MUTATION AND PARAMUTATION AT THE *R* LOCUS IN MAIZE[1]

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THE *R* locus in maize exhibits two different kinds of genetic variability, mutation and paramutation (BRINK 1960). Mutation is a rare event, not predictable in an individual gamete, and produces a limited number of visually distinct mutant phenotypes. In contrast, paramutation is a directed change in the pigmenting ability of an *R* allele which invariably occurs in certain genotypes, and results in a continuous array of genetically different forms. Presumably mutation is concerned with changes in the basic genetic material of the locus, while paramutation affects expression of the basic material during development of the individual. On this basis, the ability of an R^r allele to undergo paramutation should not be related to the ability of the allele to mutate. Alternatively, if paramutation affects the basic structure or composition of the locus, the abilities to mutate and to undergo paramutation might be interrelated.

The paramutability of an *R* allele is readily measured by passing the factor through a heterozygote with an overtly paramutagenic allele, and mutation of *R* to colorless or near-colorless forms provides one measure of mutability. Quantitative data on mutation and paramutation for a series of *R* alleles provide a basis for determining whether any general relationship exists between the levels of expression of the two processes. Another approach is to examine the sequential effect of one process on the other (i.e., paramutation of mutant *R* alleles, and mutation of paramutant *R* alleles). BROWN (1963) has shown that the paramutational capacity of an R^r allele may or may not be changed by mutation, depending on the class of mutant, and BRINK (1958) made a preliminary study on mutation of a paramutant *R* allele. Further data on the interrelationship between mutation and paramutation are presented in the present paper.

MATERIALS AND METHODS

Genetic stocks: An extensive series of naturally occurring *R* alleles provides numerous different genetic entities for a study of this kind. The 44 *R* stocks conditioning colored aleurone that were used may be arranged in four groups:

(i) Standard R^r. Five lines of this so-called standard, red-anthered Wisconsin genetic stock were included; also, three lines carrying standard R^r in coupling with the *T2–10a* reciprocal translocation, and two lines carrying standard R^r in abnormal chromosome 10 (*K10*, containing

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a large heterochromatic knob distal to the *R* locus). The R^r gene shows reduced sensitivity to paramutation in these chromosomally aberrant stocks (BRINK and WEYERS 1960; BRINK and BLACKWOOD 1961; BRINK and VENKATESWARLU 1965).

(ii) Ten R^g (green anthered) mutants derived from standard R^r by mutation.

(iii) Ten R^{scm} (self-colored aleurone) mutants derived from R^{mb} (marbled aleurone) by mutation.

(iv) An assemblage of *R* (colored aleurone) alleles of widely separated geographic origins (BRINK 1960). These alleles came mainly from maize varieties indigenous to North, Central, and South America. They are referred to throughout the paper as "geographic alleles," or by their country or area of origin. Four groups are recognizable in terms of plant color: (a) Red roots, red coleoptile, and red anthers (R^r alleles designated as Argentina, Turkey, Ethiopia, North Dakota, Cornell, India, or Ecuador). (b) Red roots, red coleoptile, and green anthers (R^g alleles designated as Arizona, Oklahoma, South Dakota, Canada, Iowa, Washington, or New Mexico). (c) Green roots, red coleoptile, and green anthers (R^g alleles designated as Bolivia 1160, Bolivia 1520, Harvard, or Peru). (d) Green roots, green coleoptile, and green anthers (R^g alleles designated as Argentina, Guatemala, or India).

BRINK (1958) has described groups i, ii, iii, and also the commonly used R^{st}, r^r, and r^g alleles.

All the so-called geographic alleles that conditioned green anthers were designated as R^g. The phenotypes encompass the range of *R* alleles studied by STADLER (1948) and the collection is considered to be representative, with minor exceptions, of *R* alleles as a whole. Previous to testing all the alleles were incorporated into W22 inbred background by backcrossing for at least four generations.

Techniques. Mutation: All mutation studies were carried out in detasseling plots, where, by suitable choice of the male parent, and subsequent testing of putative mutants, any event other than an *R* locus mutation leading to the production of a mutant phenotype could be detected. Populations of gametes tested were estimated from the weight of shelled kernels, and corrected by a factor based on the proportion of putative mutants from which successful progeny tests were obtained.

Paramutation: Standard techniques involving testcrosses on colorless (*rr*) plants, (BRINK 1960) were used for determining aleurone pigmenting ability. Inbred W23 $r^g r^g$ plants served as pistillate parents in the testcrosses and a seven-class scoring scale was employed, ranging from 1 (colorless) to 6 (dark mottled) and 7 (self-colored). Fifty kernels were scored from each ear, and all ears were coded before scoring.

