DEPENDENCE OF THE *R*-MOTTLED ALEURONE PHENOTYPE IN MAIZE ON MODE OF SEXUAL TRANSMISSION[1]

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TYPICAL *R*-mottling, the irregular distribution of anthocyanin in the aleurone layer of maize endosperm, occurs upon crossing appropriate *R R* plants with *r r* (colorless) only when *R R* is the pollen parent (EMERSON 1918; KEMPTON 1919). When *R R* is used in the mating as female, the aleurone is solidly colored. From a conventional point of view the mottled and solidly colored phenotypes suggest the dosage effects associated with the unequal numbers of chromosome sets contributed to the triploid endosperm, two from the female and one from the male parent. This explanation assumes equal pigmenting action of *R* from the two origins. Furthermore, if parental source were to affect *R* expression, such an influence ordinarily would be confounded with the effect of dosage.

ROMAN's (1947) discovery that a given chromosome segment translocated to the centromeric portion of an accessory B chromosome in maize is transmitted by the male in duplicate provides an opportunity to vary the dosage of genes in such a segment independently of parental origin. Accordingly, his translocation B–10a, with the point of interchange in chromosome-10 between *R* and the centromere, permits introduction of two *R* factors into the endosperm via pollen. Unexpectedly on the *R* dosage interpretation, *R R* from the male when combined with *r r* from the female gave mottled, rather than solidly colored, kernels (ROMAN, cited by SCHWARTZ 1965; KERMICLE 1963). This observation called into question sufficiency of the dosage explanation and prompted further investigation. The evidence presented here shows the basis of the disparity in aleurone phenotype following reciprocal crosses between *R R* and *r r* plants is a pronounced difference in level of action at which *R* is transmitted to the endosperm through male and female gametophytes.

MATERIALS AND METHODS

The various alleles of *R* involved affect anthocyanin pigmentation of the aleurone and vegetative parts of the plant as follows:

R^r—Pigmented aleurone, red seedling and anthers; representative of those alleles which give darkly mottled aleurone in crosses to *r^g r^g* as female but solidly colored aleurone when *r^g r^g* is male. The particular *R^r* employed in experiments involving translocation B–10a was that present in the TB–10a stock obtained from the Maize Genetics Stock Center, University of Illinois, Urbana. A second source of *R^r*, that designated standard *R^r* in the Wisconsin collection, was

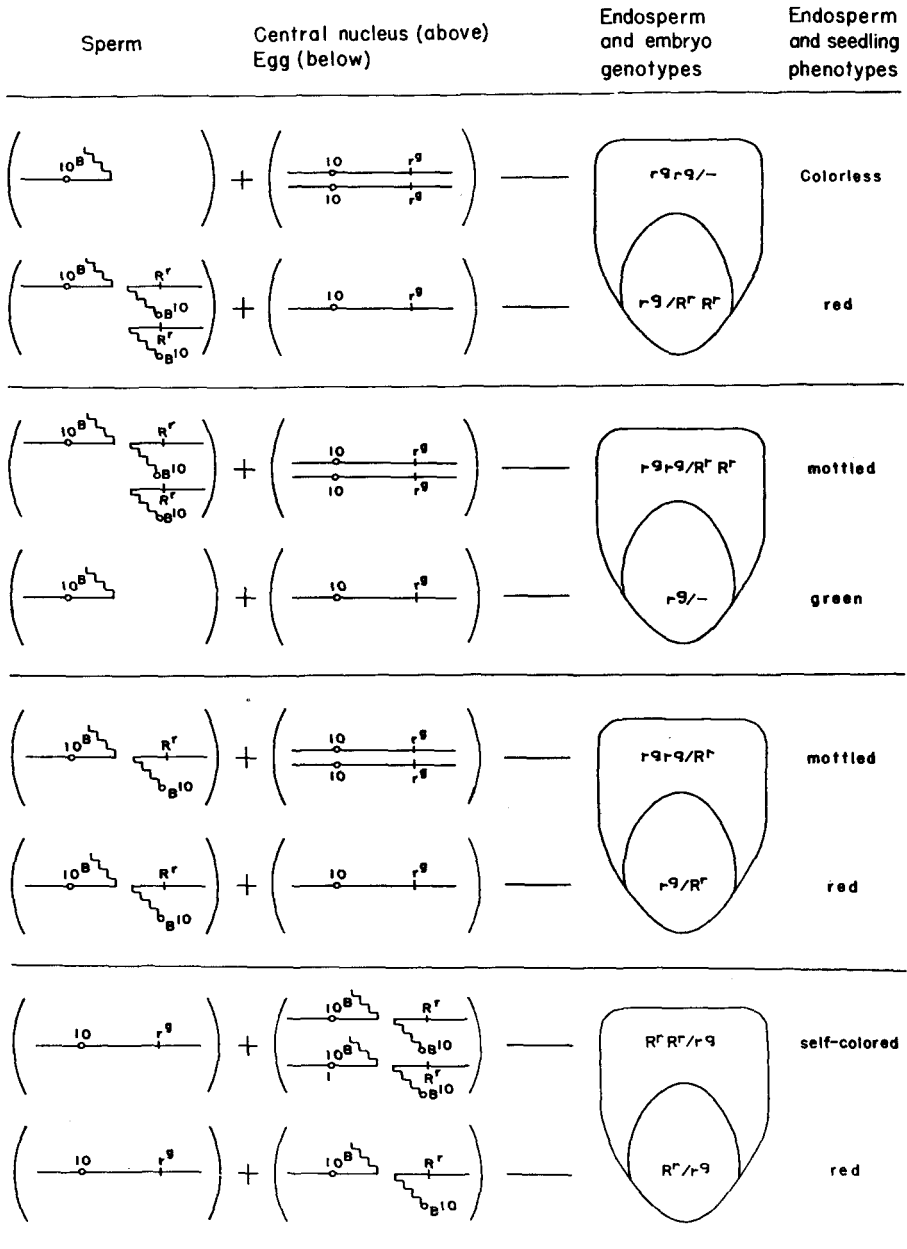| Sperm	Central nucleus (above) Egg (below)	Endosperm and embryo genotypes	Endosperm and seedling phenotypes

FIGURE 1.—Kernel classes resulting from reciprocal crosses between $r^g r^g$ and plants carrying R^r on translocation B–10a. As male, the B^{10} chromosome of TB–10a usually fails to disjoin in the sperm-forming division within the microspore, yielding $R^r R^r$ and R-deficient male gametes. Double fertilizations involving such sperm pairs are illustrated by the first two categories. If the B^{10} chromosomes disjoin normally, kernels of the third class are produced. The fourth class of seeds results from the reciprocal cross, where mitotic disjunction is regular.

employed in those experiments not involving TB–10a. No difference between the R^r alleles from the two sources has been observed.

