blotch character and the regions marked by the translocations. Linkage was observed between the bloch character and wx with all of the 17 translocations used. This indicates that at least one of the factors is probably located in chromosome 9. An average of 22% recombination was obtained from pooled data involving all of the translocations. The translocations may have caused some pseudo-linkage in regions proximal to the breakpoints. Moreover, the location of wx with regard to the breakpoint differs from translocation to translocation. Finally, the independent segregation of two more factors makes it difficult to estimate the actual linkage. Therefore, other tests are underway to place the factor in chromosome 9 more accurately. The tentative symbol for this factor is Bl_4.

Similar characters, controlled by single factors, were described by various authors. A blotch leaf factor was first described by Emerson (1923) as a recessive (bl), occasionally behaving as a dominant. Another factor (bl_2) was investigated by R. C. Wiggans (unpublished, cited by J. Weijer, 1952). The bl_3 factor was described by N. W. Simmonds (1950) and assigned to chromosome 10, but was later placed in chromosome 2 by E. M. Clark. Other factors controlling similar characters include: a recessive factor reported by J. W. Cameron (1964); a necrotic leaf spot factor, recessive, allelic to zn_1 (A. R. Hornbrook and C. O. Gardner, 1970); and a leaf fleck factor (lf_1), recessive (J. L. Brewbaker, 1970). Recently M. G. Neuffer (1973, and personal communication) described two mutants induced by EMS treatment, producing necrotic leaf spots of different size; both factors apparently are single dominant genes, segregating 1:1 in outcrosses, and possibly characterized by lethality in the homozygous condition.

A. Ghidoni

4. Constitution of nonparental strands isolated from R intralocus recombinants.

The data to be presented have been obtained in an effort to explain a quite unexpected result we observed while studying recombination within the R region. Our previous work (Gavazzi and Calati, 1972) indicated that it is possible to isolate, in the progeny of testcrosses

of $\underline{R}^{st}\ \underline{r}^{r}$ and $\underline{R}^{sk}\underline{r}^{r}$ heterozygotes with an $\underline{r}^{g}\ \underline{r}^{g}$ tester, exceptional seedlings carrying properties of both parental markers, i.e., variegated (stippled or smoky) aleurone and red seedling or colorless aleurone and green seedling.

The parental genotype expressed in terms of the P and S components of the R locus is symbolized $\underline{p}\ \underline{S}^{var}/\ \underline{P}\ \underline{s}$, where $\underline{S}^{var}$ stands for either stippled or smoky seed component, while the lower case letters p and s, as used in this context, do not distinguish between presence of a recessive allele and absence of the gene component. The genetic analysis of the exceptional progeny derived from $\underline{p}\ \underline{S}^{var}/\ \underline{P}\ \underline{s}$ parents indicated that they carry nonparental $\underline{P}\ \underline{S}^{var}$ and $\underline{p}\ \underline{s}$ strands. The event leading to nonparental $\underline{P}\ \underline{S}^{var}$ strands is quite uncommon (2.69×10^{-4}) and it is confined, judging from our own experience, to those parental genotypes where one of the two chromosomes 10 carried the large knob (K 10) in the distal portion of the long arm.

On the other hand, when $\underline{P}\ \underline{S}^{var}/\ \underline{p}\ \underline{s}$ plants are mated to $\underline{p}\ \underline{s}$ males and progeny kernels germinated, seedlings with a recombinant phenotype are observed with a frequency as much as 200 times greater than the frequency of the original event producing the $\underline{P}\ \underline{S}^{var}$ strand.