RESULTS

R^r and R^g alleles exhibit widely different mutation patterns (STADLER 1948) and studies on these two classes will be considered separately.

R^r *alleles.* (i) *Mutation:* The data obtained for mutation of R^r alleles to colorless forms are summarized in Figure 1. The populations of gametes tested ranged from 28,000 (standard R^r, line 3) to 136,000 (R^r N. Dakota), with an average of about 56,000. All putative mutants were tested to determine their authenticity. Most mutants verified proved to be r^r, (colorless aleurone, red anthers) although rare r^g mutants and a few deficiencies for *R* also were found (Table 1). The R^r *K10* stocks produced deficiencies at a higher rate than did structurally normal lines. Many kernels showing chromosome breakage events in the aleurone also were observed in the R^r *K10* lines.

The rates shown in Figure 1 lie between 0.60×10^{-4} and 19.07×10^{-4}, and are within the range observed by STADLER (1948). The detailed results are given by BRAY (1964). Considerable variability occurs between different lines carrying

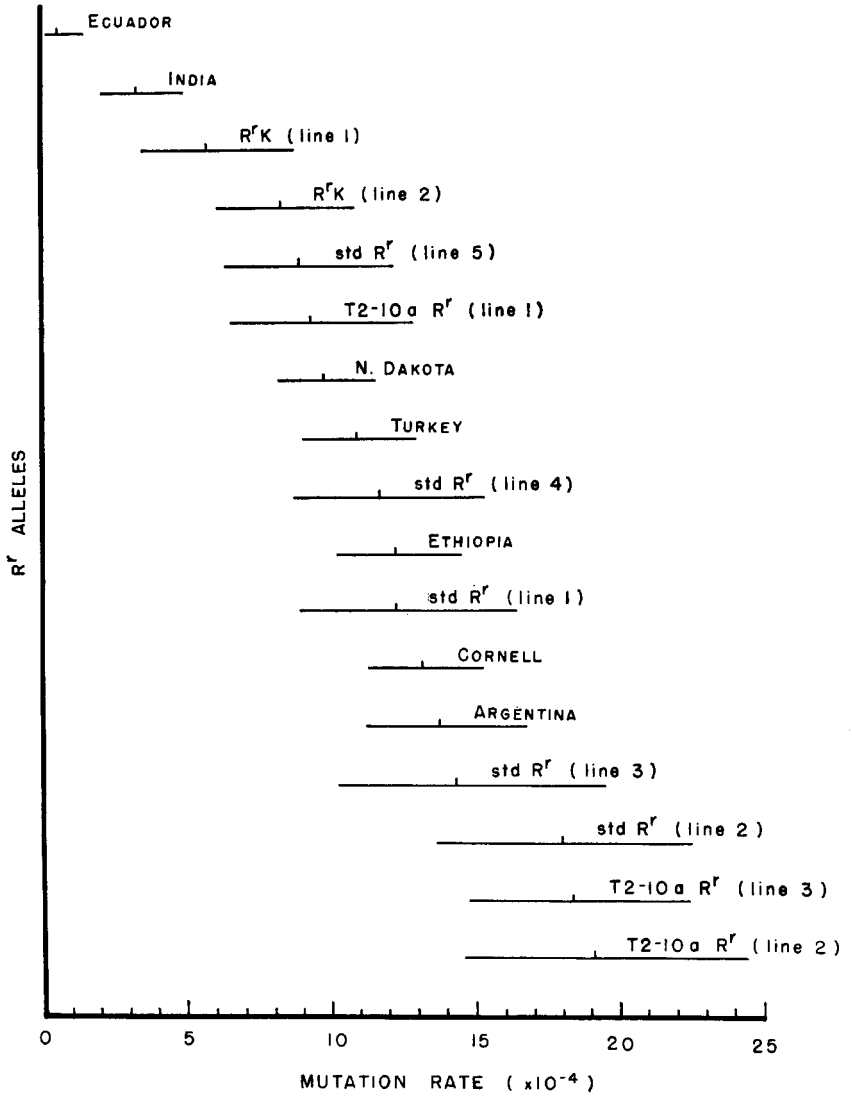

FIGURE 1.—Mutation rates and 95% Limits of Expectation for the series of R^r alleles. Horizontal lines indicate the extent of the Limits of Expectation; vertical marks show the observed mean mutation rates to *r*. The Limits of Expectation have been calculated by the method of STEVENS (1942) from the tables of FISHER and YATES (1957).

the same allele, as illustrated, for example, by the significant difference in R^r mutation rate between lines of *T2–10a* R^r and also between lines of standard R^r. Subsequent tests confirmed the reality of these differences. Divergence with respect to one or another character between separately maintained lines of inbred maize stocks is known not to be uncommon (FLEMING, KOZELNICKY, and BROWNE 1964). The basis of the divergence affecting R^r mutation rate in the present instance remains unknown.