$R^{r'}$—Paramutant forms of R^r derived from $R^r R^{st}$ heterozygotes (for a general discussion of paramutation see BRINK 1964). In crosses to $r^g r^g$ as female, aleurone pigmentation is greatly reduced relative to parental R^r; in crosses to $r^g r^g$ as male, a few kernels may be mottled, but most are solidly colored.

R^g—Aleurone pigmented as with R^r; seedling tissues and anthers lack anthocyanin. Both the standard and the paramutant forms of R^g used here derive from the sixth R^g mutation isolated from Wisconsin R^r.

R^{st}—Stippled aleurone; green seedling and anthers; paramutagenic to R^r and R^g in hetero-zygotes.

R^{scm}—Self-colored mutation (WEYERS 1961) from the paramutagenic allele R-marbled. Solidly colored aleurone results from crosses to $r^g r^g$ both as male and female. The scutellum of the embryo is intensely pigmented whereas roots and anthers are devoid of anthocyanin.

r^r—Colorless aleurone; red seedlings and anthers; nonparamutagenic.

r^g—Colorless aleurone, green seedling and anthers; recessive to the pigmented condition; nonparamutagenic.

Inbred W22 strains carrying the above alleles were furnished by R. A. BRINK, and transloca-tion B–10a was introduced similarly into W22 by backcrossing. All the experiments utilized strains of the one genetic background in order to minimize extraneous variation.

TB–10: Reciprocal translocation between chromosome 10 and the accessory B chromosome of maize. The point of interchange in 10 is L.35 (ROMAN and ULLSTRUP 1951), a position proximal to R and g_1 (golden plant), and that in B is in the euchromatic segment adjacent to the centromere (LONGLEY 1956). B^{10} denotes the derived chromosome consisting of the centromeric portion of B joined to the distal two-thirds of the long arm of 10, including the g_1–R region. 10^B, the re-ciprocal element of the interchange, consists of the centric piece of 10 with the heterochromatic segment of B attached. Three-point linkage data based on a population of 307 individuals gave the genetic map T–9–g_1–19–R, with 24.4% recombination between T and R.

The behavior of TB–10a conforms closely to ROMAN's (1947, 1948) description of TB–4a. In developing male gametophytes containing either 10^B or an intact B, the B^{10} chromosome fails to disjoin in division of the generative nucleus, yielding one hypoploid sperm (no R genes) and one hyperploid sperm (two R genes). Depending on the pattern of double fertilization (involv-ing the egg and the central nuclei of the female gametophyte) the resulting endosperm cells con-tain either two or no R gene of pollen origin. In a given kernel the complementary situation pertains in the associated embryo. Use of this complementary relationship was made in the as-signment of R^r endosperm genotypes, as illustrated in Figure 1. Whenever the R gene on the B^{10} chromosome of TB–10a was R^{st}, which resembles r^g in seedling pigmentation, $g_1 g_1 r^g r^g$ plants were employed as ear parents. The occurrence of golden, rather than green seedlings, classified after continuous exposure to fluorescent lights at 33°C, then served to identify the embryo as B^{10} deficient. When the R gene on TB–10a was r^g, aleurone genotypes were inferred from the dis-tinctive growth habits of the resulting hyperploid and hypoploid sporophytes.

Throughout, TB–10a was employed in heterozygous condition, with the allele of R under study carried by the translocated chromosome and r^g carried by a normal chromosome-10.

The frequencies of the various seed classes produced in a representative series of $r^g r^g$ ♀ × TR r^g ♂ matings were:

| Phenotype | | Number | Genotype (♀ derived/♂ derived) | | Reference group |
Endosperm	Seedling		Endosperm	Embryo	
Colorless	green	1024	$r^g r^g / r^g$	r^g / r^g	0
Colorless	red	614	$r^g r^g / -$	$r^g / R^r R^r$	I
Colored	green	173	$r^g r^g / R^r R^r$	$r^g / -$	II
Colored	red	428	$r^g r^g / R^r$	r^g / R^r	III
			& $r^g r^g / r^g R^r$	& $r^g / r^g R^r$	
		2239			

The combined endosperm and seedling phenotypes uniquely identify the genotype of kernels carrying only r^g (group 0) and those of the nondisjunctional classes (groups I and II). Some group III kernels may originate by failure of B^{10} nondisjunction as illustrated in Figure 1. Such disjunction occurs comparatively infrequently, however. When TB–10a is heterozygous, additional kernels having a single dose of male transmitted R originate from the transfer of R to the normal homologue by crossing over, and from the formation of 10 B^{10} spores by *meiotic* nondisjunction. Such 10 B^{10} spores, except for possible instances of double reduction, yield R r^g sperm since B^{10} disjunction is normal in the absence of 10^B (LONGLEY 1956). Because r^g does not affect R expression, group III is conveniently referred to by its principal kernel type, $r^g r^g / R$. Due likewise to meiotic nondisjunction, in the reciprocal cross, TR/r^g ♀ $\times$ $r^g r^g$ ♂, a minority of kernels in the colored class (designated R R/r^g) have the composition $r^g r^g R$ R/r^g.

The frequency of B^{10} nondisjunction in the second miscrospore mitosis is estimated from the above data as follows. Of the 1215 male gametophytes containing R^r (Groups I, II and III), the excess of 191 over group 0 is attributed to the functioning of 10 B^{10} spores. Due to recombination between T and R, 249 of the remaining 1024 should carry R on a normal chromosome-10. The expectation for R transmission by sperm carrying either 10 B^{10} or 10, 440 in all, accounts completely for the 428 group III kernels observed. 10^B B^{10} sperm, therefore, are infrequent or absent, indicating the regular occurrence of B^{10} nondisjunction in such spores. The ratio of hyperploid to deficient embryo types, 614:173, reflects the degree of preferential fertilization of the egg by the hyperploid sperm (ROMAN 1948).

