GEOGRAPHIC DISTRIBUTION OF PARAMUTABLE AND PARAMUTAGENIC *R* ALLELES IN MAIZE[1]

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OBSERVATIONS made in our laboratory a decade ago indicated that paramutable and non-paramutable *R* alleles, conditioning aleurone color in maize, were distinct in geographic distribution. All *R* factors then at hand from maize varieties indigenous to Ecuador, Peru, Bolivia, and Chile were found to be insensitive to heritable reduction in anthocyanin forming potential when passed through a heterozygote with the strongly paramutagenic stippled (R^{st}) allele, and so were classified as non-paramutable. On the other hand, the few *R* alleles then available from elsewhere in South America, a larger assortment from the United States, and a small number from Canada, Guatemala, Turkey, India, and Ethiopa were found, by the same test, to be paramutable, without exception. Brief mention of the early results was made in a general article on paramutation (BRINK 1964).

Our collection of *R* alleles has since been significantly enlarged, particularly with accessions from certain Latin American countries. The data assembled here on paramutability of both the old and new *R* accessions support the earlier deduction that the varieties carrying non-paramutable *R* factors are localized in northwestern South America and suggest that the region centrally involved is the Andean highlands. Paramutable *R* alleles prevail elsewhere in South America, and are the only kind found in North America, including Mexico and Guatemala. All overtly paramutagenic *R* alleles thus far tested have been derived originally from races indigenous to the Andean highlands area.

MATERIALS AND METHODS

Most of the *R* alleles tested for paramutability were initially derived from presumably long established local varieties scattered over the maize growing regions of North America and South America. The stocks of United States origin, for example, were based mainly on collections from American Indian reservations in the Great Plains region, Arizona, and New Mexico. Geographical diversity was a primary consideration in assembling the Latin American strains. Only seeds with colored aleurone were used, that is, those which carried an *R* allele as well as the complementary factors for aleurone pigmentation. Such colored seeds often were in the minority in the original samples supplied us. It is well known, of course, that many maize races entirely lack aleurone pigmentation. Strains of the latter kind did not enter into the present study.

Eight different stocks with colored aleurone were obtained from Mexico. The South American countries represented in our tests with six or more accessions were Bolivia, Peru, Ecuador, Venezuela, and Brazil. Smaller numbers of strains were obtained from Argentina, Paraguay,

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Chile, and Colombia. From outside the Americas three stocks were obtained from India, three from Turkey, and one from Ethiopia. The geographic origins of the alleles termed "standard R^r" and "R^r Cornell" are not known but these factors are indistinguishable in the paramutation tests from R^r alleles commonly found in maize varieties grown on American Indian reservations from North Dakota to Oklahoma. Only a few of the R factors tested originated by mutation in controlled cultures. The 10 R^g alleles referred to in entry 2, Table 1, were obtained in this way from standard R^r.

The R locus in maize is characterized by great diversity. The latter is expressed as variation in the distribution and intensity of anthocyanin in aleurone and scutellum of the kernel, seedling shoot and root, and in the stem, leaves, silks, glumes, and anthers of the fully developed plant. Except that the corresponding aleurone phenotype was colored in all cases, the R alleles used in the tests for paramutability represent a random sample of this diversity. No attempt is made here to document in detail the considerable R polymorphism involved. The few R superscripts employed in the tables and text (as in R^r, R^g, and R^{ch}, for example) are significant in the present context only as convenient markers in distinguishing between major R classes of seedlings or plants in segregating families. Factors designated R^r condition colored aleurone and red anthers, whereas those symbolized by R^g condition colored aleurone and green anthers. R^r and R^g, however, are group symbols. Each represents an assortment of R factors which varies widely, and sometimes subtly, in seed and plant pigmenting potential. Diversity of phenotypes within an R^r or R^g group from a given area, or political subdivision, frequently was apparent.

Also tested were a few alleles designated R^{ch} (cherry) which, in combination with the *Pl* gene elsewhere in the genome, distinctively condition anthocyanin formation in the pericarp.

The genetic background of the R alleles was standardized by incorporating the factors into the uniform Wisconsin dent inbred W22. The backcrossing to W22 was carried far enough in most lines so that any differences in phenotypic manifestation of the alleles could reasonably be attributed to variations at, or near, the R locus, in the long arm of chromosome 10. The least uniform R stocks, listed in Table 5, were tested following only three backcrosses to W22. Because of the limited standardization of these lines the paramutability scores are reported in qualitative form only.

The usual basis for classifying a given R allele as paramutable is a reduction in degree of aleurone pigmentation in the *Rrr* testcross seeds resulting from the pollination of *rr* females by RR^{st}, as compared with corresponding *RR* (or *Rr*), males.

The amount of aleurone pigmentation in the *Rrr* seeds was measured in either of two ways in the present study. One procedure, long employed in our laboratory, was to match individual *Rrr* kernels in a random sample of 50 from the middle portion of a testcross ear against a standard set of six seeds defining seven classes ranging from 1 (colorless) to 7 (self-colored). The mean score for the sample was then used to represent the pigmenting potential of the R factor in question.

An anthocyanin extraction technique also was employed in measuring aleurone pigmentation. A sample of *Rrr* seeds of relatively uniform size following elimination of the largest and smallest seeds by screening, representing a single testcross ear, was ground in a Wiley mill to pass a 20-mesh screen. A one gram portion of the resulting, thoroughly mixed meal was extracted for 24 hrs with a 1% solution of HCl in methanol. The mixture was centrifuged for 5 min at about 2000 rpm, and the clear, colored supernate was then read at 530 mμ in a Bausch and Lomb Spectronic 20 colorimeter. The absorption spectrum of the pigment was plotted with a Beckman 12B spectrophotometer. The percentage transmittance was converted to optical density according to the formula:

$$\text{Optical density} = 2\text{-log percentage transmittance}$$

The measurements are expressed in the tables as the optical density equivalent of one gram of meal in 50 ml of solvent. This method of reporting the data was adopted after it was shown experimentally that a straight line relationship exists between optical density and volumes of solvent for 1 g samples of meal varying from 25 to 200 ml, and also for weights of meal ranging from 0.5 to 3.0 g with a given volume of solvent.

