2. Mutation of a self colored allele to a Navajo pattern.

A mutation study involving 98 self colored R alleles (designated R^{sc} from R^{st}, or R^{scl} from light stippled) derived by mutation from stippled was begun in 1959. The object was to study the mutation spectrum of R^{sc} alleles, and to determine whether mutability was related to the paramutagenic action of the allele.

Eighteen independent mutants from R^{sc} alleles were established, of which 10 were based upon the selection of mutant kernels which were initially completely colorless. The progeny tests show, however, that only one of these is a completely colorless mutant. Three are near-colorless, 3 are weakly pigmented (diffuse patches) and 3 are "pale spotted," dosage-dependent alleles.

From 8 presumed mutant kernels which initially showed some pigmentation of the aleurone, 3 weakly pigmented mutants, one "pale spotted" and 3 dosage-dependent self colored alleles were obtained. (The R^{sc} parent alleles are dosage-independent.) The remaining pigmented mutant kernel had the Navajo phenotype, and its descendants gave the Navajo pattern. This mutant was derived from the cross $R^{scl}134R^{scl}134$ ♀ X $r^r r^r$ ♂, and its origin by contamination is excluded. As expected, it is associated with green plant and seedling color (all R^{sc} alleles are R^g in Emerson's terminology).

No reverse mutations to a stippled allele were found, although the "near-colorless" and weakly pigmented alleles phenotypically resemble the r^gI alleles which Ashman (Genetics 45:18) obtained directly from stippled by mutation.

The over-all mutation rate was low; considering all mutants it was $18/1,150,746 = 0.15 \times 10^{-4}$.

There is no indication in the present data of a relation between the paramutagenic action of R^{sc} alleles and mutability of these alleles. The number of mutants recovered is insufficient, however, to provide an adequate test of this question.

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3. Phenotypes of presumed mutant kernels from stippled in relation to germinal transmissibility.

Ashman's (Genetics 45:19) studies of the mutation of stippled to self colored demonstrated two points:

a) An approximately 50% concordance between a self colored endosperm phenotype of the mutant kernel and transmissibility of self colored to offspring.

b) A mutation frequency in homozygotes of stippled about four times greater than that in heterozygotes of stippled with r. This is termed the "heterozygote effect" here.

Subsequently J. Kermicle found that kernels with the phenotype, stippled aleurone-colored scutellum, frequently gave mutant self colored progeny. It was also observed that all proven self colored mutants from stippled had a colored scutellum.

These observations prompted a study of the concordance of kernel phenotype with germinal mutation of stippled to self colored.

Kernels from the crosses

W22 $\underline{R}^{st}$(light) $\underline{R}^{st}$(light) ♀♀ X W22 $\underline{r}^{r}\underline{r}^{r}$ ♂♂

(referred to subsequently as the homozygote)

and W22 $\underline{R}^{st}/\underline{r}^{g}$ ♀♀ X W22 $\underline{r}^{g}\underline{r}^{g}$ ♂♂

W22 $\underline{R}^{st}$(light) K10/$\underline{r}^{g}$ ♀♀ X W22 $\underline{r}^{r}\underline{r}^{r}$ ♂♂

(collectively referred to as heterozygotes)

were classified into one or other of the classes of kernel phenotypes listed in Table 1. The non-parental kernel types were subsequently progeny tested. The results of the progeny test, in which only the ear genotype was determined, are included in Table 1.

The data show that, irrespective of the aleurone phenotype, there is a high concordance of germinal mutation of stippled to self colored with presence of the colored scutellum phenotype. Kernels of class 4 (mosaic scutellum) probably represent mutations occurring in early sporophyte development. A small proportion of class 3 (stippled aleurone-colored scutellum) kernels regularly give non-mutant progeny. It follows from this result that some proportion of the germinal mutants from this class of kernel may be attributed to very early somatic mutations. The germinal mutations to self colored obtained from class 5 kernels (stippled aleurone-colorless scutellum) are clearly a consequence of somatic mutation later in sporophyte development.

An explanation of the remaining classes of kernels occurring on stippled ears may be made as follows:

Table 1. Concordance of germinal mutation of stippled to self colored with the phenotype of presumed mutant kernels.