To test the effect of elevated R dosage received from the female, and in order to transmit in one gamete two different forms of R, strains were derived which carry the B^{10} chromosome as an accessory to the normal complement. The R allele on B^{10} in such 10 10 10^B trisomics used as female was transmitted to 334 of 1038 offspring (32.2%). Progeny analysis showed the R located on B^{10} was retained by it in 46 of the 47 cases studied. As male, the R on B^{10} was transmitted to 467 of 1419 offspring (32.9%). Of the 68 cases of R transmission analyzed for B^{10} content, R was retained by B^{10} in 56, in 11 it had been transferred to a normal-10, and in one it was carried both by 10 and B^{10}.

Evaluation of pigmentation levels: The intensity of aleurone pigmentation was determined by matching coded samples of kernels from test ears to a standard set ranging progressively in pigmentation from colorless, class 1, to fully colored, class 7. Different standard kernels necessarily were employed for the mottled and stippled phenotypes. All kernels from matings with TB-10a as male first were evaluated for aleurone phenotype. They were then germinated and classified for genotype according to seedling pigmentation. From these crosses, 50% more $r^g r^g / R$ than $r^g r^g / R$ R kernels were used in compiling the data in order to accommodate the more frequent occurrence of the $r^g r^g / R$ class. Efficiency was further increased by restricting the total number of kernels included from each ear only in accordance with the three $r^g r^g / R$: two $r^g r^g / R$ R ratio. When TB–10a was female, and in all other situations where progeny testing was not required, 50 kernels of the appropriate genotype were evaluated per ear.

RESULTS

Test of the R *dosage hypothesis:* Matings of r r with R r when R is borne by the B^{10} chromosome of TB–10a yield kernels with endosperms carrying the R and r factors at three levels: R R/r when TR r is the female parent, and r r $/R$ or the nondisjunctional class r r/R R when TR r is the male parent (the diagonal line is used to separate the genetic factors of maternal and paternal origin, respectively). Comparisons among the three genotypes provide two tests of R dosage. Compared with r r/R, the r r/R R class represents addition of a second R factor carried by a chromosome segment that is present in duplicate, whereas R R/r, relative to r r/R, involves allelic substitution, according to the useful terminology suggested by STERN (1943) for such cases.

TABLE 1

Distribution by aleurone pigmentation class, and mean scores of (A) $r^g r^g/R^r$ and $r^g r^g/R^r R^r$ kernels from nine $r^g r^g ♀ \times$ TB–10a $R^r/r^g ♂$ matings, and (B) of $R^r R^r/r^g$ kernels from the nine reciprocal matings

Mating	Aleurone genotype (♀/♂)	Aleurone pigmentation class						Total kernels	Mean score
		2	3	4	5	6	7		
A	$r^g r^g/R^r$	..	..	..	30	301	92	423	6.15
A	$r^g r^g/R^r R^r$	..	..	..	4	146	132	282	6.45
B	$R^r R^r/r^g$	..	..	..	..	1	449	450	7.00

Homogeneity χ^2 for $r^g r^g/R^r$ and $r^g r^g/R^r R^r = 54.8$; $P < .001$.

The data in Tables 1, 3 and 4 permit dosage comparisons of the two sorts at different levels of R^r action resulting from paramutation. In the genetic background of inbred W22 the R^r resident in ROMAN's TB–10a stock conditions darkly mottled $r^g r^g/R^r$ aleurone. As Table 1 shows, the majority of such kernels score class 6 on the 1–7 pigmentation scale but the distribution also includes several individuals in the uniformly colored class, 7. The average score, 6.15, is to be compared with 6.45 for $r^g r^g/R^r R^r$ kernels, a difference that, although small, was constant in direction in each of the nine matings studied. $R^r R^r/r^g$ kernels produced in the reciprocal matings gave an average score of 7.00. The apparent increase due to a second R^r factor in the two sorts of dosage tests is 0.30 and 0.85 pigmentation units, respectively. That the discrepancy in the two values reflects the action of a major variable other than R^r dosage is clearly evident from a direct comparison of the scores of $r^g r^g/R^r R^r$ and $R^r R^r/r^g$ seeds. Whereas more than half of $r^g r^g/R^r R^r$ kernels were in the darkly mottled categories, 5 and 6, all but one of the 450 $R^r R^r/r^g$ kernels evaluated were solidly colored (class 7).

Reducing pigmentation level of the $r\,r/R$ class by using an R allele in paramutant, rather than standard, form spreads the distribution of mottled types more widely over the several pigmentation classes (Fig. 2). The data in Table 2 on three $R^r R^{st}$ plants mated reciprocally with $r^g r^g$ illustrate the sensitivity of the R^r of the translocation stock to the paramutagenic action of R^{st}. The three plants occurred among the offspring of a $TR^r r^g ♀ \times R^{st} R^{st} ♂$ mating as instances in

TABLE 2

Distribution according to aleurone pigmentation class, and mean scores, of $r^g r^g/R^{r'}$ and $R^r R^{r'}/r^g$ kernels produced by reciprocal matings of three $R^r R^{st}$ plants with $r^g r^g$ individuals

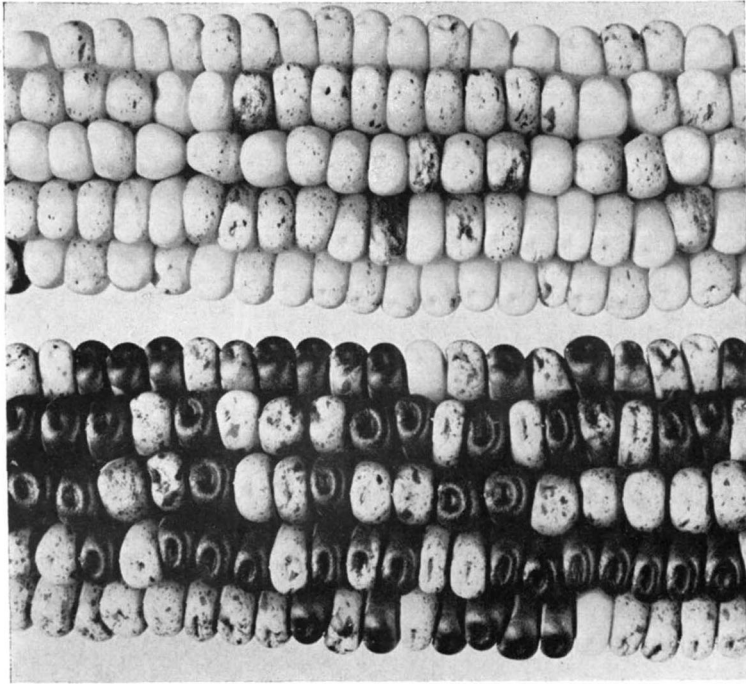| Mating ♀ ♂	Aleurone genotype (♀/♂)	Aleurone pigmentation class						Mean score
		2	3	4	5	6	7	
$r^g r^g \times R^r R^{st}$	$r^g r^g/R^{r'}$	8	61	43	31	7	..	3.79
$R^r R^{st} \times r^g r^g$	$R^r R^{r'}/r^g$	..	..	..	2	8	140	6.92