The amounts of pigment reduction in paramutant, as compared with normal, scores given by

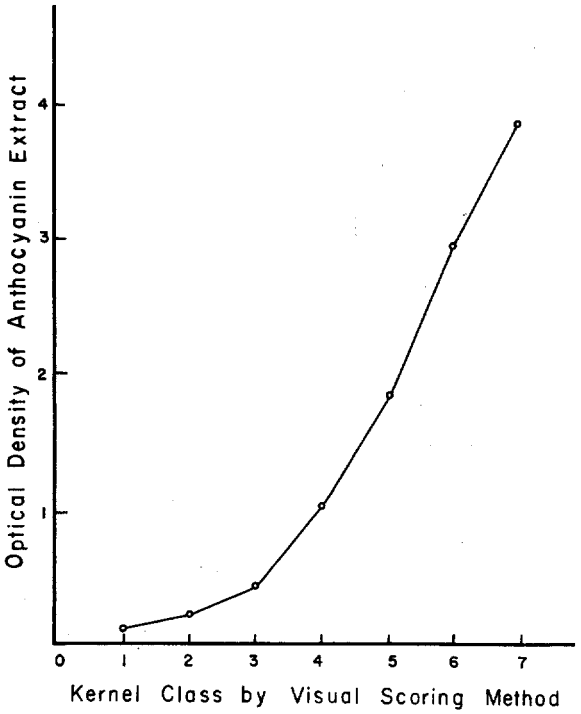

FIGURE 1.—Relative optical density measurements of anthocyanin extracts of seeds representing the standard classes used in scoring aleurone pigmentation by the kernel matching procedure.

the two procedures showed reasonably close correspondence (see Figure 1). The kernel matching procedure was found to be more discriminating in the lower than in the middle and upper portions of the pigmentation range when evaluated in terms of amount of anthocyanin present. Furthermore, the extraction method, as expected, disclosed the occurrence of alleles differing inherently in aleurone pigmenting potential within the group which scored at, or near, 7.00 (self-colored) and hence clustered at the top of the scale employed in kernel matching.

RESULTS

The data from the earliest tests for paramutability among *R* alleles of diverse geographic origin are assembled in Table 1. The kernel matching procedure was used in measuring degree of pigmentation of the initial series of testcross kernels, and the scores are reported in terms of the scale ranging from 1.00 (colorless) to 7.00 (self-colored).

Seven of the *R* alleles in this group were again tested for paramutability after the pigment extraction procedure came into use in our laboratory. The scores given by the latter method are entered in Table 1 in parenthesis beside the corresponding scores resulting from the kernel matching procedure.

The table contains 20 R^r, R^g, and R^{ch} entries from the United States, based on varieties originally collected in North Dakota, South Dakota, Iowa, Missouri, Kansas, Oklahoma, Arizona, New Mexico, and Washington. Standard R^r and R^r

TABLE 1

Testcross scores by the kernel matching (or visual) procedure of R *alleles in (1) normal form and (2) following passage through* R^{st} *heterozygotes*

(Optical density scores of anthocyanin extracts, as available, are given in parenthesis)

Entry	Identification	Geographic origin	*R r r* phenotype‡	*R* testcross score	
				Normal form	From $R\ R^{st}$
			R^r alleles		
1	Standard R^r*	U.S.A.	mot.	5.61 (0.53)	3.07 (0.08)
2	R^g mutants†	U.S.A.	mot.	5.75	2.97
3	R^r Cornell	U.S.A.	mot.	5.20	2.16
4	PI 213748	Oklahoma	....	5.53	2.91
5	PI 222889	Missouri	mot.	5.92	3.62
6	PI 222309	North Dak.	dk. mot.	5.53	2.61
7	PI 222629	Kansas	....	6.99	4.43
8	PI 161418	Argentina	mot.	4.75 (0.62)	2.31 (0.37)
9	PI 193659	Ethiopia	....	5.86	2.52
10	PI 166163	India	....	6.82	4.39
11	PI 210551	India	dk. mot.	6.79	5.49
12	PI 167989	Turkey	....	6.98	4.08
13	PI 174414	Turkey	dk. mot.	6.29	2.12
14	PI 179131	Turkey	....	6.92	3.77
15	1172	Ecuador	s. c.	7.00 (0.66)	7.00 (0.78)
			R^{ch} allele		
16	Stadler culture	U.S.A.	pale mot.	5.65	3.13
			R^g (green seedling) alleles		
17	PI 213738	Arizona	....	6.79	3.41
18	PI 218164	Arizona	....	5.97	1.90
19	PI 218175	Arizona	....	6.88	2.23
20	PI 218178	Arizona	....	6.11	4.44
21	PI 218148	New Mex.	....	6.83	3.68
22	PI 162573	Argentina	mot.	5.61 (0.60)	2.46 (0.28)
23	PI 166161	India	s. c.	6.99	5.28
24	5A 2837	Guatemala	dk. mot.	6.09	2.32
25	1160	Bolivia	s. c.	7.00 (0.84)	7.00 (0.81)
26	1520	Bolivia	s. c.	7.00 (0.66)	7.00 (0.76)
27	1304	Peru	s. c.	7.00	7.00
			R^g (red seedling) alleles		
28	PI 213729	Arizona	dk. mot.	6.79	2.69
29	PI 213755	Oklahoma	dk. mot.	6.91	2.15
30	PI 213779	South Dak.	dk. mot.	6.66 (0.41)	2.31 (0.14)
31	PI 213799	North Dak.	....	6.66	2.87
32	PI 214199	Manitoba, Canada	dk. mot.	6.86	2.71
33	PI 217411	Iowa	dk. mot.	6.73	1.81
34	PI 217488	Washington	dk. mot.	6.71	2.12
35	PI 218170	New Mex.	dk. mot.	6.33	2.23

* Visual testcross scores from R. A. BRAY, Ph.D. thesis; average of 5 standard R^r sub-lines.

