tests. R^{sc} mutants 69, 76, 111 and 128 (weakly paramutagenic) and R^{sc} mutants 63, 99, 110, 129 and 134 (very weakly paramutagenic) form nonoverlapping and significantly different groups in the second test, and both groups gave significantly higher mean scores than the F_1 $R^r R^{sc}$ control. The general significance of this fact, and of the data in the correlation diagram as a whole, is that the seriation of R^{sc} mutants on the basis of paramutagenic action is remarkably similar in the two tests. The results show unequivocally that there are innate differences among the R^{sc} mutants in level of paramutagenic action.

All 16 members of the nonparamutagenic group of R^{sc} mutants were included in both tests. Each, with the exception of $R^{sc}73$, was again found to be nonparamutagenic in the second test. The numerical scores for five of these R^{sc} mutants are included in Figure 4; the remainder were evaluated by visual examination of the intact testcross ears.

The second experiment had the dual objectives of examining the reproducibility of paramutagenic action and of determining whether paramutagenic action of the mutants was depleted by maintaining the latter in heterozygotes with R^r . The progenies testcrossed were obtained by backcrossing $R^r R^{sc}$ F_1 plants with $R^r R^r$ or $R^{95} r^r$ male parents. R^{95} is a colored aleurone, green plant-color mutant derived from standard R^r , and is closely comparable to standard R^r in aleurone-pigmenting action and paramutability (BRINK, BROWN, KERMICLE and WEYERS 1960). Remnant seed from the ear which produced the $R^r R^{sc}$ F_1 progenies and seeds from the various backcross matings were grown out, and the resulting plants were then testcrossed on W23 $r^9 r^9$ pistillate parent plants. The R^{sc} mutants tested in this experiment were $R^{sc}99$ (very weakly paramutagenic), $R^{sc}126$ and $R^{sc}132$ (strongly paramutagenic), and $R^{sc}111$ (weak and variable in paramutagenic action). The results are given in Table 4. The data for each of the four groups of progenies were examined by analysis of variance; and the F value was found to be nonsignificant in each case. Thus it was again observed that a particular level of paramutation is a specific and reproducible property of a given $R^r R^{sc}$ (or $R^{95} R^{sc}$) heterozygote. Moreover, the property of paramutagenicity, even though it may initially be very weak ($R^{sc}99$), was found not to be depleted by virtue of maintenance of the $R^r R^{sc}$ ($R^{95} R^{sc}$) heterozygous condition for two generations.

The $R^{sc}111$ mutant was previously cited as weak and variable in paramutagenic action (see Figure 3). Families Mc797 and Mc798 in Table 4 formed part of an experiment in which an attempt was made to select subfamilies differing in paramutagenic action from within a given variable family. The $R^r R^{sc}111$ pistillate parent plants from which families Mc797 and Mc798 were derived gave

TABLE 4

Mean aleurone color scores for the R^rR^sc and R^scR^sc kernels from the matings $W23\ r^gr^g \times R^rR^sc \delta$ and $W23\ r^gr^g \times R^scR^sc \delta$ involved in tests for depletion of paramutagenic action

Family no.	Staminate parent		No. of plants tested	Family mean score
	Genotype	Derived from mating:		
Mc767	$R^rR^sc99\ F_1$	$R^sc99\ r^g \times R^rR^r \delta$	8	4.66 ± 0.06
Mc751	$R^g5\ R^sc99$	$R^rR^sc99\ F_1 \times R^g5\ r^r \delta$	5	4.60 ± 0.22
Mc769	R^rR^sc99	$R^rR^sc99\ F_1 \times R^rR^r \delta$	8	4.83 ± 0.06
Mc770	R^rR^sc99	$R^rR^sc99\ F_1 \times R^rR^r \delta$	8	4.78 ± 0.05
Mc771	$R^rR^sc126\ F_1$	$R^sc126\ r^r \times R^rR^r \delta$	6	2.96 ± 0.18
Mc773	R^rR^sc126	$R^rR^sc126\ F_1 \times R^rR^r \delta$	8	2.79 ± 0.22
Mc746	$R^g5\ R^sc126$	$R^rR^sc126\ F_1 \times R^g5\ r^r \delta$	8	2.48 ± 0.08
Mc774	$R^rR^sc132\ F_1$	$R^sc132\ r^r \times R^rR^r \delta$	8	2.56 ± 0.07
Mc775	$R^g5\ R^sc132$	$R^rR^sc132\ F_1 \times R^g5\ r^r \delta$	8	2.95 ± 0.12
Mc776	R^rR^sc132	$R^rR^sc132\ F_1 \times R^rR^r \delta$	8	2.75 ± 0.17
Mc796	$R^rR^sc111\ F_1$	$R^sc111\ r^r \times R^rR^r \delta$	6	3.46 ± 0.20
Mc797	$R^g5\ R^sc111$	$R^rR^sc111\ F_1 \times R^g5\ r^r \delta$	10	3.26 ± 0.18
Mc798	$R^g5\ R^sc111$	$R^rR^sc111\ F_1 \times R^g5\ r^r \delta$	10	3.38 ± 0.19