FIGURE 2.—Ears produced by mating R^rR^{st} reciprocally with r^gr^g. When R^rR^{st} is female (lower) approximately equal numbers of solidly colored $R^{r'}R^{r'}/r^g$ and spotted, $R^{st}R^{st}/r^g$ kernels result. When R^rR^{st} is male (upper) about half the kernels are spotted (r^gr^g/R^{st}) and half are weakly mottled ($r^gr^g/R^{r'}$).

which recombination had transferred R^r from the translocated to the normal chromosome-10 homologue.

Similar tests on five TR^r/R^{st} sibs also yielded a distribution of $r^gr^g/R^{r'}$ kernels extending over the mottled classes 2 to 6, with a mean score of 3.95 (Table 3). It is especially significant that those kernels having R^r duplicated ($r^gr^g/R^{r'}R^{r'}$

TABLE 3

Distribution according to aleurone pigmentation class, and mean scores, of (A) $r^gr^g/R^{r'}$ and $r^gr^g/R^{r'}R^{r'}$ kernels totaled over five $r^gr^g ♀ \times TB–10a R^rR^{st} ♂$ matings and (B) for $R^{r'}R^{r'}/r^g$ kernels from the five reciprocal matings

Mating	Aleurone genotype (♀/♂)	Aleurone pigmentation class						Total kernels	Mean score
		2	3	4	5	6	7		
A	$r^gr^g/R^{r'}$	13	75	104	74	7	..	273	3.95
A	$r^gr^g/R^{r'}R^{r'}$	16	62	58	37	9	..	182	3.79
B	$R^{r'}R^{r'}/r^g$	..	..	1	..	6	243	250	6.96

Homogeneity χ^2 for $r^gr^g/R^{r'}$ and $r^gr^g/R^{r'}R^{r'} = 9.4$; P $= .05$.

TABLE 4

Distribution by aleurone pigmentation class, and mean scores, for (A) $r^{g}r^{g}/R^{r'}$ and $r^{g}r^{g}/R^{r'}R^{r'}$ kernels from eight $r^{g}r^{g}$ ♀ × TB–10a $R^{r'}/r^{g}$ ♂ matings, and (B) for $R^{r'}R^{r'}/r^{g}$ kernels from three reciprocal matings

Mating	No. of crosses	Aleurone genotype (♀/♂)	Aleurone pigmentation class						Total kernels	Mean score
			2	3	4	5	6	7		
A	8	$r^{g}r^{g}/R^{r'}$	12	105	83	31	6	..	237	3.64
A	8	$r^{g}r^{g}/R^{r'}R^{r'}$	4	67	56	23	8	..	158	3.77
B	3	$R^{r'}R^{r'}/r^{g}$	..	..	1	1	1	147	150	6.96

Homogeneity χ^2 for the two distributions resulting from mating A = 3.4; P = 0.47.

aleurone) covered the same range with a closely similar mean, 3.79, whereas kernels of genotype $R^{r'}R^{r'}/r^{g}$ from the reciprocal cross averaged 6.96. With paramutant $R^{r'}$ therefore, there is both an absence of a positive $R^{r'}$ dosage effect and a sharp contrast between $r^{g}r^{g}/R^{r'}R^{r'}$ and $R^{r'}R^{r'}/r^{g}$ scores even though the $R^{r'}$ dosage is constant at two.

The data in Table 4 serve to test whether the marked difference in aleurone score between $r^{g}r^{g}/R^{r'}R^{r'}$ and $R^{r'}R^{r'}/r^{g}$ seeds reflects differential paramutation in the gametes formed in the respective male and female inflorescences of $R^{r}R^{st}$ plants. The T$R^{r'}r^{g}$ plants studied derive from solidly colored, class 7, seeds of aleurone constitution $R^{r'}R^{r'}/r^{g}$. In crosses to $r^{g}r^{g}$ females each plant nevertheless gave $r^{g}r^{g}/R^{r'}$ and $r^{g}r^{g}/R^{r'}R^{r'}$ phenotypes characteristic of the weakly mottled paramutant type, with mean scores of 3.64 and 3.77, respectively. $R^{r'}R^{r'}/r^{g}$ aleurone of most of the seeds inheriting $R^{r'}$ from the reciprocal cross, T$R^{r'}r^{g}$ ♀ × $r^{g}r^{g}$ ♂, again was solidly colored, scoring 6.96. Clearly, therefore, R^{r} genes represented in megaspores of $R^{r}R^{st}$ plants are paramutant and the qualitative distinction between mottled and solidly colored aleurone depends principally on the R^{r} parentage of the immediate cross.

What alternatives to R dosage should be considered as possible bases for the uniformly colored and mottled distinction? Compared with the solidly colored phenotype of R R/r seeds, the mottled phenotype of r r/R and r r/R R might result from a dosage effect of r acting as a pigment inhibitor. Or, since R R/r kernels develop on R r plants, and r r/R and r r/R R kernels are formed on r r individuals, the R-carrying maternal sporophyte might determine the solidly colored phenotype. Thirdly, R alleles carried by the polar nuclei of the female gametophyte may be functionally differentiated from R factors borne by sperm. The results of experiments to test these possibilities follow.

