EXPRESSION AND STABILITY OF THE MARBLED ALLELE IN MAIZE[1]

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THE pigment-producing action of the R^r allele (colored aleurone, red seedling) in maize regularly is reduced among the offspring of heterozygotes carrying an allele, R^{st}, conditioning stippled aleurone and green seedling (BRINK 1956). The modified forms of R^r which arise through such intrachromosomal changes at or near the R locus are heritable. Genetic change of this kind has been termed paramutation (BRINK 1958a). The phenomenon has been demonstrated also in R^r heterozygotes in which another allele, R^{mb} (marbled aleurone, green seedling) was substituted for stippled (BRINK and WEYERS 1957).

The stippled and marbled factors are of considerable interest as sources of chromosomal determinants involved in these directed genetic changes at the R locus. An account of the stippled gene has been presented by ASHMAN (1960). The present study is concerned with the expression and stability of the marbled allele.

MATERIALS AND METHODS

The marbled gene employed in these investigations was introduced from a stock originally obtained from R. A. EMERSON into a highly uniform inbred line known as W22. After outcrossing the original R^{mb} strain to W22, the marbled segregates were backcrossed four or more times to the W22 inbred line, prior to their isolation in homozygous form by selfing.

When homozygous, the R^{mb} allele frequently causes formation in the aleurone of large, irregularly shaped, solidly colored spots on a colorless background. The degree of spotting is proportional to dosage. The scutellum is colorless. Marbled does not produce appreciable amounts of pigment in the vegetative tissues. Seedlings show traces of anthocyanin in the coleoptiles, but the roots remain colorless when exposed to light. The anthers and glumes on adult $R^{mb}R^{mb}$ plants are green.

The otherwise uniform marbled W22 stocks here employed were observed to vary strikingly in amount of aleurone spotting. To determine whether the observed variability was genetic, dark, intermediate, and colorless kernels were selected from homozygous marbled ears. Plants grown from these kernels were self-pollinated, and the progeny ears were evaluated for degree of spotting. Repetition of this procedure in successive generations resulted in the isolation of marbled sublines with distinctly different but still variable phenotypes. The families ranged from very light, in which five percent or less of the homozygous

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marbled seed was spotted, to very dark, in which nearly all the kernels were extensively pigmented (Figure 1).

To ensure a uniform genetic background, the various W22 marbled sublines were again mated to the standard W22 inbred strain. Pollen from plants belonging to the homozygous marbled families was placed on the silks of W22 R^rR^r individuals. The resulting $R^{mb}R^r$ offspring were pollinated by W22 r^gr^g (colorless aleurone, green plant), and the $R^{mb}R^{mb}r^g$ seed was used to evaluate the marbled phenotype.

Aleurone spotting was scored by matching random samples of seed from the central portions of ears against a set of standard kernels. The latter comprised seven specimens, selected in such a way that the sample populations were distributed among eight aleurone color classes. The classes ranged from class one, which was colorless, to class eight, which was almost completely pigmented. Classes between one and eight represent various degrees of marbling.

A test was made to determine whether M^{st}, a modifier of stippled aleurone, affects the expression of marbled also. According to ASHMAN (1960), the difference between light stippled and standard stippled is due to a modifier located about 5.7 crossover units distal to the R locus. R^{st} strains carry the modifier (M^{st}), whereas light stippled lines lack it, thereby representing the null condition (m^{st}) with respect to this locus. The standard W22 inbred line is homozygous m^{st}.