$W23\ r^gr^g \times R^rR^sc111 \delta$ testcross ears with mean aleurone color scores of 2.99 and 4.41, respectively. That this form of selection was ineffective in altering the level of paramutation is shown by the fact that the mean aleurone color scores in the resulting R^g5R^sc111 progenies were 3.26 ± 0.18 and 3.38 ± 0.19 , respectively. Both families, Mc797 and Mc798, exhibited wide variability between sib plants in level of paramutation, as in the parent family also. Five other progenies, of R^rR^sc111 genotype, showed similar variability between sib plants within families. This evidence leads to the conclusion that a weak and variable paramutagenic action is an inherent property of the R^sc111 mutant.

Since testcrosses using pollen from different tassel branches of the same plant occasionally give unlike results, BRINK (1960) suggested that some R^rR^sc plants are chimeras, in which paramutation had proceeded to varying levels in different parts of the staminate inflorescence. H. B. COOPER, JR. demonstrated that certain R^rR^sc117 plants (see Table 2) were chimeras of the anticipated kind. (See BRINK 1960) Chimerism for level of paramutation is thus at least a partial explanation for the variability between sib plants observed with certain R^rR^sc progenies. R^sc mutants of variable paramutagenic action are not, however, easily distinguishable in routine tests from other R^sc mutants. An analysis of the conditions promoting chimerism and extension of the studies to include other R^rR^sc heterozygotes may be expected to show whether the phenomenon of chimerism for level of paramutation is restricted to a certain class of R^rR^sc heterozygotes.

Subsequent paramutability of R^r alleles derived from R^rR^sc heterozygotes: R^r alleles extracted from R^rR^sc heterozygotes (referred to as $R^{r:sc}$) show a high degree of sensitivity to paramutation when subsequently tested in $R^{r:sc}/R^{st}$ heterozygotes. The tests upon which this conclusion is based establish the validity of the assumption that the different levels of paramutation of R^r , observed in

testcrosses of R^rR^{sc} heterozygotes, are not a consequence of prior differences in the paramutability of R^r .

$R^{r:sc}/R^{st}$ plants in which $R^{r:sc}$ was derived directly from an R^rR^{sc} heterozygote were testcrossed on W23 $r^g r^g$ females. $R^{r:sc}$ alleles from R^rR^{sc} heterozygotes involving weakly, very weakly, and nonparamutagenic R^{sc} mutants, were represented. The data obtained are reported in Table 5. Also included in Table 5 are

TABLE 5

Mean aleurone color scores for the $R^{r:sc}r^g$ and $R^r r^g$ kernels from testcrosses on W23 $r^g r^g$ females of W22 $R^{r:sc}/R^{st}$, R^rR^{sc} , $R^{r:sc}/r^g$, $R^r r$ and R^rR^{st} plants, demonstrating the uniform paramutability of $R^{r:sc}$ alleles