TABLE 1

Nature of mutants from R^r *alleles*

Source	No. of putative *r* mutants	No. tested	No. verified as			Not verified as colorless
			r^r	r^g	Deficiency	
R^r (normal chromosome)	1028	916	911	1	3	1
R^r *T2–10a*	199	192	191	..	1	..
R^r *K10*	110	84	68	1	15	..

(ii) *Paramutation:* The pigmenting abilities of all R^r alleles in normal and paramutant form were tested over two years in matings of R^rR^r (or R^rr) and R^rR^{st} plants to r^gr^g ♀♀ and the data pooled. The relationship of the color scores for the 17 R^r alleles to the respective mutation rates is shown in Figure 2. Alleles having a high (near 7.00) nonparamutant score occur at both extremes of the mutation rate scale, and weakly paramutable alleles (indicated by a small difference between paramutant and non-paramutant scores) also have a considerable range of mutation rates. R^r Ecuador, which is the only non-paramutable R^r allele in the group, also has the lowest mutation rate, falling in the upper left hand part of the figure.

Three measurements of pigmenting ability of an allele may be examined in relation to mutability of the allele: non-paramutant score, paramutant score, and the reduction in score (difference) due to paramutation. Using Spearman's rank

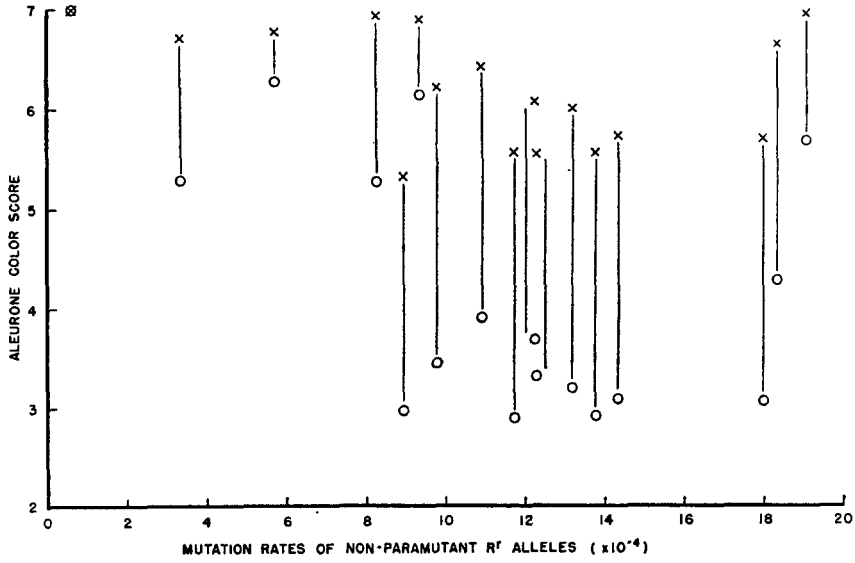

FIGURE 2.—Aleurone color scores and mutation rates for the series of R^r alleles. Each allele is represented by four measurements: non-paramutant score, (×); paramutant score, (○); reduction in score due to paramutation (length of vertical line); and mutation rate, which determines the left-to-right array of aleurone scores.

TABLE 2

Probabilities of randomness of relationship based on Spearman's rank correlation coefficient

Extent of test		Relationship between mutation rate and: Non-paramutant score	Paramutant score	Reduction due to paramutation
All alleles	r_s	0.35	0.46	0.48
	t (15 df)	1.43	1.89	1.96
	P	$0.1 < P < 0.2$	$0.05 < P < 0.1$	$0.05 < P < 0.1$
Excluding R^r Ecuador	r_s	0.21	0.33	0.35
	t (14 df)	0.82	1.29	1.36
	P	$0.3 < P < 0.4$	$0.2 < P < 0.3$	$0.1 < P < 0.2$

correlation coefficient (STEEL and TORRIE 1960), the probability of the randomness of such relationships may be established. Figures using data from all alleles are given in the upper part of Table 2. None of the probabilities is below the accepted level of significance, although not far above it.