Dosage of r^{g} and aleurone pigmentation: R^{r} in the original TB-10a stock was replaced with r^{g} in order to vary the dosage of r^{g} transmitted through the pollen. Accordingly, the cross $R^{r'}R^{r'}$ ♀ × T$r^{g}r^{g}$ ♂ produced kernels with $R^{r'}R^{r'}/(-)$, $R^{r'}R^{r'}/r^{g}$ or $R^{r'}R^{r'}/r^{g}r^{g}$ aleurones. (The symbol – represents a deficiency for the R-bearing portion of chromosome-10.) Examination of 100 kernels each from

five such matings yielded only six kernels not solidly colored, a distribution not unlike that for $R^rR^{r'}/r^g$ seeds observed in the tests referred to just above. One of the exceptional seeds was colorless, another showed a colored-colorless mosaic phenotype unlike R-motting and the remaining four kernels were intensely mottled (class 6). Only one of these four proved to have the aleurone constitution $R^rR^{r'}/r^gr^g$. This case is to be compared with 69 of that genotype among the 413 class 7 kernels successfully progeny tested. This finding renders untenable, of course, the explanation that the difference between the mottled $r^gr^g/R^rR^{r'}$ and solid $R^rR^{r'}/r^g$ groups reported in the preceding section relates to r^g dosage. Neither $R^{r'}$ dosage, r^g dosage, nor some combination of the two explains the contrasting phenotypes of the mottled $r^gr^g/R^rR^{r'}$ and solidly colored $R^rR^{r'}/r^gr^g$ classes since the two differ in constitution only in having the parentage of the r^g and $R^{r'}$ factors reversed.

The partial trisomic-10 stock $R^{r'}\ r^g\ Tr^g$, in which the B^{10} chromosome is carried as an accessory to the normal complement, was employed to test whether $R^{r'}$ expression is altered at an elevated dose of maternally transmitted r^g. When pollinated with $R^rR^{r'}$, ears of the trisomic plants gave equal numbers of solidly colored and mottled kernels (ranging from classes 3 through 6). The former produced both normal and hyperploid (10 10 B^{10}) plants, and, with three exceptions among the 161 individuals tested, carried $R^{r'}$ on both normal chromosomes ten. They represent, therefore, seed of the aleurone constitutions $R^rR^{r'}/R^{r'}$ and $R^rR^{r'}\ r^gr^g/R^r$. Likewise, all but one of 192 progeny grown from mottled kernels proved to carry only one $R^{r'}$, representing seeds having either $r^gr^g/R^{r'}$ or $r^gr^gr^gr^g/R^{r'}$ aleurone. These findings support the conclusion that the distinction between mottled and solidly colored aleurone phenotypes cannot be accounted for in terms of r^g dosage.

Autonomy of the mottled and solidly colored phenotypes: If mottled and solid patterns of pigmentation develop independently of one another, then certain crosses should yield both kernel types. Control matings of r^gr^g as female with $R^{g'}R^{g'}$ as male produced all mottled kernels ($r^gr^g/R^{g'}$). Test matings involving the same $R^{g'}R^{g'}$ males to R^rr^g plants as female gave solidly colored ($R^rR^r/R^{g'}$;

TABLE 5

Mean scores of $r^gr^g/R^{g'}$ kernels produced on two sets of R^rr^g and r^gr^g following mating to five individual $R^{g'}R^{g'}$ males

$R^{g'}R^{g'}\ \male$	R^rr^g selfed		$r^gr^g\ \female \times R^rr^g\ \male$	
	$R^rr^g\ \female$	$r^gr^g\ \female$	$R^rr^g\ \female$	$r^gr^g\ \female$
N3-20-1	5.24	5.50	4.86	5.14
-2	4.66	4.98	4.78	4.96
-3	4.52	4.84	5.10	4.78
-4	4.72	4.90	4.78	5.02
-5	5.08	5.16	4.94	4.84
Average	4.84	5.08	4.89	4.95

verified by red seedling color) and mottled ($r^g r^g/R^{g'}$; green seedlings) kernels in equal numbers. The mottled seed from the $R^r r^g$ plants scored 4.87 compared with 5.01 for the corresponding class borne on $r^g r^g$ sibs (average values from Table 5). The difference is not statistically significant. Furthermore, no difference in this respect was observed whether the $R^r r^g$ and $r^g r^g$ sib pairs used as maternal parents in the crosses to $R^{g'} R^{g'}$ derived from an $R^r r^g$ self mating or from an $r^g r^g \delta \times R^r r^g \, ♀$ cross, indicating no grandmaternal effect.

Kernels that are mosaic with respect to R constitution serve to test whether the autonomy in R expression observed for seeds on the same ear extends also to portions of single kernels. For this purpose, plants of a K10 R^r strain (K10 = abnormal chromosome-10, RHOADES 1952) described by BRAY (1964) that gives about 2% colored–colorless mosaics when pollinated by $r\ r$, a result that indicates R loss following chromosome breakage, were pollinated with $r^g r^g$ as a control and with $R^r R^{st}$ for test purposes. The resulting 1065 kernels in the control series included 22 mosaics (2.1%). The colored–colorless borders were sharply defined in the 22 cases and there was no mottling. The absence of mottling was not unexpected since the particular R allele present in this stock conditions very dark pigmentation according to BRAY's report on the phenotype of $r^g r^g$/K10 R^r kernels. The crosses of K10 R^r/K10 R^r to $R^r R^{st}$ produced mosaic seeds with about the same frequency, namely, 28 among 1202 (2.3%). Of the 15 mosaic kernels successfully progeny tested, 10 were found to have inherited $R^{r'}$ and five, R^{st}. The areas of the aleurone not solidly pigmented usually were small, however, as they were also in the crosses to $r^g r^g$. It was possible to identify typically stippled or mottled areas therefore in only a few cases. These observations, although limited in extent, nevertheless are consistent with further evidence to be presented in the following section in indicating that mottled and solidly colored areas can exist side by side even as small sectors on the same kernel.

Aleurone phenotype and dosage of maternally transmitted R'. Two approaches were taken to determine the single dose phenotype of R' of maternal origin. Both procedures were designed to lead to R' loss. In the one case spontaneous losses of a K10 chromosome were sought and in the other R loss was induced by irradiation. In the latter experiments ear shoots of $R^{g'} r^r$ plants were exposed to 500 roentgens of X irradiation (100 Kvp.; 15 ma; .25 mm. Cu − 1.0 mm. Al filter) 24–28 hr after pollination by $R^{scm} r^g$, duplicating as nearly as possible in the interval between pollination and irradiation the conditions described by STADLER (1931).

