It is apparent that hemizygosity has a marked effect on mutation of R^{st} to R^{sc}, and probably also causes an increase in mutation of mR^{nj} to R^{nj}. Further study to determine whether this is a response common to autonomous transposable elements would seem to be justified.

We do not know what property of hemizygosity is responsible for the observed effect. The mutations under study were mostly single kernel events - implying transposition at, or very near, meiosis. Conceivably then, the processes of meiotic chromosome pairing may be involved in some way. Further information on this point may be obtainable from more extensive study of the mR^{nj} system, which gives a significant frequency of somatic sectors. If only meiotic chromosome pairing is involved then no effect of hemizygosity on somatic mutation rate should be found.

W. M. Williams

5. Tandem and displaced duplications in the distal end of 10L.

The R locus, which is essential for anthocyanin pigmentation in certain plant and seed tissues, is located in the distal one-fifth of the long arm of chromosome 10. The first allele of this locus to be extensively studied, R^r (Cornell or standard), was found to be associated with a tandem duplication. The proximal member of the duplication carries P, the plant pigmenting determiner, while the distal member carries S, the seed pigmenting determiner.

Lc is the designation given to a leaf color factor extracted from the R^r-Ecuador 1172 strain. Lc maps distal to the R locus, and shows between 1 and 2% recombination with it. A large number of seedling and plant tissues are pigmented by the action of Lc: coleoptile, blade joint, roots (weakly), nodes, silks, pericarp, and leaf blade. Strong pigmentation of the first blade joint allows one to screen for the presence or absence of Lc at the seedling stage, even in the presence of P of R^r. One of the adult plant tissues, on the other hand, that is most conspicuously pigmented by Lc is the leaf blade. Hence its name Lc (leaf color). An extensive study aimed at fractionating the Lc compound phenotype yielded negative results.

Lc was first suspected of being borne on a segment sharing
homology with the R^r:standard duplicated segment when, from the
heterozygote $\pm R^{nj} \underline{Lc}/\underline{g} R^r \underline{lc}$, a $\pm$ Lc-marked derivative carrying
Nj (R^{nj}) and the S component of R^r was isolated. On subsequent
analysis this derivative was found to be Nj Lc : S lc in constitution.
This strand carries an extensive tandem duplication (the colon repre-
sents the junction between the proximal and distal members of the
duplication) and therefore is quite unstable. It arose presumably by
an exchange within the obliquely paired P-bearing segment of R^r and
Lc-bearing segment of R^{nj}.

To test for homology between the P- and Lc-bearing segments, the
following simple test was designed. The progeny from the cross
$\underline{R^r Lc}/\underline{R^r Lc}$ ♀♀ X $\underline{r^g lc}/\underline{r^g lc}$ ♂♂ was screened for $\underline{r^r lc}$ and $\underline{r^g Lc}$ excep-
tional derivatives, in addition to $\underline{r^r Lc}$ cases expected from exchange
between P and S elements of R^r. $\underline{r^r lc}$ derivatives would occur if the P-
and Lc-bearing segments pair with each other and an exchange occurs dis-
tal to the anthocyanin gene in the respective segments. An exchange
proximal to the anthocyanin genes would result in a $\underline{r^g Lc}$ derivative
(see Fig. 1).

Table 1 gives the numbers and frequencies of the different
classes of r derivatives obtained from pollinating $\underline{R^r Lc}$ homozygotes with
$\underline{r^g lc}$. The only types of derivatives recovered were $\underline{r^r Lc}$ and $\underline{r^r lc}$, which
occurred in approximately equal numbers. From these results it is possi-
ble to conclude that there is indeed a chromosomal segment distal to Lc
that is homologous with a portion of the R^r duplicated segment distal to
the anthocyanin markers. The homology between the two segments does not
appear to extend proximally beyond the anthocyanin genes, since no $\underline{r^g Lc}$
derivatives were obtained. The recombinational origin of the $\underline{r^r lc}$ deriva-
tives was confirmed in an additional experiment that incorporated M^{st} as
a marker located distal to Lc.

Peculiarly, the $\underline{r^r lc}$ derivatives from $\underline{R^r Lc}$ homozygotes were not
associated with reduced fertility. Since the region between R and Lc
has been deleted in these derivatives, one would have expected them to
show the typical transmission behavior of large deficiencies. Yet, both
male and female transmission was found to be normal. Furthermore, $\underline{r^r lc}$

Table 1. Colorless seed derivatives from $\underline{R^r Lc}$

homozygotes crosses to $\underline{r^g lc}$ males

(Population: 124,000).

Type of derivative	Number	Freq. $(\times 10^{-4})$
$r^r Lc$	69	5.6
$r^r lc$	62	5.0
$r^g Lc$	0	NIL

Figure 1. Test of homology between $\underline{Lc}$ and $\underline{R^r}$: types of

crossover colorless seed derivatives that can arise in

$\underline{R^r}$ $\underline{Lc}$ homozygotes if the $\underline{P}$- and $\underline{Lc}$-bearing segments