Because of its insensitivity to paramutation, R^r Ecuador may belong in a different class of R^r alleles than the paramutable R^r alleles. If the figures from the tests of R^r Ecuador are excluded, the probabilities rise to above 10% (see lower part of Table 2).

If the five standard R^r lines are considered independently of the other alleles, a test using Spearman's coefficient gives a probability of 6.7% for the randomness of the relationship between mutation rate and non-paramutant score. This relationship, however, is in the opposite direction to any suggested by overall consideration of Figure 2, in that within the standard R^r lines, the alleles with the higher aleurone scores have the higher mutation rates. In Figure 2, R^r Ecuador has the highest aleurone score, and lowest mutation rate. No relationship is apparent between mutation rate and paramutant scores of the five standard R^r lines, or beween their reduction in score and mutation rate.

R^g *alleles.* (i) *Mutation:* Whereas R^r alleles mutate almost exclusively to colorless aleurone forms, the 34 R^g alleles studied produced, besides 355 colorless mutants, 107 mutants with intermediate levels of aleurone pigmentation. The majority of such mutants were produced by R^g Harvard (53) and R^g Peru (44), and were of near-colorless phenotype similar to that described by ASHMAN (1960). The other ten mutants were "pale," with aleurone pigmentation at a level distinctly less than that of the parent allele. Many putative mutant kernels of the geographic R^g alleles proved to be nonmutant at the *R* locus, the original aberrant phenotypes being the result of contamination by pollen from other sources. The colorless and pale mutants from the red-rooted, green anthered R^g alleles were green rooted, having lost the ability to produce plant color concomitantly with the alteration in aleurone phenotype.

The mutation rates of all the R^g alleles are shown in Figure 3. The rate for mutation to colorless and also the total rate (colorless plus near-colorless and pale) are both shown where appropriate. Population sizes ranged from 57,000