This is true for all four of the $\underline{P}\ \underline{S}^{var}$ independent isolates so far tested. One of the four isolates, referred to as "case 2," was studied extensively with the intent of elucidating the genetic basis of the dramatic increase in recombination. This strand was originally isolated as a $\underline{g}\ \underline{P}\ \underline{S}^{sk}$ recombinant from a heterozygous $\underline{G}\ \underline{P}\ \underline{s}\ \underline{K}\ /$ $\underline{g}\ \underline{p}\ \underline{S}^{sk}$ female. The appropriate genotypes for studying recombination were obtained following the mating: $\underline{G}\ \underline{p}\ \underline{S}^{sc}/\ \underline{G}\ \underline{p}\ \underline{s}\ \times\ \underline{g}\ \underline{P}\ \underline{S}^{sk}/$ $\underline{g}\ \underline{p}\ \underline{S}^{sk}$ (where $\underline{p}\ \underline{S}^{sc}$ designates a self-colored derivative of $\underline{R}^{st}$). Progeny kernels with either genotype $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) or $\underline{G}\ \underline{p}\ \underline{S}^{sc}/$ $\underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) were grown in the field and the resulting (A) females were pollinated with $\underline{G}\ \underline{p}\ \underline{s}$ and the (B) females were pollinated with $\underline{g}\ \underline{p}\ \underline{s}$ males.

Kernels produced from these matings were germinated and the seedlings scored for pigment production. Individuals with nonparental phenotype (i.e., red seedling and colorless aleurone or green seedling and smoky aleurone from A parents; red seedling and self colored

aleurone or green seedling and smoky aleurone from B parents) appeared (see Table 1) with a frequency of 4.1% (192/4672) and 5.9% (282/4736) in the progeny of (A) and (B) parents, respectively. A sample of the exceptional plants was progeny-tested to ascertain the validity of the screening procedure.

As can be seen from the results in Table 2, the procedure is quite effective for the identification of nonparental strands. Only a very few seedlings of (B) parentage appear misclassified. They can be accounted for by one of the following two events:

1. the lack of pigment development in a $\underline{P}\ \underline{S}^{sk}/\ \underline{p}\ \underline{s}$ seedling or
2. the reversion of smoky to self-colored occurring during embryo sac development and giving rise to a kernel with colored endosperm and smoky embryo. The former affects the estimate of $\underline{p}\ \underline{S}^{sk}$, the latter of $\underline{P}\ \underline{S}^{sc}$ nonparental strands.

A third factor interfering with a correct estimate of the P-S recombination in the "case 2" strand is the reversion of smoky to self-colored occurring during meiosis of (B) parental females leading to a $\underline{P}\ \underline{S}^{sc}$ strand indistinguishable from a recombinant. The rate of meiotic reversion has been estimated in the progeny of (A) females crossed with $\underline{p}\ \underline{s}$ males and it amounts to 0.96×10^{-3}, a frequency almost negligible when compared to the total yield of $\underline{P}\ \underline{S}^{sc}$ strands from (B) parents.

The frequency of recombination between $\underline{P}$ and $\underline{S}^{sk}$, as estimated from the data of Table 1 after correction for misclassification (see progeny test), is 4.1% and 5.7% in A and B genotypes, respectively. Such a high frequency suggests that one of the two $\underline{R}$ components has been dislocated from its original position so that $\underline{P}$ and $\underline{S}^{sk}$ are now 4-5 map units apart.

Proximal marker constitution of recombinant strands

Determination of the proximal marker ($\underline{g}$) constitution of nonparental strands isolated from testcrosses of $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) plants with $\underline{G}\ \underline{p}\ \underline{s}$ males required a further generation of selfing while in testcrosses of $\underline{G}\ \underline{p}\ \underline{S}^{sc}/\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) heterozygotes with $\underline{g}\ \underline{p}\ \underline{s}$ males the constitution was established directly by classifying green vs golden plant color in the progeny seedlings (in the latter case the classification

Table 1

Frequency of exceptional seedlings with a presumed recombinant strand in the progeny of testcrosses of $\underline{G}\ \underline{p}\ \underline{s}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (A) and $\underline{G}\ \underline{p}\ \underline{S}^{sc}\ /\ \underline{g}\ \underline{P}\ \underline{S}^{sk}$ (B) heterozygotes

R constitution of parental genotype	Gametes tested	Presumed strand constitution			
		(1)	(2)	(3)	(4)
$\frac{\underline{p}\ \underline{s}}{\underline{P}\ \underline{S}^{sk}}$ (A)	4672	2299	2181	95	97
$\frac{\underline{p}\ \underline{S}^{sc}}{\underline{P}\ \underline{S}^{sk}}$ (B)	4736	2269	2185	140	142