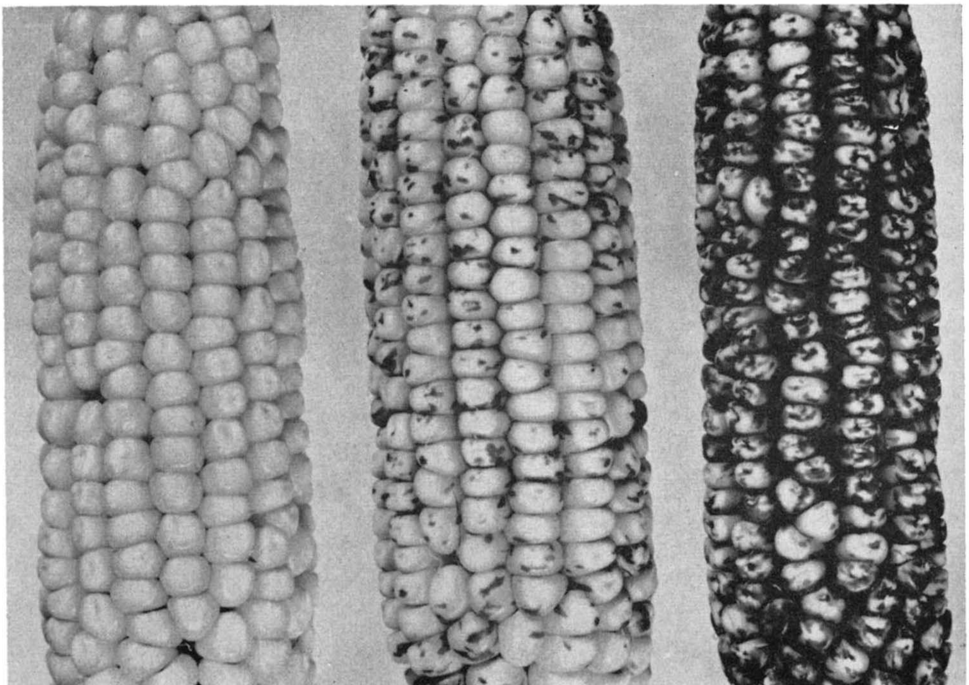

FIGURE 1.—The diversity of marbled phenotypes. The ears represent three different homozygous marbled sublines in the same W22 inbred background.

Several light and dark marbled families were pollinated with both light and standard stippled. The heterozygous $R^{st}R^{mb}$ plants were pollinated with W22 r^gr^g (m^{st}), and the resulting ears were examined for recombinants differing in grade of aleurone spotting.

Marbled mutates characteristically to germinally transmissible self-colored aleurone. The mutants occur either as single kernels with self-colored aleurone or, less frequently, as single kernels with marbled or colorless endosperm but colored scutellum. About one third of the former and all of the latter class of kernels represent germinal mutations to self-color.

Since preliminary observations had suggested that the frequency of self-colored mutants might be greater in dark than in light marbled lines, three very light families (1–5 percent pigmented kernels) and their dark relatives (80 percent or more kernels pigmented) were analysed separately with respect to mutation rate.

Homozygous $R^{mb}R^{mb}$ plants were pollinated with W22 r^rr^r (colorless aleurone, red seedling), and the putative mutants were selected from the resulting $R^{mb}r^r$ ears. Upon selfing the plants in question, presumed mutants were considered valid only if they segregated both the mutant and r^r phenotypes. Loss of supposed mutants through failure of the seed to germinate, or death of the plants, was compensated for by a proportionate adjustment of the total kernel number in computing the mutation rate.

The $R^{mb}r^r$ seed on the ears bearing the mutant kernels was used to evaluate the aleurone phenotype of each of the marbled lines by the procedure previously explained.

The mutation frequency for one of the sublines also was determined in heterozygotes with r^r. $R^{mb}r^r$ progeny from the mating $R^{mb}R^{mb}$ ♀ × W22 r^rr^r ♂ were pollinated with W22 r^gr^g and the presumptive mutants were selected for further testing.

EXPERIMENTAL RESULTS

Variations in aleurone spotting pattern in W22 marbled sublines: The average aleurone color scores of $R^{mb}R^{mb}r^g$ kernels from the mating R^rR^{mb} ♀ × W22 r^gr^g ♂ are assembled in Table 1. Each score represents a 50-kernel sample taken from the central portion of each ear. Family W189 was obtained by placing pollen of a medium marbled subline on W22 R^rR^r plants. Its light marbled sib is represented by family W201.