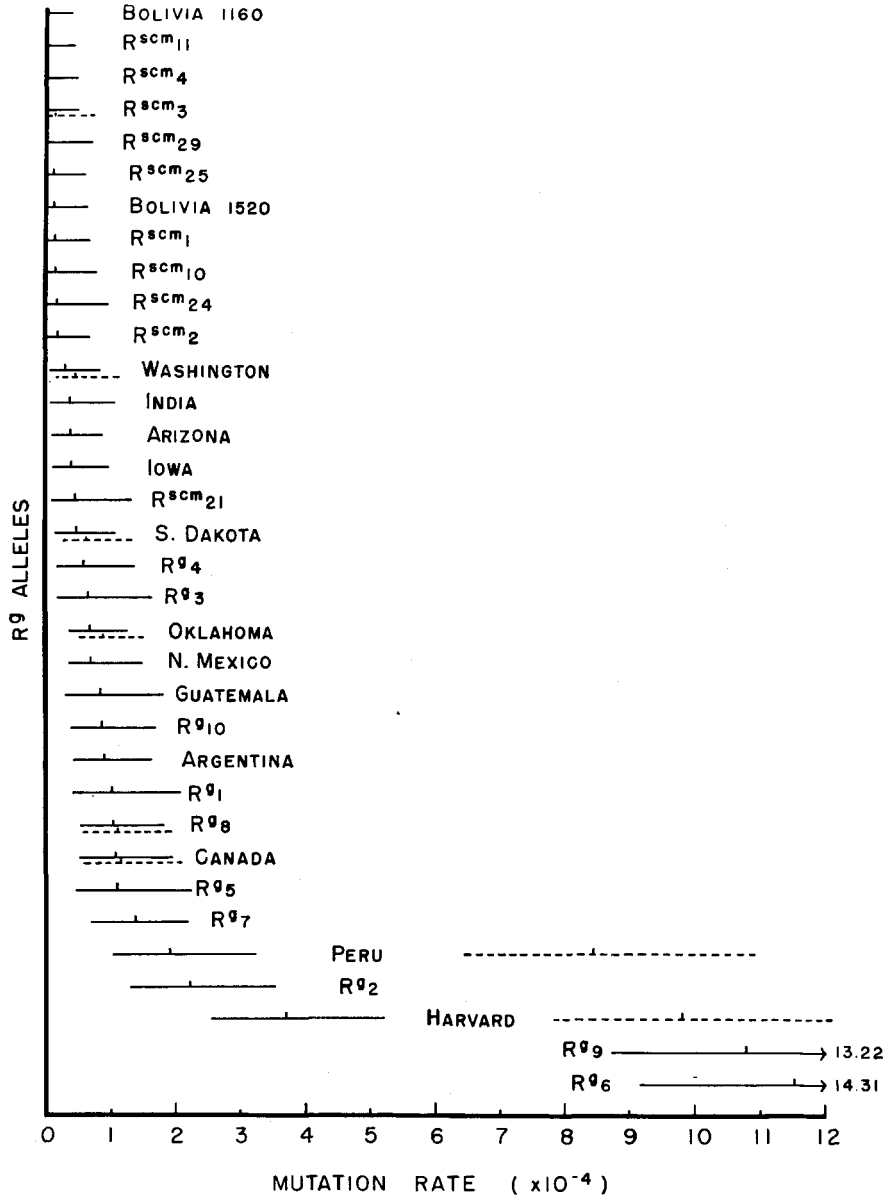

FIGURE 3.—Mutation rates and 95% Limits of Expectation for the series of R^g alleles. Horizontal lines indicate the extent of the Limits of Expectation; vertical marks show the observed mean mutation rates. Solid lines represent mutation to *r* (colorless), and the dotted lines show total *R*-locus mutation rate. The observed mutation rates of the first five alleles entered at the top of the figure are zero.

gametes ($R^{scm}24$) to 147,000 (R^g Oklahoma) with an average of about 87,000. Considering total mutation rates as indicative of *R* locus mutability, it is clear that two distinct groups of alleles exist, one with low mutation rates (below 2×10^{-4}) and the other with high rates (above 8×10^{-4}).

(ii) *Paramutation:* The R^g alleles may be considered in three groups as follows:

(a) R^{scm} alleles. By definition (and by experimental test) these alleles are all non-paramutable, and give aleurone color scores of 7.00 (self-color) both before and after passage through R^{st} heterozygotes.

(b) R^g 1–10: BROWN (1963) has shown that no differences exist in pigmenting ability between these ten mutants, either before or after they have undergone paramutation. This observation was confirmed in the present studies.

(c) Geographic R^g alleles: The scores obtained for the pigmenting abilities of these alleles are given in Table 3. Results for the four alleles R^g Bolivia 1160, R^g Bolivia 1520, R^g Peru, and R^g Harvard are not included, since these factors behaved as R^{sc} alleles, scoring 7.00. All alleles listed in the table condition darkly mottled aleurone in non-paramutant form, and are highly paramutable, with the exception of R^g India, which is weakly paramutable. Thus, of 34 R^g (green anthered) alleles tested, 14 were non-paramutable, 19 were highly paramutable, and one was weakly paramutable. These classifications were confirmed by F_2 data, based on testcrosses of the alleles after two generations of heterozygosity with R^{st}.

(iii) *Paramutagenic action of non-paramutable* R^g *alleles:* The alleles which were non-paramutable (or weakly paramutable) were tested to see if they themselves were paramutagenic. Non-paramutable R^g alleles were crossed to standard (std.) R^r, and the R^gR^r heterozygotes were testcrossed on r^gr^g ♀♀. The $R^rr^gr^g$ kernel scores were then compared with those from r^gr^g ♀ × std. R^r/std. R^r ♂ control matings. The results are summarized in Table 4. All four non-paramutable R^g alleles were paramutagenic, and showed differences between themselves in paramutagenic action.

The R^{scm} alleles were tested for paramutagenic activity by testcrossing R^{scm}/std. R^r heterozygotes on r^gr^g ♀♀. All ten alleles were found to be paramutagenic, and differences existed between alleles in the induced paramutant R score ($F = 5.03$, 9 and 47 df, $P < .01$). This result parallels the finding of MCWHIRTER and

TABLE 3

Pigmenting ability of the geographic R^g *alleles. Mean aleurone color scores from the crosses: (a) W23* r^gr^g ♀♀ × R^gr^g *or* R^gR^g ♂♂ *and (b) W23* r^gr^g ♀♀ × R^gR^{st} ♂♂

Allele	Non-paramutant scores		Paramutant scores	
	No. of plants tested	Mean	No. of plants tested	Mean
R^g Argentina	18	6.01	28	3.44
R^g Arizona	8	6.68	15	3.62
R^g Oklahoma	8	6.87	23	3.76
R^g Canada	10	6.66	23	3.49
R^g S. Dakota	10	6.69	25	3.23
R^g Iowa	5	6.75	23	3.39
R^g Washington	9	6.64	24	2.87
R^g N. Mexico	9	6.71	24	3.18
R^g Guatemala	10	6.37	17	3.54
R^g India	9	6.99	25	5.05

TABLE 4

Paramutagenic action of non-paramutable R^g *alleles. Aleurone color scores of* $R^r r^g r^g$ *kernels from the cross W23* $r^g r^g$ ♀ ♀ × R^g/*std.* R^r ♂ ♂

Test allele	No. of plants tested	Mean $R^r r^g r^g$ score
Std. R^r (control)	10	5.73 a*
R^g India	5	5.58 a
R^g Peru	6	4.64 b
R^g Bolivia 1160	5	4.26 b
R^g Harvard	6	3.56 c
R^g Bolivia 1520	7	3.06 c

* Means followed by the same letter are not significantly different at the 5% level, as determined by SCHEFFE's (1953) test.