† Visual testcross scores from R. A. BRAY, Ph.D. thesis; average of 10 mutants, $R^g{}_1$ to $R^g{}_{10}$.

‡ mot. = mottled; dk. mot. = dark mottled; s. c. = self-colored.

Cornell which are indistinguishable from some other members of the R^r group, also are included, as is one R^{ch} factor probably of United States origin. The R^g (green anther) group of alleles comprises two subgroups characterized by green and red seedlings, respectively. This distinction, however, appears to be irrelevant for the present investigation.

All the alleles of United States origin proved to be paramutable, as the data in Table 1 show. Considerable variation in score between the respective normal forms of the alleles in each main group, R^r, R^g (green seedling), and R^g (red seedling) occurs. Furthermore, the amounts of reduction in aleurone pigmenting potential following passage through the R^{st} heterozygotes varied widely from one accession to another. All the alleles, however, share the common property of undergoing a significant, heritable reduction in anthocyanin forming capacity when subjected to the action of the stippled factor in an RR^{st} heterozygote.

Two other *R* factors of North American origin, one from Guatemala (entry 24) and one from Manitoba, Canada, (entry 32) listed in Table 1 also were paramutable. Thus all the *R* factors in this group derived from North American varieties gave positive results in the test for paramutability.

A markedly different relationship was encountered with respect to the occurrence and distribution of paramutable *R* alleles in the relatively small group of South American varieties represented in Table 1. The two R^g factors from Bolivia, the one R^g entry from Peru, and the single R^r allele originally derived from Ecuador remained stable in aleurone pigmenting potential on passage through RR^{st} plants. One R^r and one R^g allele from Argentina, on the other hand, were found to be highly sensitive to paramutation.

The six alleles derived from maize varieties from outside the Americas (three from Turkey, two from India, and one from Ethiopa) all were paramutable.

It is noteworthy that the four non-paramutable *R* alleles from South America entered in Table 1 gave the self-colored aleurone phenotype in single dose, and thus scored 7.00 on the 1–7 scale. This property is characteristic of non-paramutable *R* factors. Paramutable *R* factors, in contrast, usually give darkly mottled aleurone in single dose. This criterion, however, is not fully dependable for diagnostic purposes because, as STYLES (1967b) has demonstrated, the single dose aleurone phenotype of paramutable alleles may be raised from mottling to near self-color by appropriate breeding procedures.

Scores based on both the kernel matching method and the anthocyanin extraction procedure were obtained for entries 1, 8, 15, 22, 25, 26, and 30, in Table 1. Three of the alleles, Ecuador 1172, Bolivia 1160, and Bolivia 1520, are in the non-paramutable group, and score 7.00 on the scale used in kernel matching. It will be noted that in none of these cases did the anthocyanin extraction procedure reveal a decrease in pigment content following passage of the allele through a stippled heterozygote. This result provides some assurance that in the case of an *R* allele of high pigmenting potential the kernel matching procedure would not lead to misclassification for paramutability by virtue of the occurrence of a subliminal *R* change in the RR^{st} heterozygote. The optical density scores clearly show that the three alleles in question, conditioning self-colored aleurone in single

dose and placed in the non-paramutable category by the kernel matching method, are unresponsive in any degree to R^{st} action.

Entries 1, 8, 22, and 30, in Table 1, provide additional evidence on the correspondence between kernel scores obtained by the two evaluation procedures. The amount of reduction in score for the paramutable *R* alleles represented as a result of R^{st} action in the heterozygotes varies rather widely from case to case. The average reduction in score by the kernel matching procedure, however, is 55%, as compared with 60% by the pigment extraction method.

Tests of additional R *alleles from northwestern South America:* The preliminary data assembled in Table 1 indicating occurrence of a region in northwestern South America in which maize varieties possessing colored aleurone were characterized by non-paramutable *R* alleles prompted us to assemble and to test additional stocks from this area. Four new *R* accessions were obtained from Bolivia, two from Chile, two from Ecuador, and eight from Peru. These factors were introduced into the W22 inbred strain and then tested for reduction in aleurone pigmenting potential in response to R^{st} action in RR^{st} heterozygotes. The results of the tests for paramutability are summarized in Table 2.

TABLE 2

Optical density scores of anthocyanins extracted from measured amounts of R r r *testcross kernels carrying* R^g *alleles originally collected in Bolivia, Chile, Ecuador, and Peru*

Entry	Identification	Locality	Optical density score for *R r r* kernels	
			Normal form	From *R* R^{st}
		BOLIVIA		
1	473	Kulli	0.46	0.43
2	716	....	0.70	0.58
3	724	Paru	0.49	0.47
4	1004	Kulli	0.83	0.77
		CHILE		
5	370	....	0.71	0.67
6	406	....	0.49	0.54
		ECUADOR		
7	592	....	0.55	0.52
8	635	....	0.35	0.43
		PERU		
9	568	Ancash; Huarmey	0.73	0.80
10	1083	Junín: Huancayo	0.38	0.41
11	1182	Cajamarca: Celendín	0.45	0.45
12	120*	Ancash: Corongo	0.36	0.35
13	120–907*	Ancash: Corongo	0.32	0.36
14	150	Ancash: Corongo	0.40	0.39
15	Huarmey	Ancash: Huarmey	0.37	0.38
16	San Miguel	Ayacucho: San Miguel	0.36	0.46

* These alleles also carry a cherry component (SASTRY 1965).