(1) $\underline{p}\ \underline{s}$ or $\underline{p}\ \underline{S}^{sc}$; (2) $\underline{P}\ \underline{S}^{sk}$; (3) $\underline{p}\ \underline{S}^{sk}$; (4) $\underline{P}\ \underline{s}$ or $\underline{P}\ \underline{S}^{sc}$

Table 2

Progeny test of the exceptional seedlings isolated as presumed recombinants in the progeny of testcrosses of plants with (A) and (B) genotype

R constitution of parental genotype	Presumed recombinant strands	Presumed recombinants			
		Isolated	Tested	Conf.	Nonconf.
(A) $\underline{p}\ \underline{s}\ /\ \underline{P}\ \underline{S}^{sk}$	$\underline{p}\ \underline{S}^{sk}$	95	26	26	-
"	$\underline{P}\ \underline{s}$	97	71	71	-
(B) $\underline{p}\ \underline{S}^{sc}\ /\ \underline{P}\ \underline{S}^{sk}$	$\underline{p}\ \underline{S}^{sk}$	140	53	51	2 ($\underline{P}\ \underline{S}^{sk}$)
"	$\underline{P}\ \underline{S}^{sc}$	142	69	65	4 ($\underline{P}\ \underline{S}^{sk}$)

for golden was extended to parental seedlings.) Classification for the g marker of nonparental strands of (A) parentage is given below:

P s		p S^{sk}	
G	g	G	g
60	4	1	18

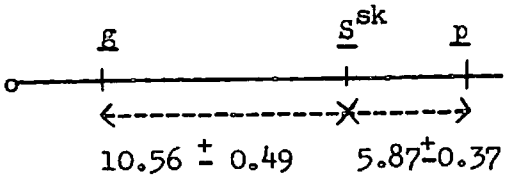
If one assumes that P is the proximal and S the distal R component (Stadler and Neuffer, 1953), the data would indicate that the majority of the recombinants (78/83) are associated with a second exchange in the region proximal to R. As to the three-point testcross data, involving G p S^{sc} / g P S^{sk} heterozygotes, recombinant strands with an additional exchange in the proximal region comprise 207 out of 232.

The results of this testcross are shown below:

P S^{sk}		p S^{sc}		p S^{sk}		P S^{sc}		total
g	G	g	G	g	G	g	G	
1616	191	201	1708	108	11	14	99	3948

The double exchange interpretation is at variance with the recombinational values for the long arm of chromosome 10 reported in the literature. A survey of published results clearly shows that in this chromosomal segment there is positive interference.

As an alternative one could interpret the results by assuming that the order of the P and S components of the "case 2" strand is inverted. According to this interpretation, the strand constitution together with the map length of each crossover region, as obtained from the three-point testcross data, would be represented as follows:



Distal marker constitution of nonparental strands isolated from (A) parents

The smoky derivative of stippled carries M^{st} (stippled modifier) as a distal marker. The constitution with regard to M^{st} can be ascertained only on nonparental strands of (A) but not (B) parentage, since only the former are heterozygous for M^{st}.

Since $\underline{M}^{st}$ affects specifically the expression of the $\underline{R}^{st}$ allele, detection of its presence or absence in nonparental strands required the matings outlined below:

1. $\underline{P}\ \underline{s}$ strands

   $\underline{P}\ \underline{s}\ /\ \underline{p}\ \underline{s}$ seedlings isolated in the progeny of testcross (A) were grown and selfed. Kernels from the self pollination were grown in the field and plants with red anthers (genotypically $\underline{P}\ \underline{s}\ /\ \underline{P}\ \underline{s}$ and $\underline{P}\ \underline{s}\ /\ \underline{p}\ \underline{s}$) were pollinated with a $\underline{g}\ \underline{R}^{st}\ \underline{m}^{st}$ male. Production of ears segregating for dark and light stippled or uniformly dark stippled indicated presence of $\underline{M}^{st}$ in the $\underline{P}\ \underline{s}$ strand, while production of ears uniformly light stippled indicated its absence.

