The data used in making these calculations are based on selected crossover seeds from the $\underline{a}_2$-$\underline{bt}_1$ region, a distance of 5 crossover units. They are, therefore, equivalent of testing 22,200 (20 x 1,110) unselected gametes from 2 four point test crosses, and indicate the following linkage map: $\underline{a}_2$ - 3.91 - $\underline{vp}_2$ - 1.06 - $\underline{bm}_1$ - 0.08 - $\underline{bt}_1$.

Donald S. Robertson

2. Genetic and biochemical studies of $\underline{cl}_1$ and its modifiers.

In the Maize Genetics Cooperation News Letter of 1963 (37:74-76) the results of allele tests were reported that suggested the dominant $\underline{Cl}^2_M$, $\underline{Cl}^3_M$, and $\underline{Cl}^4_M$ modifiers of the albino seedling phenotype of the white endosperm-albino seedling mutant $\underline{cl}_1$ were allelic. Since then more extensive data have been collected and the dominant modifier $\underline{Cl}^5_M$ which was found in our genetic stocks was also tested for allelism. The data reported in Table 1 lends further support to the conclusion that all known modifiers of $\underline{cl}_1$ are allelic. Such modifiers seem to be rather widespread in corn lines. The original $\underline{Cl}^2_M$ and $\underline{Cl}^3_M$ modifiers were found in the inbreds T1 and C106 and $\underline{Cl}^4_M$ in inbred CI31A. In crosses to transfer $\underline{cl}_1$ into the inbreds M14 and W22 they also were found to carry modifiers of $\underline{cl}_1$. These modifiers are being tested for allelism with the others. The inbreds OH43 and N25 seem to be devoid of $\underline{cl}_1$ modifiers as do some, if not all, lines of Tama flint.

The modifier locus has not been determined as yet. Early attempts to locate it were hampered by the presence of modifiers in the series of translocations which were being used as linkage testers. However, we now have a series of waxy chromosome-nine translocations converted to M14 and this series has been crossed to $\underline{cl}_1$ devoid of modifiers. If the M14 modifier turns out to be allelic to the other modifiers, it is hoped that analysis of F_2 progeny of this series of translocation crosses will reveal the location of the modifier locus.

Table 1
Summary of data from allele tests of $\underline{Cl}^2_M$, $\underline{Cl}^3_M$, $\underline{Cl}^4_M$ and $\underline{Cl}^5_M$

F_1 Cross	# F_2 seedlings	# albino	Conclusions
$\underline{cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$ x $\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$	6119	0	Allelic
$\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$ x $\underline{W}_{7716}\ \underline{W}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$	2842	0	Allelic
$\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$ x $\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$	13,571	0	Allelic
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$ x $\underline{cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$	9045	0	Allelic
$\underline{cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$ x $\underline{W}_{7716}\ \underline{W}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$	1810	0	Allelic
$\underline{Cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$ x $\underline{W}_{7716}\ \underline{W}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$	1724	0	Allelic

The allele tests of the modifiers resulted in stocks that were heterozygous for the various modifier alleles. These along with the various homozygotes and stocks which were heterozygous for the modifier and the recessive allele at this locus were analyzed for their ability to synthesize plastid pigments in the seedling stage.

Tests established that $\underline{cl}_1$ and its alleles $\underline{w}_{7716}$ and $\underline{cl}_p$ in the absence of modifiers were able to produce normal or near normal amounts of protochlorophyllide in the dark and to convert this to chlorophyll in the light (Table 2). On further exposure to light the chlorophyll is destroyed in the absence of carotenoid pigments. In this regard these mutants are similar to other white-albino mutants. This observation suggests that the genetic lesion at the $\underline{cl}_1$ locus primarily involves carotenoid synthesis and that chlorophyll is only secondarily involved. Tests of the four homozygous suppressed phenotypes ($\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}^2_M\ \underline{Cl}^2_M$, $\underline{cl}_1\ \underline{cl}_1\ \underline{Cl}^3_M\ \underline{Cl}^3_M$, $\underline{cl}_p\ \underline{cl}_p\ \underline{Cl}^4_M\ \underline{Cl}^4_M$ and $\underline{w}_{7716}\ \underline{w}_{7716}\ \underline{Cl}^5_M\ \underline{Cl}^5_M$) also establish that they possessed normal or above normal ability to make this pigment (Table 2). Since tests of effect of homozygous modifiers on the chlorophyll synthesizing system would seem to indicate that they have the ability to produce this pigment (Table 2), any variation in pigment concentration in light grown seedlings must be due to the effect of the modifiers on carotenoid synthesis.