BRINK (1962) who showed that R^{sc} mutants from R^{st} may exhibit different levels of paramutagenic action.

R^r Ecuador and R^r India were also tested for paramutagenic action. The mean scores of $R^g r^g r^g$ kernels from crosses of the type $r^g r^g$ ♀ ♀ × R^r Ecuador/R^g3 ♂ ♂ and $r^g r^g$ ♀ ♀ × R^r India/R^g3 ♂ ♂ were 5.72 and 5.52 respectively, and are not significantly different from the figure of 5.68 obtained from the $r^g r^g$ × R^g3 R^g3 controls. R^r Ecuador was also tested to see if it could acquire secondary paramutagenic action by crossing R^r Ecuador/R^{st} plants with R^g3 R^g3, and comparing scores from R^g3 gametes from $R^{r'}$ Ecuador/R^g3 heterozygotes with control R^g3 scores. The respective means were 5.82 and 5.51. R^r Ecuador therefore cannot acquire secondary paramutagenic action. Thus it is inert with respect to all known paramutation phenomena.

Mutation of paramutant R *alleles:* The rate of mutation of a series of paramutant R' alleles to r was measured in homozygous $R'R'$ plants (obtained by self-pollinating R R^{st} individuals), and then compared with that of the respective non-paramutant R alleles. The alleles tested were standard R^r, R^g2, R^g4, R^g5, R^g6, and R^g8. The results are listed in Table 5. The figures for non-paramutant standard R^r were obtained by pooling the data from the five standard R^r lines shown in Figure 1. In five out of the six pairs of rates shown in Table 5, the mutation rate of the paramutant allele is less than that of the non-paramutant form, and generally is about 60% of the higher rate. In the sixth case the rates are equal. Three of the differences are statistically significant, and it may be concluded that paramutant R alleles have lower mutation rates than the corresponding non-paramutant forms. BRINK (1958), working with small populations, did not detect this reduction in mutation rates. The larger population sizes in the present experiments establish it clearly.

The R alleles in the homozygous paramutant and non-paramutant plants differ slightly in their genetic history. The paramutant genes have been derived from plants heterozygous for R^{st}, but the non-paramutant alleles came from homozygous R R plants. To test the possibility that previous heterozygosity, *per se*, was responsible for the subsequent reduction in mutation rate, a single homozygous

TABLE 5

Mutation rates of paramutant R *alleles*

Allele	Putative *r* mutants	No. tested	Verified as *r* mutants	Corrected population	Rate of mutation ($\times 10^{-4}$)	Probability of equal rates
Standard R^r	292	233	232	182,353	12.72	< 0.01
Paramutant std. R^r	200	195	192	258,325	7.43	
R^g2	8	7	6	75,526	0.79	> 0.20
Paramutant R^g2	8	6	6	76,241	0.79	
R^g4	16	13	13	110,866	1.17	> 0.20
Paramutant R^g4	10	6	6	82,265	0.73	
R^g5	18	16	16	144,685	1.11	$0.01 < P < 0.05$
Paramutant R^g5	5	4	4	132,429	0.30	
R^g6	120	107	106	111,342	9.52	< 0.01
Paramutant R^g6	108	86	78	128,145	6.09	
R^g8	14	12	12	159,354	0.75	> 0.20
Paramutant R^g8	10	7	7	111,339	0.63	

std. R^r/std. R^r plant was crossed with: (a) Homozygous R^{st}; (b) a stock of $r^r r^r$, derived as a mutant from standard R^r, and therefore isogenic with it, except for the R locus; (c) an isogenic $r^g r^g$ stock; and (d) the same standard R^r plant (self-pollination).

Two sets of pollinations were made using two standard R^r plants. Homozygous $R^r R^r$ seed was then obtained by self-pollinating the various heterozygotes, and used to test the mutation rate to r. The results are shown in Table 6. Since only small populations were obtained, the figures represent pooled data from the two sets of crosses. It is clear that one generation of heterozygosity with r^r or r^g does not alter the mutation rate of R^r (entries 1 and 2 *vs.* entry 4), but heterozygosity with R^{st} causes a decrease in mutation rate (entry 3 *vs.* entry 4, P (equal rates) is between .05 and .10). The reduction in mutation rate observed in Table 5 therefore is due to an effect of the R^{st} gene as such.