Irradiation proved to be an effective means of inducing R loss. Among 797 colored kernels in the unirradiated, control series there were no colorless or variegated sectors involving more than one-fourth of a kernel. This compares with 263 cases among the 3,381 kernels that otherwise developed normally in the irradiated series. Seedling tests based on anthocyanin pigmentation of the coleoptile, scutellum, and roots permit assignment of 253 of the 263 exceptions to the three colored aleurone genotypes as follows:

Genotype, primary endosperm cell	Number of kernels not solidly pigmented (¼ kernel or larger sector)[*]
$r^r r^r / R^{scm}$	179
$R^{g'} R^{g'} / r^g$	69
$R^{g'} R^{g'} / R^{scm}$	5
	$\overline{253}$

[*] Ten sectored kernels were unassigned: six failed to germinate and the seedling phenotypes of four did not correspond to any of the expected progeny classes.

Because R^{scm} conditions self-colored aleurone in single dose, the 179 $r^r r^r / R^{scm}$ exceptions establish the frequency of loss per R gene as 15.9%, i.e., $\dfrac{179}{(3381)/3}$ $\times$ 100. Thirteen of the $r^r r^r / R^{scm}$ exceptions showed the finely-divided mosaic pattern indicative of breakage-fusion-bridge cycles. The others had phenotypes suggestive of nonrecurrent R^{scm} losses such as FABERGÉ (1956, 1957) has shown are attributable to segmental deficiencies and to B-F-B cycles initiated by a break proximal to the locus in question.

The number of $R^{g'} R^{g'} / r^g$ kernels expected to have (−) $R^{g'} / r^g$ sectors, whose phenotype it is desired to observe, is approximately two times 179, since $R^{g'} R^{g'} / r^g$ and $r^r r^r / R^{scm}$ kernels occur in 1:1 proportion and either of the two $R^{g'}$ factors of $R^{g'} R^{g'} r^g$ may be lost to give the (−) $R^{g'} / r^g$ genotype. Only 69 sectored kernels were obtained, however. Nonrecurrent losses, B-F-B cycles, assorted patterns of variegation other than mottling, and combinations of these accounted for 53 of the 69. The remaining 16 possessed typically mottled kernels. In 12 the mottling was very dark; only in four did the level of expression correspond to that of $r^g r^g / R^{g'}$ kernels obtained in control matings of $R^{g'} r^r$ to $r^g r^g$. From the infrequency of kernels with mottled or otherwise variegated portions observed in this experiment, it is inferred that sectors of (−) $R^{g'} / r^g$ genotype often, if not regularly, were solidly colored.

Among 1475 kernels resulting from K10 $R^{g'}$/K10 $R^{g'}$ ♀ × $r^g r^g$ ♂ crosses, where $R^{g'}$ had been substituted for R^r in the K10 stock described earlier, 8 were colored–colorless mosaics indicative of nonrecurrent loss, and 12 were of the breakage-fusion-bridge class. Since the colorless areas result from coincident but independent loss of the two dominant $R^{g'}$ alleles, the composition of much of the colored area bordering such colorless sectors should be (−) $R^{g'} / r^g$. Careful examination of the border regions, however, revealed no evidence either of mottling or of other forms of variegation. Granting autonomy of the mottled and solidly colored phenotypes, the (−) $R^{g'} / r^g$ genotype is inferred to be solidly colored. The assumption of autonomy in expression of phenotype in this material was validated by self mating K10 $R^{g'}$/K10 $R^{g'}$ sibs of those plants used in $r^g r^g$ crosses. Thirteen among the 1022 resulting kernels had distinctly mottled sectors on otherwise uniformly colored background.

TABLE 6

Pigmenting action of male transmitted $R^{r'}$ *and* $R^{g'}$ *tested singly and in combination with each other following* $r^g r^g\, ♀ \times R^{g'} r^g\, TR^{r'}\, ♂$ *matings*

(a) $R^{r'}$ and $R^{g'}$ both derived from first generation heterozygotes with R^{st}

(b) $R^{r'}$ from first, and $R^{g''}$ from second generation R^{st} heterozygotes

Aleurone genotype ($♀/♂$)	Aleurone pigmentation class						Total kernels	Average score
	2	3	4	5	6	7		
(a) $r^g r^g/R^{r'}$ and $r^g r^g/r^g R^{r'}$	..	4	29	34	8	..	75	4.61
$r^g r^g/R^{g'}$	..	3	51	130	28	..	212	4.86
$r^g r^g/R^{g'} R^{r'}$	..	3	20	17	1	..	41	4.39
(b) $r^g r^g/R^{r'}$ and $r^g r^g/r^g R^{r'}$	2	6	31	30	11	..	80	4.53
$r^g r^g/R^{g''}$	109	33	16	5	2	..	165	2.53
$r^g r^g/R^{g''} R^{r'}$	2	7	24	11	1	..	45	4.04

Since $R^{g'}/r^g$ and $R^{g'}R^{g'}/r^g$ aleurone are solidly colored, this phenotype also should be observed when the maternal R' dosage is increased to four. This expectation was verified by pollinating partial trisomic $R^{g'}r^g\, Tr^{r'}$ plants with $r^g r^g$. The major classes of colored kernels expected from this mating are $R^{g'}R^{g'}/r^g$, $R^r R^{r'}$ $r^g r^g/r^g$ and $R^{g'}R^{g'}R^{r'}R^{r'}/r^g$. When medium grade paramutant $R^{r'}$ and $R^{g'}$ alleles were used all but an occasional one of the resulting kernels were solidly colored.

Do two R' *factors transmitted to the endosperm together* via *a pollen grain function independently?* A question to which an answer was sought in this series of experiments is whether the absence of a detectable dosage effect of R' in the $r^g r^g/R'$ and $r^g r^g/R'R'$ comparisons considered earlier can be ascribed to a mutually exclusive function of the R' factors carried on B^{10} chromosomes. Do the B^{10} chromosomes behave in such a way that R' action is dosage compensated in a manner similar for example to that of X-linked factors in female mammals? Assembled in Table 6 are the measures of pigmenting action of the $R^{g'}$ and $R^{r'}$ alleles determined singly in $r^g r^g/R^{g'}$ and $r^g r^g/R^{r'}$ (including $r^g r^g/r^g R^{r'}$) seeds and in combination with each other in $r^g r^g/R^{r'}R^{g'}$ kernels. These genotypes were produced by mating $R^{g'}r^g\, TR^{r'}$ ($R^{g'}$ carried on a normal chromosome-10; $R^{r'}$ carried by B^{10}) plants as males to $r^g r^g$ individuals as females. In experiment (a), $R^{g'}$ and $R^{r'}$ were matched so as to have about equal pigmenting potentials; $R^{g'}$ gave an average score of 4.86, compared with 4.61 for $R^{r'}$. The combination scored 4.39, about a half a class interval below $R^{g'}$. This small reduction in pigmentation relative to the genotypes involving $R^{r'}$ and $R^{g'}$ individually accords with the trend reported in Table 3 where the two R' factors in the $r^g r^g/R'R'$ genotype both were borne on B^{10} chromosomes.