Figure 1 indicates the percentage of plastid pigments (chlorophyll, carotene and xanthophyll) that mutant seedlings have when compared to their normal siblings from the same ear. The data are expressed in this form since the various genotypes were not in a homogeneous background and there is considerable variation in pigment level among normals of the various lines tested. The genotypes are arranged along the abscissa in descending order, with those giving the closest approximation to normal on the left. The determination of how closely a given mutant approximates normal was largely subjective. In making this judgment, visual comparison of normal and mutant plants from the same segregating ear was made using such criteria as plant height at maturity, date of flowering and differences in plant pigmentation obvious to the eye. Such judgments are easier to make in hybrid material that produced the plants heterozygous for two different modifiers since these populations tended to be more uniform. This was also true for homozygous $\underline{cl}^4_M$ plants which were in an inbred background. However, for stocks like $\underline{Cl}^3_M$ $\underline{Cl}^3_M$ and $\underline{Cl}^5_M\ \underline{Cl}^5_M$ differences were more difficult to determine accurately because the progeny of the self pollination that produced them showed considerably more variation in both the normal and mutant individuals. This is particularly true for the $\underline{Cl}^2_M\ \underline{Cl}^2_M$ line which for the most part has been perpetuated in the homozygous condition

Table 2

The formation of protochlorophyllide and chlorophyll in normal and mutant seedlings from self-pollinated ears of plants carrying cl_1 alleles with and without modifiers.

Genotype of self-pollinated plant	Seedlings tested	O. D. 630 mu.	Protochlorophyllide mg./gm.	Protochlorophyllide Mutant ÷ Normal	O. D. 667 mu.	Chlorophyll mg./gm.	Chlorophyll Mutant ÷ Normal
Dark Grown Seedlings							
$Cl_1\ cl_1\ cl_M\ cl_M$	N	.063	.00106	3.3	0.0	0.0	--
	M	.102	.00348				
$W_{7716}\ W_{7716}\ cl_M\ cl_M$	N	.037	.00144	0.5	0.0	0.0	--
	M	.055	.00074				
$Cl_p\ cl_p\ cl_M\ cl_M$	N	.053	.00182	1.9	0.0	0.0	--
	M	.079	.00350				
$Cl_1\ cl_1\ Cl_M^2\ Cl_M^2$	N	.062	.00240	1.1	0.0	0.0	--
	M	.078	.00254				
$W_{7716}\ W_{7716}\ Cl_M^5\ Cl_M^5$	N	.070	.00272	0.7	0.0	0.0	--
	M	.056	.00185				
$Cl_p\ cl_p\ Cl_M^4\ Cl_M^4$	N	.052	.00187	1.3	0.0	0.0	--
	M	.063	.00240				
$Cl_1\ cl_1\ Cl_M^3\ Cl_M^3$	N	.047	.00153	1.4	0.0	0.0	--
	M	.064	.00216				
	Seedlings exposed to 1 min. of light at 1000 ft. c. and harvested after 1 hour of dark						
$Cl_1\ cl_1\ cl_M\ cl_M$	N	.030	.00091	1.9	.036	.00112	3.9
	M	.058	.00170		.146	.00440	
$W_{7716}\ W_{7716}\ cl_M\ cl_M$	N	.024	.00080	2.0	.024	.00082	3.9
	M	.056	.00162		.107	.00319	
$Cl_p\ cl_p\ cl_M\ cl_M$	N	.047	.00185	0.8	.012	.00049	5.7
	M	.044	.00150		.078	.00276	
$Cl_1\ cl_1\ Cl_M^2\ Cl_M^2$	N	.045	.00139	0.9	.023	.00073	2.2
	M	.037	.00121		.048	.00161	
$W_{7716}\ W_{7716}\ Cl_M^5\ Cl_M^5$	N	.037	.00140	0.9	.011	.00042	1.3
	M	.042	.00131		.017	.00055	
$Cl_p\ cl_p\ Cl_M^4\ Cl_M^4$	N	.038	.00111	1.6	.022	.00066	2.4
	M	.043	.00177		.037	.00157	
$Cl_1\ cl_1\ Cl_M^3\ Cl_M^3$	N	.033	.00091	2.0	.010	.00028	4.8
	M	.056	.00182		.040	.00135	