Preliminary experiments have been made on the mutation rates of other forms of paramutant alleles. The alleles studied and the observed mutation rates were: (a) std. R^r which had been heterozygous with R^{st} for two generations, and was then selfed: mutation rate 5.96×10^{-4}. (b) std. R^r which had been heterozygous with R^{st} for one generation and was then selfed for two generations: mutation

TABLE 6

Mutation rates of homozygous standard R^r *after extraction from heterozygotes with different alleles*

Entry	History of R^r	No. of *r* mutants	Population	Mutation rate ($\times 10^{-4}$)
1	$r^g R^r$ selfed	11	7362	14.9
2	$r^r R^r$ selfed	19	14276	13.3
3	$R^{st} R^r$ selfed	5	8871	5.6
4	$R^r R^r$ selfed	28	20751	13.5

rate 10.51×10^{-4}. (c) std. R^r which had been heterozygous with R^{st} for one generation and was then selfed for seven generations: mutation rate 13.56×10^{-4}.

Since all the standard R^r alleles did not come from the same line, the three results are not directly comparable with each other. It is evident, however, that in advanced generations following heterozygosity with R^{st} the mutation rate of $R^{r'}$ increases towards that of the non-paramutant form of standard R^r. This parallels the behavior of paramutant alleles with respect to aleurone pigmenting level as described by KERMICLE (1963). The reversion of mutation rate also provides strong evidence that the original depression of the mutation rate of paramutant R is not due to the action of modifier genes derived from the R^{st} genome, since such modifiers would still be present in unselected descendants in which presumably they would continue to have a depressing effect. The number of mutants obtained in case (a) above was too small (15) to allow any conclusions to be drawn regarding a possible progressive reduction in mutation rate.

Genetic constitution of R^g4 *and* R^g6: It has been postulated (STADLER 1951; STADLER and EMMERLING 1956) that the R locus contains at least two synaptically homologous units, P (the dominant form of which conditions plant color formation) and S (the dominant form of which conditions aleurone color formation). An R allele producing colored aleurone and green plant color (i.e., R^g) could then be either (p S) or (– S) where (–) represents a deficiency for the P component of the locus. EMMERLING (1958) proposed a genetic test to distinguish between these two possibilities. In an R^r R^g heterozygote, where R^r is (P S), if the R^g allele were (p S), two types of colorless crossover mutants, (P –) and (p –), could result, due to oblique synapsis and subsequent exchange. If the R^g allele were (– S) only (P –) colorless crossover types could be produced.

Within the group of ten R^g mutants from standard R^r there exist two distinct orders of mutation rates to r, the rates of R^g6 and R^g9 being high, comparable to that of standard R^r, whereas those of the other eight R^g mutants are low, being similar to those of many other R^g alleles (Figure 3). It might be expected that an R^g allele of constitution (p S) would, by virtue of more frequent oblique pairing and subsequent exchange, produce more colorless mutants than one of constitution (– S), assuming a common rate of production of noncrossover colorless types.

One allele from each group (R^g6 and R^g4) was selected, and crosses made, following EMMERLING's plan, to determine the status of the P component in each allele. Abnormal chromosome 10 (*K10*) served as distal marker. Plants of genotype R^gk/R^gk were crossed to R^rK/R^rK (i.e., P S K / P S K) stocks, and the resulting R^gk/R^rK (i.e., p (?) S k/P S K) heterozygotes were pollinated with r^gr^g. Colorless kernels from this mating were selected and analyzed to determine their R-locus constitution and the presence or absence of *K10*. The results are shown in Table 7.

The R^g4 k/R^rK genotype produced only one type of colorless crossover mutant, and the 5:0 ratio observed is significantly different ($P < .01$) from the expected 1.5:3.5 (r^gK crossovers should segregate preferentially, since they carry *K10*). Both types of crossover mutants are formed in the R^g6 k/R^rK plants. Although

TABLE 7

Mutation to r of R^{g}4k/R^{r}K *and* R^{g}6k/R^{r}K

Parent R^g allele	Population	Putative r mutants	No. tested	Noncrossover types		Crossover types		Overall mutation rate ($\times 10^{-4}$)
				r^rK (=PsK)	r^gk (=p(?)sk)	r^rk (=P(s)k)	r^gK (=p(s)K)	
R^g4	133,520	23	22	13	3	5	0	1.57
R^g6	133,192	27	24	13	2	1	7	1.73

the numbers of mutants are small, it is clear that R^g4 behaves as if it were deficient for the P component (– S) whereas R^g6 carries the recessive p (p S).