The optical density scores showed that all the newly acquired *R* alleles from Bolivia, Chile, Ecuador, and Peru were non-paramutable. On the assumption that some of the factors might be weakly paramutable and not detectable as such on testcrossing F_1 RR^{st} plants on *rr* females, the alleles were carried through R^{st} heterozygotes for two additional generations and then tested in matings on *rr* females. Inspection of the resulting testcross ears confirmed the conclusion based on the first test, namely, that the factors were non-paramutable throughout. The confirmatory test for non-paramutability included entry 2 (Bolivia 716) whose sensitivity to R^{st} action was left in some doubt by the results of the first trial.

It is obvious from the optical density scores for the respective normal forms that the alleles in this group are heterogeneous with respect to aleurone pigmenting action. All conditioned green anthers and so were symbolized by R^g, and all gave color over the entire surface of the aleurone in single dose. In general, however, the alleles that gave a low optical density score conditioned a paler aleurone phenotype, especially in the crown region of the kernel, than those giving high scores. The scores for aleurone pigment content ranged from 0.83 for the Bolivia 1004 allele to 0.32 for Peru Corongo 120–907. The diversity holds in some cases for alleles originally collected in the same locality. The normal forms of the two factors from the Kulli district of Bolivia, for example, gave optical density scores of 0.46 and 0.83, respectively. Similarly three accessions from Ancash, Peru, gave values of 0.73, 0.38, and 0.36. SASTRY (1965) observed that two of the Peru Corongo R^g alleles, namely, 120 and 120–907, when introduced into plants carrying the *Pl* factor, conditioned cherry pericarp. Other differences in phenotypic effects in seedlings and adult plants between certain of the alleles in this group, but not recorded here, also were in evidence. In spite of their diversity in these other respects, however, all the alleles proved to be non-paramutable.

Recent Latin American R *accessions:* Preliminary data on paramutability of 51 additional *R* accessions from Latin America are assembled in Table 3. The *rr* ♀ × RR^{st} ♂ testcrosses involving these alleles were made after only three backcrosses to the W22 inbred strain, and because of the residual variability present the scores are reported in qualitative form. If the *R* allele under test clearly showed a reduction in aleurone pigmenting potential on passage through a heterozygote with stippled it was rated "+" for paramutability. Correspondingly, the *R* alleles that were insensitive to R^{st} action in heterozygotes were scored as "—". Only one *R* factor among the 52 new accessions tested gave an equivocal result on this basis; it is omitted from Table 3.

The data in Table 3 provide further evidence that non-paramutable *R* alleles are limited in distribution to a region in northwestern South America, and that *R* alleles from outside this area are paramutable. All eight R^g accessions from Mexico were paramutable. Likewise, six of the seven R^g factors derived from Brazil, one from Paraguay, one from Colombia, and 10 of the 12 (mostly R^r) alleles from Venezuela were paramutable. In contrast, the five R^g alleles derived from races indigenous to Bolivia, the three from Ecuador, the two from Chile, and the 10 from Peru were non-paramutable throughout.

These data, taken in conjunction with those in Tables 1 and 2, reveal a well

TABLE 3

Results of preliminary tests for paramutability and paramutagenicity of recently acquired R *alleles representing maize varieties indigenous to Mexico, Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, and Venezuela*

Entry	Allele	Identification	Geographic source and variety	*R r r* phenotype*	Paramutability†	Paramutagenicity‡
			MEXICO			
1	R^g	J–27	Aguas-Calientes	mot.	+	NT
2	R^g	J–39	Aguas-Calientes	mot.	+	NT
3	R^g	J–10	Colima	mot.	+	NT
4	R^g	J–97	Guanajuato	mot.	+	NT
5	R^g	J–27	Mexico State	mot.	+	NT
6	R^g	J–33	Mexico State	mot.	+	NT
7	R^g	J–40	Mexico State	mot.	+	NT
8	R^g	....	San Juan del Rio	mot.	+	NT
			ARGENTINA			
9	R	Oke 60/62	Jujuy	s. c.	—	NT
10	R^g	Azul	Jujuy	s. c.	—	NT
			BOLIVIA			
11	R^g	473–7551	Kulli	s. c.	—	NT
12	R^g	494–7539	Tacacoma, La Paz	s. c.	—	NT
13	R^g	705	Cholito	s. c.	—	NT
14	R^g	707–6769	Cholito	s. c.	—	NT
15	R^g	716–6759	Inquisive, Cajuata, La Paz	s. c.	—	—
			BRAZIL			
16	R^g	CMI 54	Ceremonial, Mato Grosso	s. c.	+	NT
17	R^g	CMI 56	Ceremonial, Mato Grosso	mot.	+	NT
18	R^g	1963	Entrelaçado, Mato Grosso	s. c.	+	NT
19	R^g	4980	Avati Jacaira, Mato Grosso	s. c.	+	NT
20	R^g	5011	Caingang	mot.	+	NT
21	R^g	5042	Rio Grande do Sul	mot.	+	NT
22	R^g	3359	Caingang, Paraná	s. c.	—	NT
			CHILE			
23	R^g	370	Toconce Overo	s. c.	—	+
24	R^g	406	Capio Negro Chileno	s. c.	—	+
			COLOMBIA			
25	R^r	1818	....	mot.	+	NT
			ECUADOR			
26	R^r	731 (PI 302327)	Santiago Zamora, Macas, Bomboisa	s. c.	—	NT
27	R^r	929 (PI 302341)	El Oro, Machala	s. c.	—	NT
28	R^g	887–6732	....	mot.	—	NT