Experiment (b) comprised a parallel series of crosses employing the $TR^{r'}$ allele common to experiment (a) but an R^g factor which previously had been maintained heterozygous with R^{st} for two generations ($R^{g''}$) and thereby reduced to a low level of expression. Individually, $TR^{r'}$ scored 4.53 in this test and $R^{g''}$ 2.53. The $r^g r^g/R^{g''}R^{r'}$ combination scored 4.04, again approximately one-half a

TABLE 7

Influence of dosage on R^{st} *expressions*
(A) Distributions and mean scores of stippled kernels from three $r^g r^g$ ♀ $\times$ r^g TR^{st} TR^{st} ♂
matings and (B) the reciprocal matings

Mating	Aleurone genotype (♀/♂)	Kernel class					Total kernels	Mean score
		2	3	4	5	6		
A	$r^g r^g / R^{st}$ and $r^g r^g / r^g R^{st}$	..	76	74	26	4	180	3.77
A	$r^g r^g / R^{st} R^{st}$	..	6	37	69	8	120	4.66
B	$R^{st} R^{st} / r^g$	..	1	63	76	10	150	4.63

Homogeneity χ^2 for one compared with two doses of male transmitted R^{st} (mating A) = 84.2; $P < .001$.
Homogeneity χ^2 for $r^g r^g / R^{st} R^{st}$ and $R^{st} R^{st} / r^g$ = 1.36; $P = .07$.

class interval below the darker member of the pair. Had $R^{g''}$ and $R^{r'}$ functioned alternatively, bimodality in the kernel distribution or sectoring for level of R action should have occurred. The $r^g r^g / R^{g''} R^{r'}$ distribution is monomodal and although sectoring for level of mottling would be difficult to identify, its net effect should have been to give a mean score more nearly the average of $R^{g''}$ and $R^{r'}$ than that observed.

Dosage effect of R^{st} *with respect to seed spotting:* The intensity of aleurone spotting increases markedly in the series $r\,r / R^{st}$ through $R^{st} R^{st} / r$ to $R^{st} R^{st} / R^{st}$ (R^{st} is used here to represent the unstable allele of R and an enhancer of its expression, M^{st}, located 5.7 crossover units distal to the R locus, ASHMAN 1960). If the level of action were due strictly to dosage of the spotting factor and its modifier, then $r\,r / R^{st} R^{st}$ should be more intensely pigmented than $r\,r / R^{st}$ and equal to $R^{st} R^{st} / r$, whereas these relationships would not be expected to hold if R^{st} behaves like R' in the corresponding genotypes.

The mating of hyperploid r^g $TR^{st} TR^{st}$ males to $r^g r^g$ females gave $r^g r^g / R^{st} R^{st}$ kernels which scored 4.66, as compared with 3.77 for the $r^g r^g / R^{st}$ and $r^g r^g / r^g R^{st}$ genotypes from the same cross (Table 7). $R^{st} R^{st} / r^g$ from the reciprocal cross scored 4.63, which is very close to the score for two doses of R^{st} derived from the male. Although the effects of the spotting factor and its M^{st} modifier are not distinguishable in this experiment, equivalence of the two dose R^{st} combinations hardly could have resulted unless both factors acted in a conventional dosage-dependent manner.

DISCUSSION

Because each genotype evaluated in this study gave principally mottled or solidly colored aleurone, the evidence presented on the relations between phenotype and genotype may be summarized qualitatively in the following categorical manner:

Aleurone genotype (♀ derived/ ♂ derived)	Aleurone pigmentation	
	Mottled	Solid
r r/R	+	
r r/r R	+	
(−) (−)/R*	+	
r r r r/R	+	
r r/R R	+	
R R/R		+
r r R R/R		+
R R R R/r		+
R R/(−)		+
R R/r		+
R R/r r		+
(−) R/r		+

* From Brink, Kermicle and Brown (1964); (−) designates a chromosome-10 deficient for the R region.

The gene symbols used in the above listing have been abbreviated by omitting reference to those features shown not to affect the mottled and solidly colored distinction. The r r/R genotype, to use the first entry as an example, gave mottled aleurone whether the R allele used was R^r or R^g, and whether R was carried on the B^{10} chromosome of translocation B–10a or on a structurally normal chromosome-10. And for the purpose of this summary classification, in which the aleurone phenotypes are collected into two groups only, both standard and paramutant forms of the R alleles in r r/R seeds are properly designated as mottled even though the two cases involve quite different levels of pigmentation.

From the relationship between phenotype and genotype shown in the above summary there is no indication that r dosage contributes to the mottled and solidly colored distinction. Neither can a valid generalization be made based on dosage balance between R and r alleles. All the evidence, in fact, points to the conclusion that the r^g allele used is neutral in this relation. This finding is not surprising since this factor has proved to be amorphic also in respect to other R properties (Brink. Styles and Axtell 1968). A result of these experiments that was not foreseen, however, is that R dosage as such also fails to account for the marked differences in R phenotype. Comparison between the r r/R R (mottled) and R R/r r (solidly colored) genotypes, summarized in Figure 3, provides definitive evidence on the point. This finding excludes interpretation of the difference in aleurone phenotype resulting from reciprocal crosses between R R and r r plants in terms of R or r dosage.

Proof that the dosage hypothesis was invalid stimulated inquiry in a new direction and eventually led to an explanation in terms of an unusual genetic phenomenon, namely, a difference in action of a given R in the endosperm accord-

ing to the kind of gametophyte, female or male, from which the R allele is derived. The relationship between aleurone phenotype and the mode of R transmission proposed is basically simple. The evidence shows that when R is received from a sperm, whether in one or two doses, the phenotype is mottled. In contrast, if R is transmitted to the endosperm through the female gametophyte, whether in one, two, or four doses, the aleurone phenotype is solidly colored. $R\ R/R$ and $r\ r\ R\ R/R$ kernels are solidly colored, showing that solid coloration is dominant to mottled.