Staminate parent			No. of plants tested	Mean score	Significance of differences
Family no.	Genotype	Derived from mating:			
A. <i>R^{sc99}</i> —very weakly paramutagenic*					
Mc233	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc99} F₁ ♀ × Rst<i>r^g</i> ♂</i>	6	3.24	All differences significant at one percent level‡
Mc233	<i>R^{r:sc}<i>r^g</i></i>		6	4.94	
Mc234	<i>R^rR^{sc99} F₁</i>	<i>R^{sc99} r^g ♀ × R^r<i>r^g</i> ♂</i>	7	4.33	
Mc234	<i>R^r<i>r^g</i></i>		8	5.54	
B. <i>R^{sc110}</i> —very weakly paramutagenic*					
Mc398	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc110} F₁ ♀ × Rst<i>r^g</i> ♂</i>	4	3.16	All differences significant at one percent level‡
Mc399	<i>R^rR^{sc110} F₁</i>	<i>R^{sc110} r^g ♀ × R^rR^r ♂</i>	7	4.74	
Mc399	<i>R^r<i>r^g</i></i>		6	5.72	
C. <i>R^{sc89}</i> —weakly paramutagenic*					
Mc390	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc89} ♀ × Rst<i>r^g</i> ♂</i>	5	3.01	Significant at one percent level <i>t</i> ₃ = 8.04
Mc390	<i>R^{r:sc}<i>r^g</i></i>		4	4.79	
D. <i>R^{sc128}</i> —weakly paramutagenic*					
Mc426	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc128} ♀ × Rst<i>r^g</i> ♂</i>	5	2.29	Significant at one percent level <i>t</i> ₃ = 5.85
1-24	<i>R^rR^{sc128}</i>	<i>R^{sc128} r^r ♀ × R^rR^r ♂</i>	4	3.40	
E. <i>R^{sc106}</i> —nonparamutagenic*					
Mc240	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc106} ♀ × Rst<i>r^g</i> ♂</i>	6	2.98	Difference significant at one percent level <i>t</i> ₄ = 7.7
Mc239	<i>R^rR^{sc106}</i>	<i>R^rR^{sc106} ♀ × R^rR^r ♂</i>	5	5.30	
Mc240	<i>R^{r:sc}<i>r^g</i></i>	<i>R^rR^{sc106} ♀ × Rst<i>r^g</i> ♂</i>	3	5.54	
F. <i>R^{sc65}</i> —nonparamutagenic					
Mc200*	<i>R^rR^{sc65} F₁</i>	<i>R^{sc65} r^r ♀ × R^rR^r ♂</i>	4	5.96	Difference significant at five percent level <i>t</i> ₆ = 3.7
Mc200*	<i>R^r<i>r^r</i></i>		4	5.86	
Mc780‡	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc65} F₁ ♀ × RstRst ♂</i>	8	2.85	Difference not significant <i>t</i> ₁₄ = 0.78
Mc781‡	<i>R^rRst</i>	<i>R^r<i>r^r</i> ♀ × RstRst ♂</i>	8	2.98	
G. <i>R^{sc83}</i> —nonparamutagenic					
Mc204*	<i>R^rR^{sc83} F₁</i>	<i>R^{sc83} r^r ♀ × R^rR^r ♂</i>	5	5.93	Difference not significant <i>t</i> ₇ = 0.9
Mc204*	<i>R^r<i>r^r</i></i>		4	5.98	
Mc782‡	<i>R^{r:sc}/Rst</i>	<i>R^rR^{sc83} F₁ ♀ × RstRst ♂</i>	10	3.52	Difference significant at one percent level <i>t</i> ₁₈ = 3.90
Mc793‡	<i>R^rRst</i>	<i>R^r<i>r^r</i> ♀ × RstRst ♂</i>	10	2.59	

* 1959 data.

† 1960 data.

‡ By Duncan's multiple range test.

the results of testcrosses of (1) R^rR^{sc} plants which serve to establish the level of the initial alteration in R^r phenotypic effect, (2) $R^{r:sc}/r^g$ plants, showing the level of phenotypic effect of the paramutant $R^{r:sc}$ alleles and (3) R^rr plants which provide for a comparison with the pigment-producing action of unchanged standard R^r .

The data in Table 5 show that the $R^{r:sc}$ alleles extracted from the heterozygotes R^rR^{sc65} , R^rR^{sc83} , R^rR^{sc106} , R^rR^{sc99} , R^rR^{sc89} , R^rR^{sc110} and R^rR^{sc128} , were reduced to a fairly uniform, low level of pigment-producing action by subsequent passage through a heterozygote with R^{st} . Initially, in the R^rR^{sc} heterozygotes, R^r was either not altered, or was only slightly reduced, in pigment-producing action. Thus the initial differential behavior of R^r in various R^rR^{sc} heterozygotes was not reflected by differences in the paramutability of the $R^{r:sc}$ alleles in $R^{r:sc}/R^{st}$ descendants. Since there were no differences in paramutability these data show that paramutation of R^r in a given instance is not necessarily terminal, in the sense that weak paramutation does not preclude a strongly paramutagenic allele such as stippled from subsequently further reducing the pigment-producing potential of R^r in an $R^{r:sc}/R^{st}$ heterozygote. A direct comparison of the paramutability of $R^{r:sc}$ alleles extracted from nonparamutagenic R^rR^{sc} heterozygotes with standard R^r is provided by the data in sections F and G of Table 5. R^rR^{sc65} and R^rr sibs, and also R^rR^{sc83} and R^rr sibs, were crossed with $R^{st}R^{st}$ plants from a common subfamily. The respective $R^{r:sc}/R^{st}$ and R^rR^{st} progenies were then testcrossed on W23 $r^g r^g$ females. It is apparent from the results that there was no paramutation of R^r in either of the R^rR^{sc} heterozygotes. Testcrosses of $R^{r:sc}/R^{st}$ plants, however, showed that $R^{r:sc}$ in both instances was sensitive to paramutation. $R^{r:sc}$ and R^r (control) were equally paramutable in the case of $R^{r:sc}$ extracted from an R^rR^{sc65} plant.