are coextensive and can pair with each other

(xxxxxxx = dispensable chromosome segment).
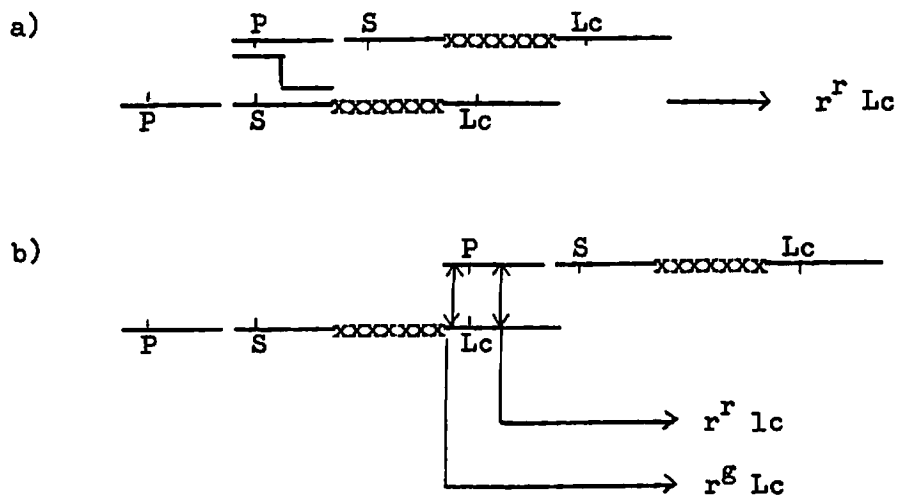
homozygotes occurred in the expected frequency among the progenies of
the original $r^r lc/r^g lc$ selections, ruling out the possibility of a
zygotic recessive lethal being associated with the deficiency. There-
fore, it appears as if the region between R and Lc—one to two units
long—is completely dispensable; its deletion from the genome seems to
have no discernible effect.

The R-Lc segment could be duplicated elsewhere in the genome. If
one thinks of Lc as an R factor, then this segment could conceivably be
duplicated either immediately proximal to R or immediately distal to Lc
in a standard chromosome 10. If the R-Lc segment occurs also proximal
to R, then one would have expected several $r^g Lc$ derivatives from $R^r Lc$
homozygotes (see Fig. 2a). Yet none were found. If the segment is also
present distal to Lc one would expect frequent losses of Lc in Lc homozy-
gotes (see Fig. 2b). $R^g_1 Lc$ ($R^g_1 = S$) homozygotes were pollinated with
$r^g lc$ and the seedling progeny screened for the absence of Lc traits.
From a population of 12,400 seedlings, 7 $R^g_1 lc$ derivatives were found:
the lc phenotype of these selections was confirmed in the respective
mature plants. This fraction represents a frequency of 5.6×10^{-4}, which
compares favorably with the frequency of $r^r lc$ derivatives obtained from
$R^r Lc$ homozygotes (5.0×10^{-4}), suggesting that the region of exchange in
$R^g_1 Lc$ homozygotes is small and very possibly restricted to that half of
the R^r duplication present in R^g_1.
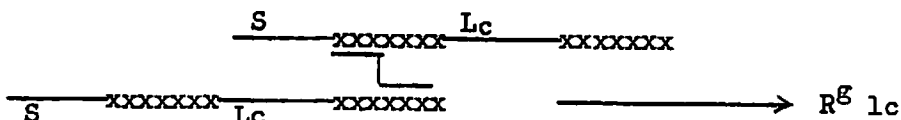
Figure 2. Types of crossover derivatives expected from
(a) R^r Lc homozygotes if the R-Lc region occurs also
proximal to R, or (b) R^g_1 Lc homozygotes if the
R-Lc region is duplicated distal to Lc.

a) R-Lc region duplicated proximal to R
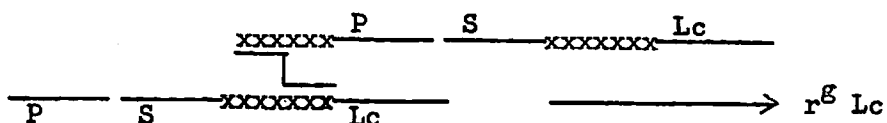
b) R-Lc region duplicated distal to Lc

In conclusion, the R-Lc region does not appear to be duplicated in the immediate vicinity of the R locus. The Lc-marked segment and either the P- or the S-marked segment would therefore constitute a direct but displaced duplication, just as the P- and the S-marked segments comprise a direct, tandem duplication.

<div align="right">Hugo K. Dooner</div>

6. Induction and maintenance of maize callus tissue.

Yamada, et al. (Proc. Japan Acad. 43: 156, 1967) and Carter, et al. (Nature 214: 1029, 1967) demonstrated that callus could readily be induced in rice and wheat by germinating the seeds on a medium which contained greater than 5 mg/l 2,4-D. We have used this method for maize and have obtained nearly 100% success regardless of the strain used.

Maize seeds are surface sterilized by stirring in detergent and 5% Chlorox, rinsed, and soaked overnight in aerated water. The seeds are again sterilized with 5% Chlorox and the embryos with a portion of the scutellum are removed under sterile conditions. These are planted on Murashige and Skoog medium (Physiol. Plant. 15: 473, 1962) containing 10 mg/l IAA, 0.04 mg/l kinetin, 25 mg/l 2,4-D and 10 g/l agar. The cultures are grown under continuous low light at 25° to 30°C. On this medium the primary root of the germinated seedling thickens and grows only several millimeters. The shoot grows a few centimeters and dies. The mesocotyl swells and unorganized callus proliferates from this region. Under the best conditions a cubic centimeter of callus is formed in six weeks. This callus may be divided and subcultured on Murashige and Skoog solid medium without 2, 4-D. Callus induced last March is still growing after being subcultured several times on this medium. Particularly rapid growth of maize callus can be obtained in liquid shake culture. Again, Murashige and Skoog medium is used, but without agar. The cultures are shaken at 120 rpm at 30°C.

We have not obtained complete differentiation of the callus in culture. Under the levels of hormone employed, normal appearing roots are often initiated, but other than occasional green buds, no shoots are formed.