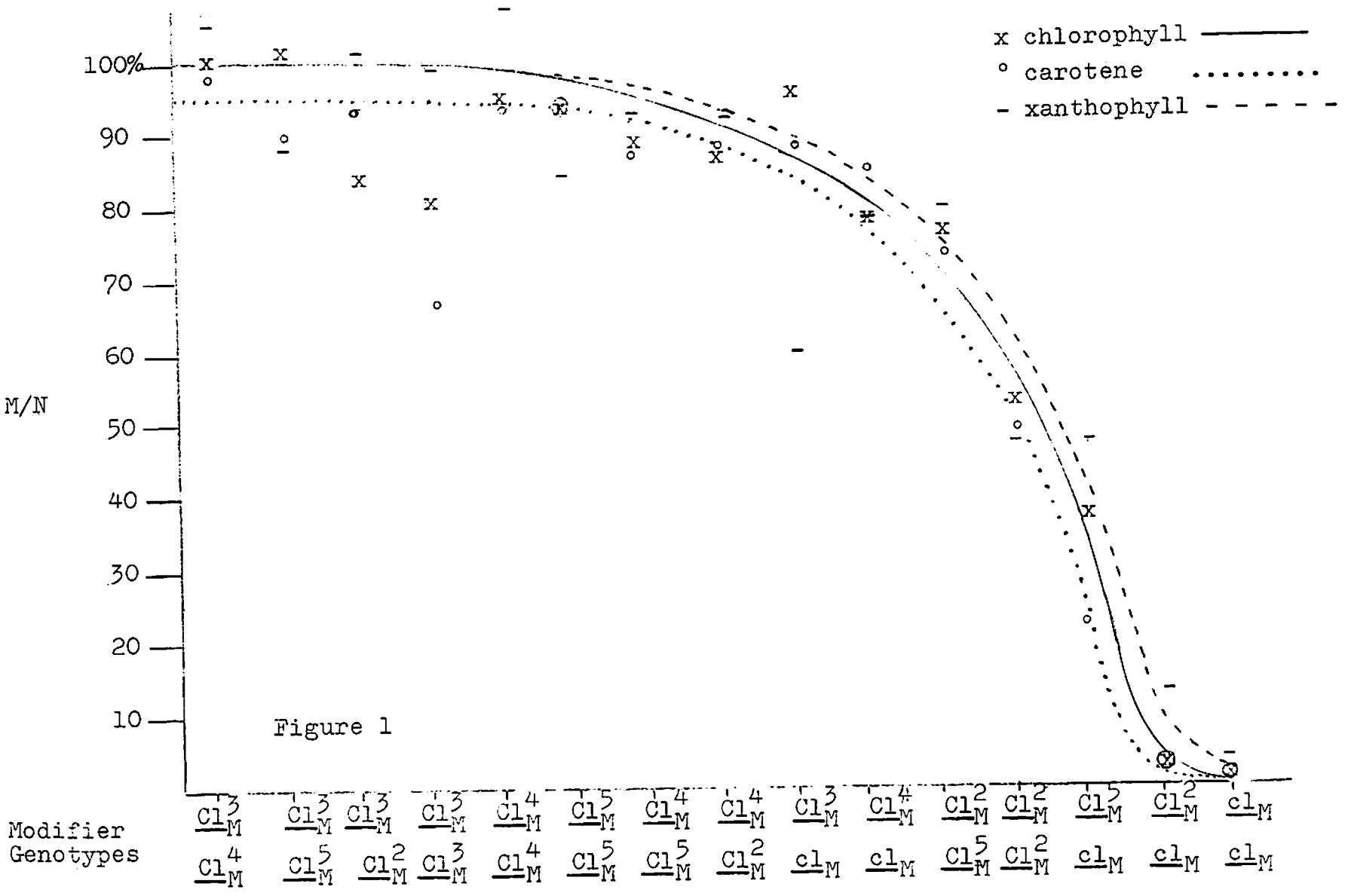
x chlorophyll
° carotene
- xanthophyll
100%
90
80
70
60
M/N
50
40
30
20
10
Modifier Genotypes
Cl3M Cl3M Cl3M Cl3M Cl4M Cl5M Cl4M Cl4M Cl3M Cl4M Cl2M Cl2M Cl5M Cl2M clM
Cl4M Cl5M Cl2M Cl3M Cl4M Cl5M Cl5M Cl2M clM clM Cl5M Cl2M clM clM clM