The result entered in section G in Table 5 with $R^{r:sc}$ extracted from an R^rR^{sc83} plant is noteworthy in that the highly significant difference between the scores for testcrosses of $R^{r:sc}/R^{st}$ and R^rR^{st} plants suggests that the paramutability of $R^{r:sc}$ was slightly reduced by virtue of prior association of R^r with the nonparamutagenic R^{sc83} mutant. Reproducibility of this result, however, has not been tested. The only clearly established fact is that $R^{r:sc}$ extracted from the nonparamutagenic R^rR^{sc83} heterozygote was paramutable to a high degree. Reduced paramutability was clearly not characteristic of all $R^{r:sc}$ alleles extracted from R^rR^{sc} heterozygotes involving nonparamutagenic R^{sc} mutants, as the data in Table 5 for $R^{r:sc}$ alleles extracted from R^rR^{sc65} , and R^rR^{sc106} heterozygotes show. Moreover, there was no suggestion of reduced paramutability of $R^{r:sc}$ alleles extracted from heterozygotes with eight additional nonparamutagenic R^{sc} mutants similarly tested, but not scored in detail. Without direct, confirmatory data, therefore, it cannot safely be concluded that the R^{sc83} mutant tends to make R^r somewhat refractory to paramutation in R^rR^{sc} heterozygotes.

The heritable nature of paramutation in R^rR^{sc} heterozygotes: Transmission through subsequent sporophytes of the changes in R^r arising in R^rR^{st} and R^rR^{mb} heterozygotes has been well established earlier (BRINK 1958, 1960; BRINK and WEYERS 1957). In the present series of experiments tests with $R^{r:sc}$ alleles were

made to determine whether (1) paramutation in these cases was similarly heritable, and (2) the considerable diversity of $R^r r^g r^g$ kernel phenotypes observed in individual $r^g r^g \times R^r R^{sc}$ matings persisted through the next following sporophyte generation.

The tests were based on the progeny from four ears resulting from different W22 $r^g r^g \times$ W22 $R^r R^{sc}$ matings. That is, both parents in the first testcross were W22 inbred strains. The distributions and mean scores obtained for the $R^r r^g r^g$ kernels from these matings are given in Table 6. Kernels from the several color classes in each mating subsequently were grown out, and samples of the resulting group of $R^{r:sc}/r^g$ plants were then testcrossed on W23 $r^g r^g$ females for evaluation of the pigment-producing action of the respective $R^{r:sc}$ alleles in the second generation. The data obtained from scoring the $R^{r:sc}/r^g r^g$ kernels from the latter W23 $r^g r^g \times R^{r:sc}/r^g$ testcrosses are entered in Table 7.

A complicating factor in interpreting the results is the partial reversion of a paramutant allele toward the standard R^r level of pigment-producing action that occurs after the first testcross generation, provided $R^{r:sc}$ is heterozygous with a nonparamutagenic R allele. This phenomenon, which is an established feature of $R^{r:st}$ and $R^{r:mb}$ paramutants (BRINK 1958) is in evidence with $R^{r:sc}$ paramutants also, and leads to more overlapping of phenotypes in the second than in the first, testcross generation.

The results in Table 7 show that the changes in R^r pigment-producing action arising in $R^r R^{sc}$ heterozygotes, like those in $R^r R^{st}$ plants, persist in some degree through the immediately following sporophytic generation. Ten $R^r r^g$ control plants, derived from the mating W22 $r^g r^g \times R^r R^r$, used in testcrosses on W23 $r^g r^g$ females gave a mean aleurone color score of 5.91 ± 0.03 (Table 7). All the $R^{r:sc}/r^g$ progenies gave values significantly lower than this figure when the data were tested by Dunnett's procedure, with adjustment for unequal family size (STEEL and TORRIE 1960, page 114). Thus, in the case of the initial matings on W22 $r^g r^g$ females, disclosing weak or very weak levels of paramutation all, or nearly all, of the $R^{r:sc}$ alleles involved reacted in the W23 $r^g r^g \times R^{r:sc}/r^g$ testcrosses as paramutants. The data are not sufficiently extensive to prove, however, that paramutation invariably occurs in all such instances.

TABLE 6

Distributions of $R^r r^g r^g$ kernels in aleurone color classes and mean aleurone color scores for four W22 $r^g r^g \times$ W22 $R^r R^{sc}$ matings

Mating	Staminate parent		Aleurone color classes							Mean score
	Plant no.	Genotype	1	2	3	4	5	6	7	
A	Mc462-3	$R^r R^{sc*}$	..	40	39	19	2	..	..	2.83
B	1-16-11	$R^r R^{sc76}$	..	16	28	38	18	..	..	3.60
C	1-16-16	$R^r R^{sc76}$	..	..	10	32	52	6	..	4.54
D	1-29-17	$R^r R^{sc134}$	..	..	..	5	37	58	..	5.53

* The R^{sc} mutant used in this mating had not been assigned a number.