TABLE 3—Continued

Entry	Allele	Identification	Geographic source and variety	*R r r* phenotype*	Paramutability†	Paramutagenicity‡
			PARAGUAY			
29	R^g	CMI 128	Avaticambá	mot.	+	NT
			PERU			
30	R^g	568	Ancash; Santa, Huarmey	s. c.	—	+
31	R^g	1083–53A	Junín: Sisibamba, Huancayo	s. c.	—	—
32	R^g	1182	Cajamarca; Celendín	s. c.	—	—
33	R^g	1304–2993	Huancavelica	s. c.	—	+
34	R^g	1497	....	....	—	—
35	R^g	Corongo 120	Ancash	s. c.	—	—
36	R^g	Corongo 120–907	Ancash	s. c.	—	—
37	R^g	Corongo 150	Ancash	s. c.	—	—
38	R^g	Anc 9	Huarmey	s. c.	—	—
39	R^g	Aya 59	San Miguel	s. c.	—	+
			VENEZUELA			
40	R^r	412–4478 (PI 302347)	Aragua; Girardot, Cata	lt. mot.	+	NT
41	R^r	497–4263 (PI 302351)	Monagas; Piar, Chaguaramas	lt. mot.	—	NT
42	R^r	530–4238 (PI 302354)	Guárico; Miranda, Calabozo	lt. mot.	+	NT
43	R^r	559–4199 (PI 302355)	Guárico; Zaraza, Tacalito	mot.	+	NT
44	R^r	567–4218	Guárico; Infante, Malquerida	lt. mot.	+	NT
45	R^r	590–4284	....	lt. mot.	+	NT
46	R^r	594–4479 (PI 302363)	Cojedes; Tinaquillo	lt. mot.	+	NT
47	R	628–16038 (PI 302369)	Anzoátegui; Aragua, La Torti	pale	—	NT
48	R^r	702–4405	Anzoátegui; Freites, El Perú	lt. mot.	+	NT
49	R^r	760–16029 (PI 302383)	Monagas; Maturín, El Catito	pale	+	NT
50	R^r	760–4181	....	lt. mot.	+	NT
51	R^g	753–4242 (PI 302381)	....	lt. mot.	+	NT

* mot. = mottled; lt. mot. = light mottled; s.c. = self-colored.
† "+" = paramutable; "—" = non-paramutable.
‡ NT = not tested; "+" = paramutagenic, and "—" = non-paramutagenic.

defined pattern in the geographic distribution of paramutable and non-paramutable *R* alleles. The pattern is illustrated in Figure 2 for North America and Figure 3 for South America. The focus of maize races carrying non-paramutable *R* alleles obviously is the Andean highlands. Outside this area the *R* alleles present

FIGURE 2.—Geographic distribution of North American *R* alleles tested for paramutability. Each hollow circle represents an *R* factor found to be sensitive to heritable reduction in aleurone pigmenting potential on passage through a stippled heterozygote.

in varieties exhibiting colored aleurone in one or another frequency are paramutable.

The observed exceptions to this pattern are few indeed. There were none in the samples from the United States, Canada, Mexico, Guatemala, and from outside the Americas. Two Argentina alleles (Table 1, entries 8 and 22) from Misiones and Buenos Aires, respectively, were found to be paramutable in the early tests. Two *R* alleles from Argentina (Table 3, entries 9 and 10) among the later accessions, on the other hand, proved to be non-paramutable. These non-paramutable

FIGURE 3.—Geographic distribution of paramutable and nonparamutable South American R alleles. Hollow circles represent alleles sensitive to R^{st} action in heterozygotes, and solid circles represent R alleles insensitive to R^{st} action in heterozygotes.

factors were derived from collections made in Jujuy province, northern Argentina, adjacent to Bolivia. They may properly be included, therefore, in the Andean highland group. The one non-paramutable allele among seven tested from Brazil (Table 3, entry 22) may be accounted for in the same way. It was extracted from a variety native to Caingang, Paraná, near the Bolivian boundary.

Ten of the 12 R alleles from Venezuela were found to be paramutable. The exceptions in this case (Table 3, entries 41 and 47) did not come from stocks collected in the Andean highlands, but from Monagas and Anzoátegui provinces in eastern Venezuela. Possibly maize carrying these particular non-paramutable R alleles was transported at some earlier time to the regions in question from the Andean highlands.

The presently available data are inadequate to establish the northern boundary of the area characterized by non-paramutable R alleles. It may lie somewhere in Colombia, a country represented in our collection by a single R allele only, and which proved to be paramutable (Table 3, entry 25).

Paramutagenicity of non-paramutable R *alleles:* A systematic attempt was not made to characterize the *R* factors used in the present study for paramutagenicity. The fact was established, however, that within the non-paramutable group of self-colored factors from the Andean region of South America alleles occur that are more highly paramutagenic than stippled (R^{st}). Self-colored alleles either equal to, or less than, R^{st} in intensity of paramutagenic action have long been known (McWHIRTER and BRINK 1962) whereas there has been uncertainty whether alleles exceeding stippled in this respect occur.

The conventional test for paramutagenic action of an *R* factor involves introducing the latter into a heterozygote with a sensitive allele, usually either standard R^r or an R^g mutant from standard R^r. Pollen from the heterozygotes is then applied to colorless (rr) females, and the resulting R^r (or R^g) testcross kernels are examined for possible reduction in aleurone pigmenting potential. This procedure is useful, of course, only when the two equally frequent classes of kernels on the testcross ears can be definitively separated. If the scoring method is based on anthocyanin extracts from ground seeds, as in some of the present experiments, such separation can be made only in those cases in which the phenotypes of the two kinds of kernels in question do not overlap. This condition limited the present study of paramutagenicity to *R* alleles at the upper end of the scale for this property.