The data in Table 1 show that phenotypic differences between closely related marbled sublines tend to persist when these lines are again backcrossed to the W22 inbred strain. The two sib families are distinct with regard to aleurone phenotype.

Noneffect on marbled of a modifier of the stippled aleurone pattern: The $R^{mb}R^{st}$ heterozygotes from the matings $R^{mb}R^{mb}$ ♀ × $R^{st}R^{st}$ ♂ invariably yielded some light stippled progeny when pollinated with W22 r^gr^g. Thus both the light and dark marbled families tested must regularly have carried m^{st}. Moreover, the $R^{mb}M^{st}$ counterparts of the light stippled recombinants ($R^{st}m^{st}$) were indistinguishable

TABLE 1

Aleurone color scores of $R^{mb}R^{mb}r^{g}$ *kernels following matings of W22* $R^{r}R^{mb}$ ♀ × *W22* $r^{g}r^{g}$ ♂

Female parent	Average aleurone color score	Female parent	Average aleurone color score
W189– 1	1.82	W201– 1	0.48
– 2	2.31	– 2	0.14
– 3	5.16	– 3	0.87
– 4	3.78	– 4	0.22
– 5	3.50	– 5	0.74
– 6	4.40	– 7	0.06
– 9	1.26	– 8	0.16
–12	1.86	– 9	0.30
–13	3.42	–11	0.22
–14	3.16	–12	0.12
–16	3.44	–13	0.78
Mean	3.1		0.37

phenotypically from the $R^{mb}m^{st}$ segregates on the same ear. These results show that the stippled modifier is not responsible for the phenotypic differences between the various marbled sublines.

Mutation of marbled to self-colored aleurone: The frequencies of mutation to self-color of the R^{mb} alleles associated with different aleurone phenotypes are presented in Table 2. As can be seen from the table, the light marbled families failed to produce any self-color mutants among 24543 gametes tested. The average mutation frequency to self-color for the corresponding medium and dark marbled lines was 11.0 per 10^4 gametes. Thus marbled lines which exhibit extensive aleurone spotting also show relatively high frequencies of germinally transmissible mutations to self-color.

The mutation frequency for one of the sublines (W200) in heterozygotes with r^r is given in Table 3. The data show a marked increase in the mutation rate of R^{mb} when the particular marbled line was made heterozygous with r^r ($\chi^2 = 10.18$, P <.01,1 d.f.). This finding is the reverse of that reported for stippled, in which the frequency of R^{sc} (self-color) mutations is greater in homozygotes than in heterozygotes with r^r (ASHMAN 1960).

TABLE 2

Frequency of mutation of R^{mb} *to* R^{scm} *(self-color) in various W22* $R^{mb}R^{mb}$ *sublines*

Subline	Mean aleurone color score of marbled parent strain	No. of gametes tested	No. of R^{scm} mutations per 10^4 gametes
W186	0.22	4,576	0.0
W200	0.27	14,967	0.0
W229	0.31	5,000	0.0
W249	2.13	4,410	6.8
W188	2.60	5,774	8.6
W226	4.90	2,834	17.6

TABLE 3

Frequency of mutation to self-colored aleurone in a light marbled family when homozygous and heterozygous with r^r

Genotype	Number		Number of R^{scm} mutations per 10^4 gametes tested
	R^{mb} gametes tested	R^{scm} mutants	
$R^{mb}R^{mb}$	14,967	0	0.0
$R^{mb}r^r$	11,751	8	6.8

DISCUSSION

The main question raised by the present findings concerns the source of the genetic variability in the marbled pattern in W22 stocks. Following the initial outcross of marbled to the standard W22 stock, the marbled segregates were backcrossed four times to the foundation inbred line, prior to recovery of the R^{mb} factor by self-pollination. During this period, there was no selection for particular marbled phenotypes. Consequently, the W22 marbled line so obtained and the W22 inbred strain would be expected to be closely similar, except at and in the vicinity of the *R* locus. Nevertheless, the aleurone spotting density in the recovered W22 marbled stock subsequently was readily shifted by selection. Moreover, the resulting marbled sublines, although distinct with regard to aleurone phenotype, continue to exhibit considerable dispersion for aleurone color index (data of Table 1) when further backcrossed to the W22 inbred strain. Whether the striking diversity of ear phenotypes within the two sublines represents a continuance of genetic variation in the marbled allele itself is a question to which further experiments should provide an answer.