2. $\underline{p}\ \underline{S}^{sk}$ strands

   $\underline{p}\ \underline{S}^{sk}\ /\ \underline{p}\ \underline{s}$ seedlings in the progeny of testcross (A) were grown and selfed; heterozygous $\underline{p}\ \underline{S}^{sk}\ /\ \underline{p}\ \underline{s}$ individuals from the selfed ears were grown in the field and crossed with $\underline{g}\ \underline{R}^{st}\ \underline{m}^{st}$ males. The presence or absence of about 6% dark stippled seeds from such crosses indicates the respective presence or absence of $\underline{M}^{st}$ in the $\underline{p}\ \underline{S}^{sk}$ nonparental strand.

The outside marker constitution of nonparental strands, as determined in the way just outlined, is given below:

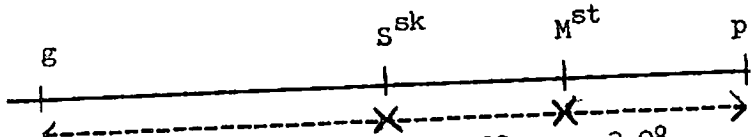
$\underline{P}\ \underline{s}$				$\underline{p}\ \underline{S}^{sk}$			
$\underline{G}$ $\underline{M}^{st}$	$\underline{G}$ $\underline{m}^{st}$	$\underline{g}$ $\underline{M}^{st}$	$\underline{g}$ $\underline{m}^{st}$	$\underline{G}$ $\underline{M}^{st}$	$\underline{G}$ $\underline{m}^{st}$	$\underline{g}$ $\underline{M}^{st}$	$\underline{g}$ $\underline{m}^{st}$
14	30	1	1	2	0	14	5

It can be seen, from these data, that among the strands selected as $\underline{P}$ - $\underline{S}$ crossovers, 47 had the exchange distally and 20 proximally to $\underline{M}^{st}$.

Since the $\underline{P}$ - $\underline{S}$ frequency of exchange in this mating (see Table 1) is 4.1%, $\underline{M}^{st}$ must then lie between $\underline{S}^{sk}$ and $\underline{P}$, 1.2 map units distal to $\underline{S}^{sk}$ (20/67 of 4.11).

By combining these data with the previous information the arrangement of the four markers on the "case 2" strand can be envisaged as shown below:

Linkage map:



Recombination values:

The main results of this analysis can be summarized as follows:

1. When P S^{var} / p s females, carrying a nonparental chromosome ("case 2" strand) derived from a p S^{var} / P s parent, are mated to p s males, P - S recombinants are observed in the progeny with a frequency as much as 200 times greater than the frequency of the original event producing the P S^{var} nonparental strand.

2. Linkage relations of the P component of the "case 2" strand have been determined in testcrosses involving the S component of R^{sk}, M^{st} and G_1. The recombination values obtained are best interpreted by assuming that the P component of the "case 2" strand is removed from its standard position and is relocated at a new position about 5 recombination units to the right of the R locus.

3. The strand with P in its new position shows a decrease of recombination in the g - S^{sk} and S^{sk} - M^{st} intervals. There is some indication that the dislocation of P is associated with physiological and morphogenetic effects, to be reported.

An interpretation of similar effects as well as a tentative hypothesis on the origin of the "case 2" strand is postponed to the time when the analysis of the other three strands will be completed. This analysis is presently underway.

G. Gavazzi
G. Galli

5. A new mutant affecting amino acid metabolism.

The problem of isolating auxotrophic mutants in eucaryotic organisms to use as experimental tools in dissecting the metabolic control processes has been faced by geneticists for some time (Nelson, 1967). Nevertheless, the success of their isolation in higher plants has been very limited when compared to the many results obtained in procaryotes, algae and fungi. Different hypotheses have been offered to explain this failure (Li and Rédei, 1969; Neuffer, 1974). However,