DISCUSSION

It has been shown that no clear quantitative relationship exists between pigmenting ability and mutation rate for R^r alleles, or between paramutability and mutation rate (Figure 2). The possibility of a qualitative relationship however, cannot be excluded, since the R^r Ecuador allele, which is inert with respect to paramutation, also has the lowest mutation rate. There is a definite lack of entries in the lower left quadrant of Figure 2 (low mutation rate, low aleurone color score). It is uncertain whether this is merely a consequence of the particular sample of R^r alleles studied, or is evidence that all R^r alleles with low mutation rates are darkly pigmented and either weakly paramutable, or non-paramutable. Only further experiments on a wider range of R^r alleles can clarify this point.

Within the 34 R^g alleles tested the evidence is clearer. There are two alleles in this group which are non-paramutable and have a high mutation rate, two which are paramutable and highly mutable, 18 which are paramutable and have a low mutation rate, and 12 which are non-paramutable and have a low mutation rate. The probability of independence in this 2 × 2 table is over 80%. No relationship is apparent therefore between paramutability and mutation rate of the R^g alleles. It is also clear that there is no association between non-paramutant pigmenting ability of the R^g alleles and mutation rate, since R^g6 and R^g9 have aleurone pigment producing potentials indentical with those of the other R^g mutants from standard R^r, but have distinctly different mutation rates, whereas R^g Harvard and R^g Peru are similar to R^g Bolivia 1160, R^g Bolivia 1520 and R^{scm} alleles in pigment producing potential, but again have different mutation rates.

Within the R^g group of alleles it is possible to examine the relationship between paramutagenic action of an R allele and its mutation rate. The data of Table 4 demonstrate that alleles with similar high mutation rates (R^g Peru and R^g Harvard) may possess different paramutagenic capacities. Also, alleles with widely differing mutation rates (e.g. R^g Bolivia 1160 and R^g Peru) may be similar in paramutagenic action. The spectrum of paramutagenic activities shown by the R^{scm} alleles, all of which have similar mutation rates, also suggests the absence of a clear-cut relationship between paramutagenic activity and mutation rate.

The data on mutation of paramutant R alleles show however, that mutation

and paramutation are not completely independent of each other. Many "mutations" at the *R* locus are ascribable to intralocus recombination (STADLER and EMMERLING 1956). Any factor which influences or alters either the regularity of pairing or frequency of crossing over within the *R* locus therefore, would be expected to alter the mutation rate. Investigation of the nature of mutants from paramutant *R* alleles (crossover or noncrossover) would assist in determining the paramutational mechanism.

It has been shown that R^g6 is of constitution (p S), and presumably many of the mutants from this allele are the products of crossing over within the locus. R^g Peru and R^g Harvard have mutation rates that are similar to that of R^g6, but the mutants are mainly faintly pigmented, *r* (I) types. ASHMAN (1965) has shown that from R^rR^{st} heterozygotes, most *r* (I) products are associated with genetic recombination within the *R* locus. It is probable that the *r* (I) mutants from R^g Peru and R^g Harvard also are recombination products, the parent alleles being two unit systems (p S), similar to R^g6, but not paramutable, and paramutagenic. The stable, paramutable R^g alleles are presumed to lack the P component (– S), while the nature of the stable, non-paramutable R^g alleles is still obscure.

The present data do not allow the formulation of a general statement regarding the relationship between mutation and paramutation. The levels of expression of the two processes in any one allele appear to be independent, but the mechanisms cannot be completely unrelated.

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SUMMARY

No clear relationship was found between mutation rate and pigmenting ability either before or after paramutation in a collection of R^r alleles exhibiting a wide range of mutation rates. Within R^g alleles two distinct orders of mutation rates were observed, with paramutable and non-paramutable alleles occurring in both groups. Paramutagenic and non-paramutagenic alleles may or may not have similar mutation rates.—The mutation rate of a paramutant *R* allele is less than that of the corresponding non-paramutant allele. The reduction is not due to previous heterozygosity *per se*, or to modifying genes. The mutation rate of paramutant alleles appears to revert, in advanced generations, towards that of the non-paramutant form, simulating behavior in regard to aleurone color.—With respect to the plant color component of the *R* locus, P, two mutants from standard R^r, R^g6 (high mutation rate) and R^g4 (low mutation rate) have been identified as carrying the recessive p, and as deficient for P, respectively.

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