Figure 1

so that we have had very little opportunity to make accurate comparisons between normals and mutants from a given ear. Those that have been made would indicate that mutants are slightly less vigorous than normal mature plants. However, the chemical data would suggest that the phenotype of this genotype should fall below that of $\frac{Cl^3_M}{}\ \frac{cl_M}{}$ plants. It is obvious from visual observation that this cannot be the case since even to the casual observer mature $\frac{Cl^3_M}{}\ \frac{cl^3_M}{}$ are decidedly pale-green plants with a tendency to have white sheaths and zebra striping while $\frac{Cl^2_M}{}\ \frac{Cl^3_M}{}$ plants are a definite dark green and closely approximate normals. The explanation for the low values for $\frac{Cl^2_M}{}\ \frac{Cl^2_M}{}$ could be due to an increased effeciency of the modifiers as the plants mature so that the seedling values do not accurately reflect performance in mature plants. However, this is not observed to be the case for the other genotypes. Perhaps the low value for $\frac{Cl^2_M}{}\ \frac{Cl^2_M}{}$ is due to some peculiarity in the particular background of the material used for these determinations which came from lines of rather low vigor due to several generations of inbreeding. We are in the process of crossing this gene out to inbreds that do not possess modifiers and re-extracting what we hope to be a more vigorous $\frac{Cl_1}{}\ \frac{cl_1}{}\ \frac{Cl^3_M}{}$ $\frac{Cl^3_M}{}$ line for further pigment tests.

The outstanding characteristic of Figure 1 is that the levels of the three plastid pigments vary together. Since it is known that both the albino mutants and the modified genotypes have a chlorophyll producing mechanism that, as far as has been tested, appears to be normal, it is strongly suggestive that the marked parallelism between chlorophyll content and the carotene and xanthophyll levels is dependent on the amount of one or both of the latter two pigments that can be produced under the influence of a given modifier. This is just what would be expected if carotene is acting here to protect chlorophyll from photodestruction. At low levels of carotene productions only small amounts of chlorophyll can be protected; at higher carotene levels more chlorophyll is protected. These results are in agreement with those of other workers that suggest that one of the functions of colored carotenoids is to protect chlorophyll from photo-auto-oxidation.

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3. Electron microscopy studies of plastid development in mutants at the white endosperm - albino seedling W_3 locus.

This past year we have begun an electron microscopy study of plastid development in normal and mutant plant material. In these studies seedlings were grown for 10 - 14 days in the dark at 26.6°C. (80°F.). Others were grown under