TABLE 7

Mean aleurone color scores for the $R^r r^{sc}$ kernels from testcrosses on W23 $r^{sc} r^g$ females of $R^r r^{sc} r^g$ plants derived from the W22 $r^{sc} r^g \varnothing \times R^r R^{sc} \delta$ matings described in Table 5, and $R^r r^{sc}$ plants (control) derived from the mating W22 $r^{sc} r^g \varnothing \times R^r R^r \delta$

$R^r r^{sc} r^g$ staminate parents derived from mating:		Aleurone color class of parental $R^r r^{sc} r^g$ kernels			
		2	3	4	5
A	No. plants tested	6	6	6	6
	Mean score	3.54	4.55	4.44	...
	Significance test*	± 0.32	± 0.20	± 0.14	...
	(five percent level)				
B	No. plants tested	8	8	8	8
	Mean score	3.80	4.35	4.94	4.97
	Significance test*	± 0.14	± 0.14	± 0.08	± 0.04
	(one percent level)				
C	No. plants tested	...	5	5	5
	Mean score	...	4.49	4.51	4.74
	Significance test*	...	± 0.15	± 0.32	± 0.17
	(five percent level)				
D	No. plants tested	...	...	...	10
	Mean score	...	...	...	4.87
	Significance test	...	...	...	± 0.11
			$t_{18} = 2.55$	P 0.05	± 0.08
E (control)					
	No. plants tested	...	...	...	10
	Mean score	...	...	...	$5.91 \pm 0.03^\dagger$

* Means underscored are not significantly different by Duncan's multiple range test.

† All $R^r r^{sc} r^g$ progeny mean scores were significantly lower than the control by Dunnett's procedure.

Comparison of groups of $R^r r^{sc}/r^g$ sib plants within the progeny of individual W22 $r^g r^g \varnothing \times R^r R^{sc} \delta$ matings shows that in three of the four sets of data in Table 7 there were significant differences between groups. The mean scores for the group of $R^r r^{sc}/r^g$ progeny are in the same relation as the aleurone phenotypes of the parental $R^r r^g$ kernels. This result shows that the $R^r r^{sc}$ alleles from an individual W22 $r^g r^g \varnothing \times R^r R^{sc} \delta$ mating are not homogenous with respect to pigment-producing action. Thus at least part of the diversity of phenotypes of $R^r r^g$ kernels in the W22 $r^g r^g \varnothing \times R^r R^{sc} \delta$ matings was due to differential alteration of R^r in each of the respective $R^r R^{sc}$ male plants.

Comparison of the data from the coordinate matings A to D in Tables 6 and 7 shows that the differences in level of R^r paramutation observed when $R^r R^{sc}$ plants carrying particular R^{sc} mutant alleles are directly testcrossed on $r^g r^g$ females are also detectable when the $R^r r^{sc}/r^g$ progeny possessing the respective paramutant $R^r r^{sc}$ factors are similarly evaluated for aleurone-pigmenting action. It is evident, however, that the $R^r r^{sc}/r^g$ progeny tests are less discriminating in

disclosing different levels of paramutation than are direct tests of the respective R^rR^{sc} heterozygotes.

DISCUSSION

The most significant observation in the present studies is that the R^r allele, in heterozygotes with a series of self-colored mutants from stippled, gives rise to paramutant forms varying continuously over a wide range in level of pigment-producing action. This variation in the single R^r gene under test was primarily disclosed by quantitative differences in aleurone-pigmenting effect between the R^r paramutants extracted from different R^rR^{sc} heterozygotes. The variation clearly relates to the degree to which R^r aleurone color expression is altered, and not to the frequency with which a given single kind of change occurs. This is evident from the distributions of kernels according to aleurone phenotype recorded in Table 2.

The differences in level of paramutation of R^r in the various R^rR^{sc} heterozygotes, likewise, were found to be gametically transmissible. This is shown by the data in Table 4 and Figure 4, and by the data in Table 2, which demonstrate that $R^{r:sc}$ alleles extracted from R^rR^{sc} heterozygotes were heritably altered in pigment-producing potential to varying degrees. Moreover, the differential behavior of R^r in the various R^rR^{sc} heterozygotes was not due to inherent initial differences in paramutability of R^r (Table 5).

Thus the evidence shows that the R^r allele has the capacity to respond to paramutagenic factors in a wide variety of ways. A corollary of this result is that the R^{sc} mutants in question vary continuously in paramutagenic action.

BRINK, BROWN, KERMICLE and WEYERS (1960) inferred that when paramutation occurs a chromosome component affecting R^r expression located at, or near, the R^r locus is altered. The present studies show that the alteration is not of an all-or-none kind, but can vary continuously in magnitude. The degree of alteration that occurs in a given case is a specific reflection of the level of paramutagenic action characteristic of the particular R^{sc} mutant with which R^r is associated in an R^rR^{sc} heterozygote.