The influence of mode of transmission is a special case of the general category of "maternal effects" or, with reference to plants, of a phenomenon that BATESON (1926) termed *anisogeny*. Extrachromosomal inheritance is not involved since matings between parents of appropriate genotypes produced kernels of both phenotypes.

The study of sectored kernels also provided convincing evidence that the differential expression of R according to sexual origin is chromosomal. In kernels inheriting R from both parents mottling was observed after the maternally received R's were removed, even when removal occurred at a stage of endosperm development so advanced that the mottled sector formed was small. The potential for mottling persisted, therefore, even after passage through numerous mitotic cycles in nuclei also carrying R factors that condition solid coloration.

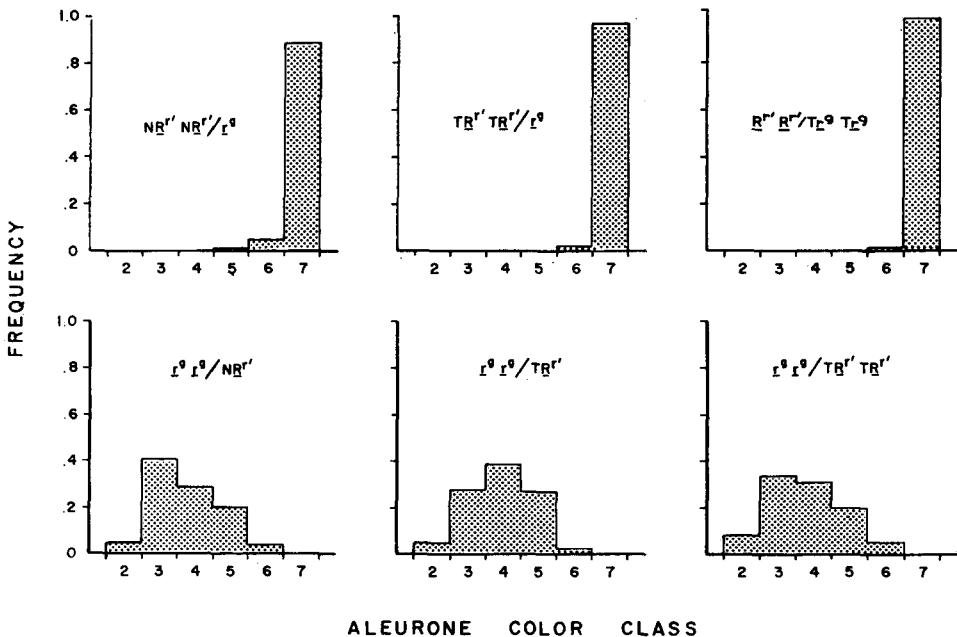No second generation effects were detected. The distinction between mottled

FIGURE 3.—Frequency distributions by aleurone color class of those aleurone genotypes dealt with in the RESULTS section which provide critical comparisons concerning the effects on aleurone pigmentation level of $R^{r'}$ and r^g dosage, of translocation TB–10a, and of mode of $R^{r'}$ transmission.

and solidly colored is a function therefore of the immediate parentage and is independent of the grandparentage. This fact could mean that the chromosome differentiation responsible for the distinction is reversible such that the effect is effaced at some later stage in the life cycle. Reversibility would not be a necessary condition if the differentiation were restricted to the endosperm; R in cells of the germ line, in this event, could remain undifferentiated. Experiments specifically designed to delimit the stage in the life cycle at which the differentiation takes place are a main consideration in the article that follows (BRINK, KERMICLE and ZIEBUR 1970).

It is difficult to account on a simple basis for the virtual absence of a dosage influence of a second male transmitted R. The darkly mottling standard form of R did give a positive, although barely perceptible, dosage response. Paramutant forms gave no detectable response in one test and definite indications of a negative dosage response in two other experiments. If R factors that condition mottling are simply hypomorphic forms of the gene, an increase in their number in a system in which R action is the factor limiting genetic expression should have given increased pigmentation.

Exact tests for an effect of dosage may be complicated in the present material by paramutational interaction between the two or more R factors present in the nuclei of endosperm cells. When passed through a sporophyte together, two paramutable alleles tend to mutually stabilize each other so that each attains a lower average pigmenting potential than if passed individually through heterozygotes with r^g (BRINK, STYLES and AXTELL 1968). It is known also that paramutable alleles are sensitive to paramutagenic influences in the endosperm (McWHIRTER and BRINK 1963). Possibly, therefore, male transmitted R's have a potential for a positive dosage expression but this influence is offset by paramutation. The decrease in dosage response observed with increasing levels of paramutation is consistent with this view.

The property of mottling and the potential for paramutational change among R alleles may be intimately related. In a recent survey of R alleles of diverse geographic origin, VAN DER WALT and BRINK (1969) showed that all of the mottling alleles in their sample were paramutable, i.e., they were sensitive to heritable reduction in pigmentation in heterozygotes with R-stippled. If the relation between mottling and sex-dependent expression also proves general, a close relationship between paramutability and sex-dependent expression would be indicated. The evidence already available suggests, in fact, that the sex-dependent behavior of R here described is a paragenetic, rather than a conventional genetic, phenomenon (cf. BRINK 1962). That is to say, the change in question affects the expression but not the constitution of the R gene.

SUMMARY

A class of R alleles of which R^r and its mutant R^g derivatives are representative

regularly gives a mottled distribution of anthocyanin in $r\ r/R$ aleurone following crosses of $R\ R$ plants on $r\ r$ females, but solidly colored $R\ R/r$ aleurone after the reciprocal mating. Expressions of the mottled and solidly colored phenotypes were shown to be autonomous, both as kernel classes on individual ears and as sectors within individual kernels. Hence the genetic basis of mottling is not extrachromosomal in nature. The phenotypes of the various aleurone genotypes tested do not support an interpretation of the mottled and solidly colored distinction in terms of R dosage, r dosage, or an R to r dosage balance. In each of the several allelic combinations tested, kernels were mottled if they received one or two doses of R paternally, and solidly colored if they received one, two, or four doses of R maternally. Furthermore, a test for a single active, paternal R in $r\ r/R\ R$ aleurone gave a negative result. The various observations support the view that a paternally inherited R conditions mottled whereas a maternally inherited R conditions solidly colored aleurone. This chromosomally based response of R to mode of sexual transmission is allele specific, moreover, since the unstable spotting allele R-stippled, in contrast to R^r and R^g, varied in expression in accordance with dosage but independently of sex of the parent.

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