Optical density scores are presented in Table 4 for R^rrr testcross kernels from heterozygotes carrying standard R^r and seven highly paramutagenic alleles from Bolivia, Chile, or Peru. The controls are standard R^rR^r of stock culture origin and F_1 R^rR^{st} plants. It will be noted that the scores for entries 3 and 9, involving the Bolivia 724 and the San Miguel, Peru, alleles, are low, as compared with that for entry 2, namely, R^r derived for the R^rR^{st} control. The question of interest is

TABLE 4

Optical density scores of anthocyanins for the standard R^r *allele in* R^rrr *testcross kernels in normal form (from an* R^rR^r *stock culture) and following passage through heterozygotes with stippled or a paramutagenic self-colored* R^g *allele originally derived from a strain indigenous to Bolivia, Chile, or Peru*

Entry	Staminate parent in testcross	Optical density score for $R^rr^rr^r$ kernels
1	standard R^rR^r (stock culture; control)	0.48
2	R^r R^{st}	0.11
3	R^r/R^g Bolivia 724	0.01
4	R^r/R^g Bolivia 1160	0.20
5	R^r/R^g Bolivia 1520	0.06
6	R^r/R^g Chile 370	0.05
7	R^r/R^g Chile 406	0.12
8	R^r/R^g Peru 568	0.12
9	R^r/R^g San Miguel, Peru	0.03

The testcross score for each entry from 2 to 9, inclusive, is significantly lower, at the $P < .01$ level, than that for standard R^r in entry 1.

TABLE 5

Optical density scores of anthocyanins extracted from measured amounts of R^{r}rr *testcross kernels carrying one or the other of two* R^r *alleles, North Dakota (PI 222309) or Argentina (PI 161418) following passage through heterozygotes with stippled and two self-colored, paramutagenic alleles, Bolivia 724 and Peru, San Miguel, respectively*

Entry		Staminate parent in testcross on $r^g r^g$ ♀ ♀	Optical density score for R^r
1		R^r No. Dakota/R^{st}	0.06
2		R^r No. Dakota/R^g Bolivia 724	0.01
3		R^r No. Dakota/R^g San Miguel, Peru	0.14
4		R^r Argentina/R^{st}	0.25
5		R^r Argentina/R^g Bolivia 724	0.12
6		R^r Argentina/R^g San Miguel, Peru	0.24
Differences in score			
1 and 2	0.05	$P < 0.01$	
1 and 3	0.08	$P < 0.01$	
2 and 3	0.13	$P < 0.01$	
4 and 5	0.13	$P < 0.01$	
4 and 6	0.01	n.s.	
5 and 6	0.12	$P < 0.01$	

whether these self-colored alleles are significantly stronger in paramutagenic action than stippled.

Two paramutable R^r alleles which gave somewhat higher aleurone pigmentation scores than that for standard R^r when tested in R^rR^{st} heterozygotes were chosen for further tests of the relative paramutagenicities of Bolivia 724, San Miguel, Peru, and R^{st}. It was postulated that these R^r alleles, namely, North Dakota (PI 222309) and Argentina (PI 161418), would increase the degree of resolution for paramutagenicity in tests with strongly acting factors. The testcross data are summarized in Table 5, in which each mean value in the last column is based on matings involving two families of male parents.

The Bolivia 724 allele caused a significantly greater reduction in aleurone pigmenting potential of both the North Dakota and Argentina paramutable factors than either R^{st} or the San Miguel, Peru, R^g factors. The reduction in pigmenting potential of the Argentina R^r factor by the San Miguel, Peru, R^g allele, on the other hand, was not significantly different from that caused by R^{st}. The reduction in the case of the North Dakota R^r factor was significantly less for the San Miguel, Peru, than for the R^{st} allele. The results of these tests bear out the conclusion suggested by the data in Table 4 that the Bolivia 724 self-colored allele is more strongly paramutagenic than stippled. The inference that the San Miguel, Peru, R^g factor also is more highly paramutagenic than stippled, however, is not supported.

It should be borne in mind that the sensitive R^r factors on which the data in Tables 4 and 5 are based are unlike, and that the difference in the two sets of results may be related to this fact. A still unexplored possibility is that a differ-

ential interaction occurs between paramutable and paramutagenic alleles in heterozygotes. One paramutable factor might be more sensitive to repression by paramutagenic allele X than by Y, whereas a second paramutable allele might be more responsive to the action of Y than to that of X.

A new class of paramutable R *alleles:* A previously unrecognized class of *R* alleles came to light during the course of the present tests for paramutagenicity. These factors are insensitive to R^{st} action in heterozygotes and so are classifiable as non-paramutable by this criterion. They do not alter the pigmenting potential of standard R^r in heterozygotes, and so are non-paramutagenic. The distinctive property which they exhibit is a reduction in pigmenting potential on passage through heterozygotes with standard R^r (or an R^g mutant from standard R^r). Only preliminary data are available on the distribution and properties of this new category of *R* factors.

The reduction in pigmenting potential of an *R* allele of this kind following heterozygosity with standard R^r for a single generation often is so small that an accurate separation cannot be made of the two classes of kernels on a testcross ear. A more conspicuous change in appearance occurs after two generations of such heterozygosity. The affected testcross seeds were not mottled but were pale, whereas the standard R^rrr kernels on the same ear characteristically were darkly mottled.

All the alleles tested in this experiment but two, gave green anthers (R^g), and so were tested in heterozygotes with standard R^r. The two remaining factors, namely, R^r (Ecuador 635) and R^r (Ecuador 1172) gave red anthers, and so were tested in heterozygotes with R^g, a green-anthered mutant from standard R^r.

Sensitivity to repression by standard R^r of this group of non-paramutagenic alleles was not anticipated, so that controls were not included in the test. The single dose scores following corresponding $rr♀ \times Rr♂$ matings made in an earlier year, therefore, were used as controls. These values and the testcross scores of 13 alleles tested after two generations of heterozygosity with standard R^r for the property in question are shown in Table 6.

The alleles listed in Table 6 fall into two well defined groups. One is reactive to standard R^r and the other is non-reactive. All the non-paramutagenic Peru alleles tested showed a statistically significant reduction in pigmenting potential, as did Bolivia 473 and Ecuador 592.

Entry 9 in Table 6 is possibly an anomaly. According to our records, this R^g allele is of South Dakota origin (PI 213779). It is highly sensitive to R^{st} action in R^gR^{st} heterozygotes, in accordance with entry 30 in Table 1. Verification is needed before it is concluded that any *R* alleles that are paramutable, as defined by the test in heterozygotes with R^{st}, react in the same way as the non-paramutable South American alleles in question in heterozygotes with standard R^r.