Class	Phenotype of the presumed mutant kernels	Origin(1)	Number of presumed mutant kernels tested(2)	Progeny test result (ear classified only) Mutant ($\underline{R}^{sc}\underline{r}$)	Non mutant $\underline{R}^{st}\underline{r}$	Mosaic
1	Colored aleurone colored scutellum	Homozygote	38	38	-	-
		Heterozygotes	4	4	-	-
2	Colored aleurone colorless scutellum	Homozygote	22	-	22	-
		Heterozygotes	16	3	13	-
3	Stippled aleurone colored scutellum	Homozygote	14	12	2	-
		Heterozygotes	12	10	2	-
4	Stippled aleurone mosaic scutellum	Homozygote	18	5	13	-
5	Stippled aleurone colorless scutellum	(3)	757	3	753	1(4)

(1) "Homozygote" and "heterozygotes" refer the kernels to origin from the crosses listed in the text.

(2) 2 proven contaminants and 4 kernels not tested were omitted from the tabulation.

(3) All these kernels were from the crosses $\underline{R}^{scl}/\underline{R}^{st}$(light) K X $\underline{r}^r\underline{r}^r$

$\underline{R}^r/\underline{R}^{st}$(light) K X $\underline{r}^r\underline{r}^r$

$\underline{R}^{st}$(light) K/$\underline{r}^g$ X $\underline{r}^r\underline{r}^r$

(4) Sectored ear bearing a patch of 18 R^{scl} kernels and 10 colorless kernels, remaining kernels were $\underline{R}^{st}$(light) or colorless.

<u>Class 1 kernels</u>: colored aleurone-colored scutellum

These are invariably germinally transmissible and the majority probably represent instances in which the megaspore is mutant. Mutations occurring early in the development of the megagametophyte could also give rise to this kernel phenotype.

<u>Class 2 and class 3 kernels</u>

These may be interpreted as the reciprocal types expected if the mutation of stippled to self colored occurs during development of the megagametophyte. The embryo sac would therefore contain a mixture of mutant and nonmutant haploid nuclei. Disposition of a mutant nucleus to function in the egg, or in the development of the endosperm would result in kernels with the class 3 and class 2 phenotypes, respectively.

The relative frequencies of these kernel phenotypes are in the relation expected on this interpretation. There are several ways of deriving a class 2 type (mutant endosperm, non-mutant egg), whereas class 3 kernels (non-mutant endosperm, mutant egg) presumably can be obtained in only one way. More definitive support for this interpretation comes from a study of the relative frequencies of these types of kernels following mutation in the pollen grain (see the following note by J. Kermicle).

The high concordance of the colored scutellum phenotype with germinal mutation to self colored shows that this scutellum pigmentation is an effect of the $\underline{R}^{sc}$ allele. This conclusion is confirmed by the subsequent inheritance of the character. Colored scutellum was found to be completely associated with the self colored aleurone, green plant, color complex in a backcross test involving 3126 kernels from the cross W22 $\underline{r}^g\underline{r}^g$ ♀♀ X W22 $\underline{R}^{sc}\underline{R}^r$ ♂♂. The $\underline{R}^r\underline{R}^r$ stock in W22 background has a colorless scutellum. The only apparent exceptions to the parental types proved to be the result of heterofertilization when progeny tested. The colored scutellum character in these stocks, therefore, is not the same as that earlier described by Sprague (1932, U.S.D.A. Tech. Bull. 292).

The mutation rates calculated from these data and the appropriate significance tests are contained in Table 2. These show that, when comparison is made of the frequencies of mutant kernel types in homozygote and heterozygotes, there is a difference with respect to only one class, namely the colored aleurone-colored scutellum kernels. It follows from the above interpretation of the origin of this class of kernels that the "heterozygote effect" observed by Ashman is manifest only in terms of the mutations occurring during meiosis. Since kernels of classes 2 and 3 are interpreted as the consequences of mutation occurring during embryo sac development, a "heterozygote effect" on the mutability of stippled is not to be expected at this stage, and was not observed.

Table 2. Mutation rates and significance tests for the data in Table 1.