Paramutation of R^r to some intermediate level of pigment-producing action does not alter the sensitivity of R^r to further heritable change in the direction of reduced pigment-producing potential. This fact is evident from the data in Table 5, which show that weakly paramutant $R^{r:sc}$ alleles, extracted from various R^rR^{sc} heterozygotes, were rather uniformly reduced to a low level of pigment-producing action when subsequently tested in heterozygotes with the strongly paramutagenic stippled factor. Maintenance of R^r heterozygous with a single R^{sc} mutant through successive generations similarly has been found to result in an increase in the degree of alteration of R^r (McWHIRTER 1961). The latter experiments, in which testcrosses of F_1 , F_2 , and F_3 R^rR^{sc} heterozygotes were examined, showed that maintenance of the R^r allele heterozygous with R^{sc99} (very weakly paramutagenic) for three sporophytic generations led ultimately to a level of paramutation approximately equal to that observed after one generation of heterozygosity of R^r with R^{sc69} (weakly paramutagenic). Similarly, testcrosses

of F_2 $R^rR^{sc}69$ heterozygotes showed a level of paramutation of R^r about equivalent to that produced by F_1 $R^rR^{sc}126$ plants, $R^{sc}126$ being a strongly paramutagenic allele. These results suggest that the paramutagenic action on R^r of different R^{sc} mutants derived from R^{st} is qualitatively the same, and that the differences between them is in the rate at which a common kind of change in R^r is produced. Since paramutation occurs in vegetative tissues (BRINK 1960; MCWHIRTER 1961) this may mean that the differences between R^{sc} mutants in net paramutagenic action in R^rR^{sc} plants is referable to differences in the extent of alteration of R^r that occurs in each somatic cell generation. It is also possible that all paramutagenic R^{sc} mutants, and R^{st} , produce the same degree of alteration in R^r per somatic cell generation, and that the differences are referable to the number of somatic cell generations in which the paramutagenic component is active in a given case. The effects are averaged, however, over an entire sporophytic generation by the restriction that only the male inflorescence at the apex of the mature plant may be sampled in measuring the progress of the alteration in R^r . Neither the materials nor the techniques are available at present, however, for ascertaining the course of R paramutation during vegetative development of the plant, so that such a possibility cannot be examined in detail.

The 83 R^{sc} mutants studied for paramutagenic action were derived from the uniformly highly paramutagenic stippled allele. Comparison of the R^{sc} mutants with stippled shows that coincidentally with the mutation of stippled to self-colored the paramutagenic property associated with stippled may be unchanged, quantitatively reduced, or lost entirely. Mutation of R^{st} to R^{sc} without apparent alteration in the paramutagenic property shows that paramutagenicity is separable from the stippled phenotype at the R locus. Paramutagenicity at a high level may be associated with an unstable R allele (stippled) or a stable one (self-colored). ASHMAN's (1960) derivation of paramutagenic r^g and r^r mutants from R^rR^{st} plants earlier provided evidence of separability of paramutagenicity from the colored aleurone phenotype.

A significant fact of additional interest evident in the present data is that in a high proportion of instances, mutation of R^{st} to R^{sc} was accompanied by alteration of the paramutagenic property. A detectable reduction, or apparent loss, of paramutagenicity was found for 57 percent of the group of 83 R^{sc} mutants studied. The coincidence of alterations also appeared to be a consequence of a single mutational event (Table 1). The possibility that alteration in paramutagenicity may occur subsequently to the mutation of stippled to self-colored is discounted by the evidence that paramutagenicity of R^{sc} mutants was not depleted by successive passages through heterozygotes with R^r or R^g (Table 4).

There is evidence that this coincidence of alterations is not due to association of mutation of stippled with crossing-over in the R region. ASHMAN (1960) found no relation between mutation of R^{st} to R^{sc} and crossing-over in the region marked by golden-1, about 14 units proximal to the R locus. The possibility of an association with distal crossing-over was examined in the present study, using the closely linked knob on abnormal chromosome K10 as a marker (1-2 percent of crossing-over with R). Seven independent mutations of R^{st} to R^{sc} were obtained

following $R^{sc}K10/r^g \varphi \times r^r r^r \delta$ matings, all of which proved to be noncrossovers, $R^{sc}K10/r^r$. Significantly, three of the $R^{sc}K10$ mutants (of four tested) proved to have reduced paramutagenic action as compared with the parent $R^{st}K10$ stock. Attempts have been made also to separate paramutagenicity from the stippled allele by crossing-over in the regions proximal and distal to R in the absence of mutation of stippled. These attempts, which involved recovering several hundred crossovers between R^{st} and the outside markers, and subsequently testing them for paramutagenic action, have been unsuccessful. The possibility was not excluded, however, that paramutagenicity and the aleurone spotting pattern characteristic of stippled sometimes may be *partially* separated by crossing-over in the K region (H. B. COOPER, JR., and R. A. BRINK, unpublished). However this may be, it is clear from the present evidence that paramutagenicity of the R^{st} allele, and of R^{sc} mutants from R^{st} , is intimately associated with the R locus, and may vary independently of detectable crossing-over in that region.