The two alleles represented in Table 6 as entries 10 and 11 which normally conditioned high levels of aleurone pigmentation (0.70 and 0.83, respectively) were unaffected by association in heterozygotes with standard R^r. Strong pigmenting action alone, however, is not a sufficient criterion of non-reactivity in this test.

TABLE 6

Effect on level of pigmenting action in testcrosses on rr *plants of certain* R^g *and* R^r *alleles, insensitive to* R^{st} *action, following passage through two generations of heterozygotes with standard* R^r *or an* R^g *mutant from standard* R^r.
The control values are taken from Table 2

Entry	Allele	Optical density score for *R*	
		Control	From second generation R^r (or R^g) heterozygote
	Reactive alleles		
1	R^g Bolivia 473*	0.46	0.31
2	R^g Ecuador 592	0.55	0.40
3	R^g Peru 1083	0.38	0.26
4	R^g Peru 1182	0.45	0.26
5	R^g Peru Corongo 120	0.36	0.24
6	R^g Peru Corongo 120–907	0.32	0.25
7	R^g Peru Corongo 150	0.40	0.27
8	R^g Peru Huarmey	0.37	0.26
9	R^g So. Dakota, PI 213779	0.41	0.29
	Non-reactive alleles		
10	R^g Bolivia 716–6759	0.70	0.71
11	R^g Bolivia 1004	0.83	0.82
12	R^r Ecuador 635	0.35	0.39
13	R^r Ecuador 1172	0.66	0.69

* All entries from 1 to 9, inclusive, show statistically significant differences from the corresponding control.

R^r Ecuador 635 which gave an optical density score of only 0.35 also was non-reactive to standard R^r in the heterozygotes.

Further work is needed on the nature and geographic distribution of *R* alleles that react to standard R^r in heterozygotes in this novel way before the significance of this aspect of the *R* paramutation phenomenon will be apparent.

A naturally occurring non-paramutagenic stippled allele: Stippled was the original paramutagenic allele used in the Wisconsin studies. It conditions a distinctive, finely spotted, aleurone phenotype, is strongly active in reducing the pigmenting potential of R^r in R^rR^{st} heterozygotes, and so was adopted as standard in the paramutagenic *R* class. This particular R^{st} factor, incorporated in our foundation stock cultures and extensively used since, was known to have come from a South American variety, but the records do not show the country of origin.

Tests for paramutagenicity have not yet been completed for additional R^{st} accessions from South America. One R^{st} allele, namely, Bolivia 494, has been found, however, that is non-paramutagenic in heterozygotes with standard R^r, although it conditions the same spotted aleurone phenotype as stippled factors of other origins. This case provides direct evidence that the component of the R^{st} allele underlying paramutagenicity is distinct from that associated with the aleurone spotting.

LINDEN and RODRIGUEZ (1965) have presented evidence for heterogeneity with respect to paramutagenic action among Andean strains of corn showing "variegated" aleurone. The factors conditioning variegation were not further identified, so it is not known whether non-paramutagenic R^{st} alleles like that in the Bolivia 494 stock were included among them.

DISCUSSION

The evidence presented shows that *R* paramutability in maize is widely distributed geographically. *R* alleles from diverse sources in the United States, Mexico, and Guatemala were paramutable throughout. Furthermore, paramutability was the prevailing, although not the exclusive, condition encountered among *R* factors originally derived from Venezuela, Brazil, and Argentina. The single accessions tested from Canada, Colombia, Paraguay, and Ethiopia, and a few from Turkey and India, also were paramutable. It is thus apparent that *R* alleles conditioning colored aleurone and the potentiality of *R* to undergo paramutation are coextensive over a major part of the wide area in which maize is grown.

The Andean region of South America marks a sharp discontinuity in the distribution of paramutable *R* alleles. No *R* factors sensitive to heritable changes in aleurone pigmenting potential in R^{st} heterozygotes (a standard test for paramutability) occurred in the maize varieties tested from Ecuador, Peru, Chile, and Bolivia. Two *R* factors from a province in Argentina bordering this area and one from a nearby part of Brazil also were non-paramutable. The only local irregularity encountered was represented by two non-paramutable *R* alleles from eastern Venezuela, a region in which most of the *R* factors present are paramutable. These exceptions may well have been the result of earlier migration of varieties from the Andean area into this part of Venezuela.

R alleles from the Andean region, although uniformly insensitive to R^{st} action in RR^{st} heterozygotes, vary importantly in other properties concerned with *R* paramutation. Many of the factors are overtly paramutagenic (e.g., Bolivia 724, Chile 370, Peru 568). They resemble in this respect standard stippled (R^{st}) and marbled (R^{mb}) which are of sporadic occurrence, and have been collected from races indigenous to this area only. Other *R* alleles from this region have proved to be non-paramutagenic (e.g., Ecuador 1172, Bolivia 473–7551, Bolivia 494, and Peru 1182).

The present study shows that the latter class is itself compound, and includes *R* factors of a kind not previously recognized. The relevant data, summarized in Table 6, show that eight R^g alleles originally derived from Bolivia, Ecuador, and Peru varieties undergo significant reduction in aleurone pigmenting potential when passed through heterozygotes with standard R^r for two generations. Two R^g factors of Bolivian origin and two R^r alleles from Ecuador, in contrast, were non-reactive in the same test.

BRINK (1964) postulated that the *R* locus originally was amorphic with respect to paramutation, and that the properties of paramutability and paramutagenicity

were acquired later. The Andean region of South America was suggested as the locale of the primitive *R* form. The present observations are compatible with these suggestions. *R* alleles that are unreactive with respect to all the paramutation tests applied have been found in the Andean region only. The number of *R* factors known to be in this group, however, is small. It is limited, in fact, to two from Bolivia, 716–6759 and 1004, and two from Ecuador, designated 635 and 1172, entered in Table 6.

