The crossover frequencies among the three loci, Amy-2, Ct, and AcP.

Crosses	Amy-2:Ct		Amy-2:AcP		Ct:AcP	
	Total	Crossover	Total	Crossover	Total	Crossover
W64A x (W64A x 6)	180	0.09± 0.02	100	0.53± 0.05	101	0.53± 0.05
6 x (W64A x 6)	118	0.05± 0.02	120	0.54± 0.05	119	0.50± 0.05
(W64A x 6) x (W64A x 6)	278	0.05± 0.01	312	0.49± 0.03	298	0.49± 0.03

The genotypes of the two inbreds are:

$$W64A = \frac{Amy\text{-}2^{B}\ Ct^{S}\ AcP^{A}}{Amy\text{-}2^{B}\ Ct^{S}\ AcP^{A}} \; ; \; 6 = \frac{Amy\text{-}2^{A}\ Ct^{F}\ AcP^{B}}{Amy\text{-}2^{A}\ Ct^{F}\ AcP^{B}}$$

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1. Transfer of instability of the P S^{st} complex to the P component.

The compoundness of the R locus allows an analysis of the manner in which the P component of R is affected by its association in coupling with an unstable S component (S^{st}). The first step of this analysis is the recovery of P S^{st} intralocus recombinants. Two of these recombinants have been previously isolated (Gavazzi and Avila, M.N.L. 1968) and reproduced.

Contrary to our earlier observations, descendants of these intralocus recombinants show pigment variegation in their sporophytic tissues. The variegation, when roots or coleoptile tissues are observed at low magnification (20x), consists of a series of contiguous red stripes.

We have observed a third case of root variegation in the descendants of a single colorless kernel. Originally this kernel was isolated as a putative seed mutant (s) on an ear produced from the cross of a p S^{st}/P S individual with a p s/p s line. Since no R outside markers were present in the heterozygous parent, the association of the "colorless" mutant with a recombinational event could not be established.

This exceptional individual and its descendants, whose structural organization at the R locus (in terms of p and s) is not clear, will be referred to as "mutant." Descendants of the "mutant" were colorless in their aleurone and devoid of pigment in their sporophytic tissues. However, in a few individuals clear stripes of anthocyanin were produced either on the roots or on the coleoptile or in the anthers.

Kernels obtained by testcrossing individuals genotypically p s/"mutant" (i.e. with one chromosome 10 marked with p and s and its homolog carrying the R "mutant") with a ps/ps line were germinated and scored for variegation in their sporophytic tissues. Out of 2044 kernels germinated, 270 were variegated in either their roots or coleoptile, 8 were completely red and the remaining 1766 were without pigment at all. If we assume that seedling variegation is controlled by the "mutant" only (as shown below), it then appears that variegation is expressed in only 26.4% (270/1022) of the seedlings that have the "mutant" in their genotype.

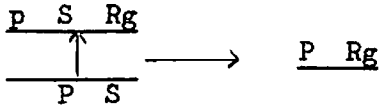
Evidence that the root variegation is associated with the R region of the "mutant" was obtained by crossing heterozygous p s/"mutant" individuals with a p S/p S homozygous line. Individuals so obtained are genotypically separable into two classes: (i) "mutant"/p S and (ii) p s/p S.

They were both testcrossed with a p s/p s line.

Ears obtained from plants of genotype (i) could be easily distinguished from those of genotype (ii) since the "mutant" had maintained the paramutagenic capacity of the S^{st} ancestor. Germination of colored and colorless seeds from the two groups of ears proved that root variegation is associated with the R "mutant" since only colorless kernels from testcross (i) were exhibiting, upon germination, pigment variegation in their sporophytic tissues.

If we assume that $\underline{S}^{st}$ is a compound structure consisting of $\underline{S}$ plus an adjacent regulatory component ($\underline{Rg}$), the most likely interpretation of these observations is that the $\underline{R}$ "mutant" originated from an intralocus recombination of the oblique type (Emmerling 1958) that gave rise to a crossover strand deficient for the $\underline{S}$ component and with the regulatory component ($\underline{Rg}$) in coupling with $\underline{P}$.

The event leading to the appearance of a similar strand is depicted below:



These observations, even though limited, seem to suggest that the mechanism leading to $\underline{S}$ instability can extend its action to the $\underline{P}$ component of $\underline{R}$ when both $\underline{P}$ and $\underline{S}$ are brought in coupling on the same chromosome.

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2. Mutability of the $\underline{S}$ component of the $\underline{R}$ locus.

As previously outlined, the $\underline{S}$ component of $\underline{R}^{st}$ is unstable. The instability consists of frequent changes of $\underline{S}$ from an inactive to an active state, leading to a variegated aleurone phenotype. If this change occurs in the germ line cells, it gives rise to a stable form of $\underline{S}$. This form is symbolized $\underline{S}^{sc}$ (self colored) to distinguish it from the $\underline{S}$ components present in the $\underline{R}^{r}$ and $\underline{R}^{ch}$ gene complexes. $\underline{S}^{sc}$ in fact determines pigmentation in both the scutellum and aleurone and it conditions a full pigmentation of the aleurone even when present in a single dose ($\underline{s}\ \underline{s}\ \underline{S}^{sc}$). The other two $\underline{S}$ components do not extend the pigmentation to the scutellum tissues and condition a mottled aleurone phenotype ($\underline{S}$ of $\underline{R}^{r}$) or a pale phenotype ($\underline{S}$ of $\underline{R}^{ch}$) when present in a single dose.

It is likely that these phenotypic differences reflect a difference in the structural organization of the genetic material at the $\underline{R}$ locus in the three different forms of $\underline{R}$.

This possibility has been tested by comparing the frequency and spectrum of mutation of the $\underline{S}$ component of the three forms of $\underline{R}$. The results obtained are reported in Table 1. The $\underline{S}^{sc}$ gametes analyzed were obtained from individuals heterozygous for two $\underline{S}^{sc}$ isolates of