The coincident alterations in aleurone pigmentation and paramutagenicity can be formally explained in terms of a transposable, nonspecific inhibitor assumed to be a regular component of the stippled allele. It might be supposed that stippled consists of an R^g gene (self-colored aleurone, green seedling) in conjunction with an inhibitor, I^R , that suppresses R^g pigment-producing action. Mutation of R^{st} to R^{sc} , on this hypothesis, arises by transposition of I^R from R^{st} to one or another site elsewhere in the genome, thus permitting full color expression of the R^g gene. ASHMAN (1960) reported that gametically transmissible mutations of R^{st} to R^{sc} occurred at the rates of 17.0×10^{-4} and 19.9×10^{-4} in two different experiments. The densely spotted aleurone pattern characteristic of stippled endosperm is assumed to reflect a high frequency of transpositions of I^R in this tissue. Except in the endosperm, however, the postulated I^R factor transposes infrequently, although at a rate still within the known range of other elements of this kind in maize (BRINK 1954; McCLINTOCK 1961).

Paramutagenicity of an R^{st} chromosome may be assumed to depend upon an element distinct from, but very closely linked with, the R^g component of stippled. The paramutagenic element is unaffected by I^R in an R^{st} chromosome, and so is fully active. When, however, I^R transposes from R^{st} , the new chromosome site at which it is inserted in many instances may be that of the paramutagenic element. In such a case I^R may be supposed to act as a suppressor, not of aleurone color, but of paramutagenicity.

A precedent for the postulate of short-distance transpositions of I^R is afforded by Modulator (Mp) in variegated pericarp of maize. VAN SCHAİK and BRINK (1959) found that of 83 independently occurring Mp transpositions from the P locus, 25 were to points within approximately one crossover unit of the initial position.

Three general classes of consequences may be visualized with reference to suppression of paramutagenicity after I^R transposes from R^{st} to give the self-colored (R^{sc}) aleurone phenotype. (1) The new site of I^R is distinct from that of the chromosomal component concerned with paramutagenicity. Paramutagenicity is then unaltered by the mutation of R^{st} to R^{sc} . Thirty-six of the

83 R^{sc} mutants tested in the present study would be in this category. (2) The new site of I^R is either adjacent to, or so close to, that of the paramutagenic factor, that the action of the latter in $R'R^{sc}$ heterozygotes is completely suppressed. This consequence of the transposition of I^R would be represented by the group of nonparamutagenic mutants, comprising 16 of the R^{sc} alleles. (3) Transposition of I^R is to sites adjacent to that of the paramutagenic component, but with a different consequence to that occurring in (2), such that partial expression of paramutagenicity occurs in $R'R^{sc}$ heterozygotes. I^R may either form a stable association with the paramutagenic factor, resulting in partial suppression of paramutagenicity, or the association of I^R with the paramutagenic factor may be unstable. In the latter event, partial suppression of paramutagenicity would be the apparent result, since paramutation would occur only in cell lineages in which retransposition of I^R restores full expression of paramutagenicity. The 31 R^{sc} mutants of weak and very weak paramutagenic action belong in this category.

The properties attributed to the postulated transposable element, I^R , and the subsequent interpretation of the variation in paramutagenic action of different R^{sc} mutants from R^{st} is reasonable by analogy with the known properties of other transposable factors in maize (McCLINTOCK 1956, 1961; BRINK 1960; PETERSON 1961). Direct evidence validating it in the case of stippled, however, is lacking. A present handicap in testing the hypothesis is that no criterion, other than paramutation itself, has been found whereby the putative transposable I^R factor is recognizable except when situated at the R locus.

The hypothesis leads, however, to certain predictions. By analogy with the behavior of Modulator in variegated pericarp (ORTON 1960) it would be expected that stippled would be reconstituted occasionally in R^{sc} plants by transposition of I^R back to the position characteristic of stippled. McWHIRTER (1961) has tested this possibility on a rather large scale, but with essentially negative results. None of the 17 mutants from 1,138,569 R^{sc} gametes tested gave the stippled phenotype, although certain of them phenotypically resembled the near-colorless mutants (ASHMAN 1960) which have been obtained directly from stippled plants. Additionally, it might be expected that coincident alteration of a stable marker gene and of stippled might occur occasionally if a suitable marker was situated close to R^{st} . No such marker is at present available, but a useful test system might be synthesized by the use of a reciprocal translocation. Finally, it may be possible to duplicate the present results by means of first obtaining a transposition of Mp from the variegated pericarp locus to the R^g component of an R^{sc} mutant of strong paramutagenic action. In the meantime, however, the chromosomal basis of the coincidence in alterations of aleurone phenotype and paramutagenicity remains unknown.