A. In the homozygote, cross $\underline{R}^{st}$(light) $\underline{R}^{st}$(light) ♀♀ X $\underline{r}^r\underline{r}^r$ ♂♂

Class of presumed mutant kernel	Mutants ($\underline{R}^{sc}/\underline{r}^r$)	Total kernels (corrected)	Mutation frequency X 10^{-4}
1. Colored aleurone (class 2 + class 3)	38	13,414	28.3
2. Stippled aleurone colored scutellum (class 3)	12	14,308	8.38
3. All classes 1, 2 and 3	50	13,575	36.8
4. Stippled aleurone mosaic scutellum (class 4)	5	4,266	11.75

B. In the heterozygote, pooled data from the crosses $R^{st}r^g$ ♀♀ X r^gr^g ♂♂ and $\underline{R}^{st}$(light) $\overline{K}/\underline{r}^g$ ♀♀ X $\underline{r}^r\underline{r}^r$ ♂♂

Class of presumed mutant kernel	Mutants ($\underline{R}^{sc}/\underline{r}^r$)	Total kernels (corrected)	Mutation frequency X 10^{-4}
1. Colored aleurone (class 2 + 3)	7	7,425	9.4
2. Stippled aleurone colored scutellum (class 3)	10	7,796	12.8
3. All classes 1, 2 and 3	17	7,560	22.5

Significance tests: 1) Frequencies of colored aleurone kernels which give mutant progeny differ significantly at P = 0.01 in homozygote and heterozygote.

2) Frequencies of class 3 mutants (stippled aleurone-colored scutellum kernels), and of class 2 kernels (colored aleurone, colorless scutellum) which give non-mutant progeny, do not differ at P = 0.2 in homozygote and heterozygote.

These data also provide some evidence showing lack of association of mutation of stippled to self colored with distal crossing over. Thirteen self colored mutants (9 in the present study and 4 previously) from the cross W22 $\underline{R}^{st}$(light) K10/$\underline{r}^{g}$ ♀♀ X W22 $\underline{r}^{r}\underline{r}^{r}$ ♂♂ were all $\underline{R}^{scl}$K10/$\underline{r}^{r}$ in constitution (i.e., noncrossovers). However, only those mutants derived from colored aleurone, colored scutellum kernels legitimately test for an association with distal crossing over. Only three mutants had this origin, and all three were noncrossovers.

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4. Mutation of stippled (R^{st}) to self-colored (R^{sc}) aleurone during microsporogenesis.

This investigation was prompted by the following two observations made in studies of mutation of $\underline{R}^{st}$ to $\underline{R}^{sc}$ in $\underline{R}^{st}\underline{R}^{st}$♀♀. In the first place, a large portion, perhaps half, of the self-colored kernels on stippled ears prove to be non-germinal (Ashman, Genetics 45:19; and McWhirter, accompanying report). The second point is the frequent occurrence of kernels with stippled aleurone and colored scutellum (the latter is a characteristic of the $\underline{R}^{sc}$ phenotype) which turn out, in fact, to be germinally transmissible self-colored mutants. The present experiment was undertaken to test the supposition that the two classes described above arise from mutation in the haploid gametophyte. Since, in the case of microsporogenesis, a single mitosis (the second nuclear division in the male gametophyte) gives rise to the two sperm which participate in double fertilization, one would expect the two reciprocal classes in equal frequencies from matings on colorless plants in which $\underline{R}^{st}\underline{R}^{st}$ was the male parent.

The categories and respective frequencies of mutants arising from the mating $\underline{r}^{r}\underline{r}^{r}$; $\underline{Y}$ $\underline{Y}$ x $\underline{R}^{st}\underline{R}^{st}$; $\underline{y}$ $\underline{y}$ are given in the following table. The recessive marker $\underline{y}$ was utilized in the male parent in order to identify pollen contaminants. All presumed mutants were grown out and then tested by pollination with $\underline{r}^{r}\underline{r}^{r}$; $\underline{Y}$ $\underline{Y}$.

Mutation of $\underline{R}^{st}$ to $\underline{R}^{sc}$ in $\underline{r}^{r}\underline{r}^{r}$♀ x $\underline{R}^{st}\underline{R}^{st}$♂ matings

Kernel phenotype of presumed mutant	Classification by breeding test	Number of mutants	Number of * R^{st} gametes tested	Mutation rate ($\times 10^{-4}$)
Colored aleurone	R^{st}	15	2,831	53.0
	R^{sc}	27	2,831	95.4
Colored scutellum (R^{st} aleurone)	R^{st}	1	3,640	2.75
	R^{sc}	13	3,640	35.7

* Initial population = 3,640. Corrected in computation where progeny were not obtained from mutant kernels.