The data show also that an increase in the amount of aleurone spotting is accompanied by an increase in the rate of mutation to self-color. Conceivably, the formation of a self-colored sector in the aleurone and the production of a transmissible self-colored mutant reflect the same kind of event, namely, the mutation of a colorless allele to a colored form. Differences between the various marbled sublines might then reside in a modifying gene closely linked to the *R* locus. This modifier could alter the stability of the R^{mb} allele, or else could change the time during development, when mutations to self-color occur. It is necessary, on this hypothesis, to assume a high rate of mutability of the modifier postulated.

An alternative interpretation which excludes the participation of a modifying gene of the conventional kind is more likely. The observed diversity might be due to occurrence of the marbled allele itself in a number of states of unlike stability. Frequent shifts between the various states in germinal and somatic tissues would account for the persistent heterogeneity in R^{mb} stocks.

Changes of state of a locus have been related to the transposition of controllers (McClintock 1951, 1956; Brink and Nilan 1952), to changes in state of controllers (McClintock 1951), and to alterations in the mode of association of such an element with its locus (Brink 1958b). The R^{mb} allele, as shown earlier, mutates at different rates to R^{scm} in $R^{mb}R^{mb}$ and $R^{mb}r^r$ genotypes. While it could be supposed that R^{scm} mutants arise through crossing over and that heterozygosity enhances the frequency of this process, such an assumption is clearly incompatible with the observation that germinal and endosperm mutants occur independently of each other. Instead, the increased incidence of germinal mutations in $R^{mb}r^{r}$ heterozygotes may represent a specific response on the part of the R^{mb} allele to the presence of a particular genetic element in the homologous r^{r}-carrying chromosome, or it may reflect an interaction between a component of the R^{mb} allele in $R^{mb}R^{mb}$ homozygotes. An operational model of the latter kind is afforded by the variegated pericarp allele in maize. WOOD and BRINK (1956) obtained cogent evidence in this case that the increased frequency of mutation of variegated to self-red pericarp in heterozygotes, as compared with homozygotes, was a function of dosage of Modulator, a transposable element which, when present at the *P* locus, but not elsewhere, suppressed the action of the gene for self-red pericarp.

The marbled allele, similarly, may comprise a gene for self-colored aleurone plus an inhibitor at the *R* locus. The occurrence of stable mutations to self-color is compatible with this idea. Proof is lacking, however, that such mutations and the heritable variations in the marbled allele itself involve shifts in position of a pigmentation inhibitor relative to the gene for self-color.

SUMMARY

The paramutagenic R^{mb} allele which, by itself, conditions spotted aleurone and green plant in maize fails to give a constant aleurone phenotype when incorporated in a highly uniform inbred strain of corn known as W22.

Attempts to modify the amount of aleurone spotting in W22 marbled ($R^{mb}R^{mb}$) plants by selection resulted in the recovery of various marbled sublines differing in degree of aleurone pigmentation. In addition, the rate of mutation to germinally transmissible self-colored (R^{scm}) aleurone was found to be much higher in heavily pigmented marbled sublines than in their lightly spotted and near-colorless relatives. Presumably the formation of a colored spot in the aleurone is due to mutation of a relatively unstable colorless allele to a stable colored form in an endosperm cell. When such a mutation occurs in a cell lineage that includes the egg, then the self-colored allele is transmitted to the offspring.

Since the diversity observed between marbled sublines has its basis at, or in the vicinity of, the *R* locus, it is suggested that the various marbled phenotypes are controlled by an allele which regularly varies between several states of unlike stability.

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