

segregating in the progeny in approximately a 1:1 ratio. In the light of these results, it is assumed that the c-m allele expresses a coarse pattern that is modified by a second factor resulting in a fine pattern. However, the nature of this second factor remains unknown.

Attempts have been made to determine if the second factor is En itself. Plants grown from the coarse kernels of the original testcross of fine plants (c-m Sh Wx/c sh wx x c sh wx/c sh wx) were sibcrossed by colorless shrunken sibs known to contain En (c sh wx/c sh wx En). The results are given in Table 2. Among the 53 tested, 26 showed segregation of coarse and fine patterns. In view of the absence of segregation for coarse and fine types in all of the progenies, even in the presence of En, there is a strong indication that the second factor modifying the coarse pattern is not En. Further tests to characterize the second factor are in progress.

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Initiation and maintenance of callus cultures of the En-Spm controlled allele, wx-m8

We are presently developing callus and cell cultures of homozygous recessive wx-m8 endosperm and scutellar tissue. Endosperm cultures were obtained by the following procedure. Immature ears were harvested (7 to 10 days post-pollination) and immediately processed for culture on solid media. The young ears were cut into small pieces and surface sterilized for 10 minutes in 0.5% Clorox solution. After rinsing three times in sterile distilled water, the tops of the kernels were cut off with a fine scalpel and the endosperm squeezed with a spatula onto the medium. The basal medium used consisted of the major and minor salts of revised Murashige and Skoog (1962): 0.5 mg of thiamine per litre of medium; 0.5 mg/L of pyridoxin HCl; 0.5 mg/L of niacin; 8 mg/L of glutamine; 2 gm/L of asparagin; 30 gm/L of sucrose; 8 mg/L of agar; 500 mg/L of yeast extract and 2 mg/L of 2,4-dichlorophenoxyacetic acid; pH 5.8 to 6; dark incubation 80 F. The callus was transferred to fresh medium every 21 days.

Callus induction was not successful either with (a) White's basal medium plus Nitsch's trace elements, 2% sucrose, 2 gm/L of asparagine; 9 gm/L of agar; and 2 gm/L of yeast extract or, (b) Linsmaier and Skoog medium: 0.4 mg/L of thiamine HCl; 100 mg/L of i-inositol; 2 mg/L of NAA; 2 mg/L of IAA; 30 gm/L of sucrose; 10 gm/L agar and 150 ml/L of coconut milk. In both of these cases, no callus growth was observed even after transferring to fresh medium.

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Further data on the location of the modifier gene of the cl locus

In the 1972 News Letter (M.G.C.N.L. 46:93-95, 1972) and the 1973 News Letter (M.G.C.N.L. 47:79-81, 1973) F<sub>2</sub> data were presented that indicated the modifier of cl (Clm) was linked to the breakpoint of T8-9(6673) in chromosome 8. The Clm locus has a series of alleles that partially or completely suppress the albino phenotype of cl seedlings. The pale yellow or white endosperm phenotype of this mutant is not suppressed. In the 1972 report, it was indicated that there were two modifiers of cl in the inbred M14. One was responsible for green seedlings (Clm-M14 gr) and the other for pale green or pastel seedlings (Clm-M14 pas). Allele tests had confirmed that Clm-M14 gr was allelic to the other modifier genes of cl. However, the Clm-M14 pas, which was involved in the 1972 linkage tests with T8-9(6673), had not actually been shown to be allelic to the other modifiers. In 1973 allele tests between Clm-3 and Clm-M14 pas were made by self-pollinating plants from the cross of cl cl Clm-3 Clm-3 X Cl cl-7716 Clm-M14 pas Clm-M14 pas.

These selfed plants were either C1 c1 Clm-3 Clm-14 pas or c1 c1-7716 Clm-3 Clm-14 pas. All of the pale yellow seeds from these selfs were seedling tested. If Clm-3 and Clm-M14 pas are allelic, no white seedlings should be observed in the  $F_2$  seedlings from the pale yellow seeds. This turned out to be the situation. No albino seedlings were observed out of 10,588 seedlings scored. Thus the linkage tests in 1972 involved the Clm locus and placed it on chromosome 8.

Observations of seedlings with Clm-M14 grown under controlled temperature conditions revealed that the inbred M14 did not have two different modifiers (i.e., Clm-M14 gr and Clm-M14 pas). The tests demonstrated that the Clm-M14 allele is actually temperature sensitive. At high temperature (95 F) the seedlings with Clm-M14 are pale green or pastel while at low temperatures they are green.

In the 1973 report the same indication of linkage was obtained with T8-9(6673) in crosses involving the Clm-3 modifier.

Last summer testcross progeny were grown of a cross between + clm T8-9(6673)/c1 Clm-3 + plants and plants of the inbred OH43 (lacks any dominant modifiers). The testcross progeny were scored for sterility and the plants self-pollinated. All the pale yellow seeds from the selfed ears that segregated for c1 were grown and scored for the presence or absence of green seedlings:

Parental		Crossovers		Total	% C.O.
<u>clm</u> T	<u>Clm-3</u> +	<u>Clm-3</u> T	<u>clm</u> +		
97	93	40	33	263	27.8

The Clm modifier undergoes 27.8% recombination with the T8-9(6673) breakpoint in chromosome 8. Since the breakpoint is at 8L.35 it is impossible to determine the chromosome arm in which this locus is located.

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#### Additional studies of a mutator locus on chromosome 10

Previous reports (M.G.C.N.L. 45:81-87, 1971 and 49:73-79, 1975) have indicated that a factor at or near the y9 locus is responsible for an increased spontaneous mutation rate. In these reports, it was noted that a given outcross family would frequently have several plants that segregated for mutants which were quite similar in phenotype. Such mutants were assumed to have arisen by individual somatic events resulting in tassel sectors. Results of a series of allele tests (Table 1) on mutants of similar phenotype within families indicate that most mutants arise by a very late somatic or a meiotic mutation, since in most cases the mutants are not allelic. In only 2 out of 11 families were positive tests observed. In family 72-3120, 2 phenotypes were noted, luteus (l) and albino (w). All allele tests for the luteus mutants were positive except that of 18(l) x 20(l). Since both 18(l) and 20(l) were allelic to 6(l), the negative results observed in the 18(l) x 20(l) cross are unexpected. These crosses will be repeated next summer. Until further data are obtained all luteus mutants of family 72-3120 are assumed to be allelic. The two albino mutants from this family also proved to be allelic. It is conceivable, since these mutants are in a heterogeneous background, that the luteus and albino mutants of family 72-3120 may be allelic. Further tests of allelism within this family are required but until such tests are performed the albino will be assumed to be different from the luteus mutants. In family 72-3125 both allelic and nonallelic mutants were observed. Thus in 2 out of 11 families, allelic mutants were observed while in 10 out of 11 families nonallelic mutants with similar phenotype were recovered. It is assumed that allelic mutants are of somatic origin while nonallelic mutants may be meiotic or the result of two independent somatic mutations which occurred late in development, resulting in very small tassel sectors.

These results indicate that in about 90% of the families tested, with two or more mutants of similar phenotype, there is evidence that mutation takes place very