It was assumed, on this view, that paramutagenicity arose as the result of transfer to the *R* locus of a particular element embodying this property from elsewhere in the genome. Another kind of element, similarly acquired, was assumed to underlie paramutability. The element conferring paramutagenicity, when present at the *R* locus, did not affect expression of the allele with which it was coupled, so that paramutagenic *R* factors regularly conditioned self-colored aleurone in single dose. The adventive element involved in paramutability, on the other hand, was assumed not only to confer this particular property on the locus but also to make the allele in question metastable so that its usual expression in single dose was darkly mottled, rather than self-colored, aleurone. The metastability has since been examined in detail and shown by STYLES and BRINK (1966) and STYLES (1967a, 1967b) to be characteristic of *R* alleles that are sensitive in heterozygotes to R^{st} action. The occurrence of a non-paramutagenic stippled allele, reported here as an exception among the factors giving this aleurone phenotype, supports the argument that paramutagenicity rests upon a distinctive element at the *R* locus.

The present evidence shows, however, that not all *R* alleles previously classified as non-paramutable are properly described as such. It is now found that some of the *R* factors that are insensitive to change in R^{st} heterozygotes undergo heritable reduction in aleurone pigmenting potential if passed through heterozygotes with the standard R^r factor. The few data available on this newly recognized class of alleles suggest occurrence at the *R* locus in these cases of an element different from that underlying sensitivity to R^{st} action in heterozygotes or conferring paramutagenicity.

We have not been directly concerned in this study with the relationship between *R* paramutation phenomena and the history and evolution of maize. In general, little information except geographic origin has been available to us concerning the various indigenous races from which the *R* alleles used were extracted and then incorporated in the W22 inbred strain. A few data are at hand, however, which suggest that *R* paramutation has had a long history in maize.

RAMIREZ *et al.* (1960) state that Kulli, from which Bolivia 473 (Table 3, entry 11) and Bolivia 1004 (Table 2, entry 4) were extracted is a "primitive" race of maize. These R^g alleles condition self-colored aleurone in single dose, are insensitive to R^{st} action in R^gR^{st} heterozygotes, and are both non-paramutable and non-paramutagenic in heterozygotes with standard R^r. That is, they appear to be paragenetically amorphic. Bolivia 724 (Table 2, entry 3), on the other hand, was extracted from Paru, a race described by these investigators as "anciently derived" and, according to their classification younger than the primitive group. Bolivia 724 gives self-colored aleurone in single dose, is insensitive to R^{st} action in R^gR^{st}

heterozygotes, but is paramutagenic in standard R^r/R^g heterozygotes. The three Peru Corongo alleles, included in Table 6 as entries 5, 6, and 7, belong to the race Sjajato which, according to GROBMAN, SALHUANA and SEVILLA (1961), also belongs to the anciently derived group. The Peru Corongo alleles are insensitive to R^{st} action but, as the data in Table 6 show, they undergo heritable reduction in aleurone pigmenting potential on passage through heterozygotes with standard R^r.

Thus within either primitive or anciently derived Andean races three main categories of R alleles occur in terms of paramutation. One group is paragenetically amorphic, another causes paramutation in standard R^r heterozygotes, and the third is sensitive to reduction in aleurone pigmenting action in response to standard R^r action.

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SUMMARY

All R^r and R^g alleles derived from North American maize varieties proved to be paramutable, that is, on passage through heterozygotes with the stippled (R^{st}) factor, they underwent heritable reduction in aleurone pigmenting potential. Several alleles from Indian reservations in the Great Plains and Southwest regions of the United States, eight from Mexico, one from Guatemala, and one from Canada were included in the sample tested. Most of the R alleles extracted from varieties indigenous to Venezuela, Brazil, and Argentina also were sensitive to R^{st} action in heterozygotes. Single accessions obtained from Colombia and Paraguay in South America, one from Ethiopia, and a few from Turkey and India were paramutable. R paramutability is thus coextensive with the alleles at this locus conditioning aleurone pigmentation over a major part of the area in which maize is cultivated.—South America differs sharply from North America, however, in containing a large, but circumscribed, area in which all the factors are insensitive to R^{st} action in heterozygotes. No R^{st} sensitive alleles were found in collections from Ecuador, Peru, Chile, and Bolivia. Two R factors from Jujuy, a province in Argentina adjacent to Bolivia, and one from Caingang, Paraná, in southwestern Brazil, also were non-paramutable. Two among 12 Venezuelan R factors likewise fall in the non-paramutable class. It is clear from this evidence that R factors carried by varieties indigenous to the Andean region of South America are characteristically insensitive to the paramutagenic action of R^{st}.—The Andean R alleles just mentioned give self-colored aleurone in single dose, whereas the contrasting class of paramutable R factors from other parts of South

America, and from North America, usually condition darkly mottled aleurone at this dosage level. Furthermore, many of the *R* factors of Andean origin are overtly paramutagenic in heterozygotes with standard R^r, and are comparable in this respect to stippled and marbled, which appear to be limited in distribution to the same region.—It was learned from the present experiments that a significant proportion of the *R* alleles from Andean sources are sensitive to heritable changes in aleurone pigmenting potential in heterozygotes with standard R^r. This newly recognized property has been little studied, but the *R* factors possessing it appear to constitute a distinct group with respect to the paramutation phenomenon.—A few *R* alleles of Andean origin proved to be insensitive to change in all the paragenetic tests applied. Such alleles are assumed to represent the primitive condition at the *R* locus. Paramutable and paramutagenic *R* alleles may be looked upon as resulting from movement to the locus of chromosomal components from elsewhere in the genome by transposition, local inversions, or other structural alterations, which conferred these respective secondary properties on the *R* locus. The evidence from this study, concerned primarily with geographic distribution, also points to a long history of polymorphism for paramutation at the *R* locus.

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