SUMMARY

Eighty-three R^{sc} mutants (self-colored aleurone, green seedling) from the strongly paramutagenic R^{st} allele (stippled aleurone, green seedling) were characterized for paramutagenic action by testcrossing the respective $R'R^{sc}$

heterozygotes (all containing standard R^r : colored aleurone, red seedling) on $r^g r^g$ females (colorless aleurone, green seedling).

The R^{sc} mutants were found to vary continuously in level of paramutagenic action from high levels not significantly different from that of the parent R^{st} factor (strongly paramutagenic) to no detectable effect on the pigment-producing action of R^r (nonparamutagenic). There were 16 mutants of the latter class, and 36 of the former. Each of the remaining 31 R^{sc} alleles was paramutagenically active at one or another intermediate level. The level of paramutation for a given $R^r R^{sc}$ combination was highly reproducible, and was not altered when the R^{sc} allele was maintained in heterozygous condition by successive backcrosses to $R^r R^r$ or $R^{95} r^r$ plants.

The results show that the aleurone pigment-producing potential of standard R^r may be reduced to any one of a graded series of levels, each of which is then gametically transmissible.

The pigment-producing potential of R^r is reduced in two steps when the allele is first passed through a heterozygote carrying a weakly paramutagenic R^{sc} mutant and then through a heterozygote with the strongly active R^{st} factor.

The occasional occurrence on single ears from $R^{st} R^{st} \text{♀} \times r^r r^r \text{♂}$ matings of two or more independent self-colored mutants from stippled that are subsequently found to differ significantly in level of paramutagenic action suggests that change in aleurone pigmentation and paramutagenicity are coincident events.

Crossing-over in the vicinity of the R locus as the basis of the coincident changes was excluded.

The simultaneous alteration in aleurone pigmentation and paramutagenicity in mutation of R^{st} to R^{sc} may be formally interpreted in terms of transposition of an assumed component of the stippled allele that inhibits aleurone color when in one position and paramutagenic action in variable degree when at one or another particular site nearby. An attempt to verify this hypothesis by screening more than a million gametes from $R^{sc} R^{sc}$ plants for back mutations to stippled (i.e., transpositions of the assumed inhibitor from its new site back to old position) gave a negative result. Seventeen mutations of R^{sc} were observed, but none conditioned the stippled phenotype.

ACKNOWLEDGMENTS

This study was supported by grants from the Research Committee of the Graduate School, University of Wisconsin, of funds supplied by the Wisconsin Alumni Research Foundation, and by grants from the National Science Foundation. The work was carried out during tenure, by K. S. McWHIRTER, of the Overseas Research Scholarship of the Sir Benjamin Fuller Trust, Government House, Sydney, New South Wales, Australia.

LITERATURE CITED

- ASHMAN, R. B., 1960 Stippled aleurone in maize. *Genetics* **45**: 19-34.
- BRINK, R. A., 1954 Very light variegated pericarp in maize. *Genetics* **39**: 724-740.
- 1956 A genetic change associated with the *R* locus in maize which is directed and potentially reversible. *Genetics* **41**: 872-899.
- 1958 Paramutation at the *R* locus in maize. Cold Spring Harbor Symp. Quant. Biol. **23**: 379-391.
- 1960 Paramutation and chromosome organization. *Quart. Rev. Biol.* **35**: 120-137.
- BRINK, R. A., and W. H. WEYERS, 1957 Invariable genetic change in maize plants heterozygous for marbled aleurone. *Proc. Natl. Acad. Sci. U.S.* **43**: 1053-1060.
- BRINK, R. A., D. F. BROWN, J. KERMICLE, and W. H. WEYERS, 1960 Locus dependence of the paramutant *R* phenotype in maize. *Genetics* **45**: 1297-1312.
- EMERSON, R. A., 1921 The genetic relations of plant colors in maize. *Cornell Univ. Agr. Exptl. Sta. Mem.* **39**: 1-156.
- McCLINTOCK, BARBARA, 1956 Intranuclear systems controlling gene action and mutation. *Brookhaven Symp. Biol.* **8**: 58-74.
- 1961 Some parallels between gene control systems in maize and in bacteria. *Am. Naturalist* **95**: 265-277.
- MCWHIRTER, K. S., 1961 Paramutation studies with self-colored mutants from the stippled allele in maize. Ph.D. thesis. Univ. of Wisconsin Library. Madison, Wisconsin.
- ORTON, E. R., JR., 1960 Reconstitution of the variegated pericarp allele in maize by return of Modulator to the *P* locus. Ph.D. thesis. University of Wisconsin Library. Madison, Wisconsin.
- PETERSON, P. A., 1961 Mutable *a₁* of the *En* system in maize. *Genetics* **46**: 759-771.
- STEEL, R. G. D., and J. H. TORRIE, 1960 *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- VAN SCHAIK, NANCY W., and R. A. BRINK, 1959 Transpositions of Modulator, a component of the variegated pericarp allele in maize. *Genetics* **44**: 725-738.