HEREDITY VOLUME 21 PART 1 FEBRUARY 1966

ALLELIC RELATIONSHIPS AND PHENOTYPIC INTERACTIONS OF FOUR DOMINANT MODIFIERS OF THE cl_1 LOCUS IN MAIZE

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Received 22.iv.65

1. INTRODUCTION

EVERETT (1949) described a series of genes controlling the formation of plastid pigments in maize. These consisted of, (1) a recessive pleiotropic gene, cl_1, that when homozygous resulted in white or pale yellow seeds which upon germination gave albino seedlings, (2) a dominant gene, Cl_2, which partially suppressed the albino phenotype of the cl_1 seedlings while not affecting the endosperm colour of this mutant, and (3) a dominant gene, Cl_3, which resulted in the partial suppression of the albino phenotype of cl_1 seedlings when heterozygous and the complete suppression of the seedling phenotype when homozygous. The Cl_3 gene also does not affect the endosperm phenotype of cl_1 mutants.

Everett (1949) considered Cl_2 to be a suppressor gene and Cl_3 to be a semi-duplicate gene. However, work to be reported in this paper would indicate that these two genes are allelic and, therefore, probably have a common mode of action. This creates a problem as to whether Cl_2 and Cl_3 should be considered duplicate or suppressor genes. It is not certain whether the distinction between these two alternatives can be made on a phenotypic level since the segregation of an independent dominant complete suppressor would result in the 15:1 genetic ratio associated with duplicate genes. Even at the chemical level of gene action, it may not be possible to distinguish between these two classes of mutants, for it has been suggested that some suppressor mutants may be due to the mutation of an independent gene which, in its mutated state, assumes the function that the gene being suppressed had lost. In reality such a suppressor would be nothing more than a duplicate gene, and the term suppressor probably should not be applied to it. The term suppressor ought to be confined to that class of mutants that affect the milieu of the cell in some unspecified manner (other than taking over the exact function of the original gene) so as to overcome the metabolic lesion produced by the original mutation. The exact mode of action will, undoubtedly, vary from suppressor to suppressor. Recent work with micro-organisms has suggested that some suppressors

* Journal Paper No. J-5066 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1381. This work was supported in part by grant G 19395 from the National Science Foundation.

act to produce changes in the primary structure of a gene product (Brody and Yanofsky, 1963, 1964; Orias and Gartner, 1964; Garen and Siddiqi, 1962; Stent, 1964). Other less direct effects of suppressors are also possible (Yanofsky and St. Lawrence, 1960).

Thus, it would seem that knowledge of gene action at the most basic level is necessary before any definite conclusions can be reached as to whether a given situation involves a suppressor or duplicate gene mutation. Since this information is not available for the *Cl* genes, it is impossible to state definitely what class of mutants Cl_2 and Cl_3 represent. Certainly Cl_2 and Cl_3 are not duplicate genes in the classical sense since they do not completely duplicate the action of Cl_1. This is evident since the presence of the dominant alleles at the Cl_2-Cl_3 locus does not affect the endosperm phenotype. Thus, these genes are not duplicating Cl_1 in its endosperm effect. Neither do these genes (Cl_2 and Cl_3) act as classical duplicate genes as far as the plant phenotype is concerned since they do not result in the 15:1 F_2 ratios which characterise such an epistatic condition. Dihybrid F_2 populations involving the segregation of the Cl_1-cl_1 and Cl_2-cl_2 genes give a seedling ratio of 12 green:3 pale green:1 albino, while dihybrid F_2 populations involving the segregation of the Cl_1-cl_1 and Cl_3-cl_3 genes give a plant colour ratio of 13 green:2 pale green (mature plant):1 albino. It is obvious that neither of these genes duplicates exactly the activity of the Cl_1 gene. In this paper, we will report on two additional alleles of the Cl_2-Cl_3 locus which also do not exactly duplicate the Cl_1 activity. These four alleles can be arranged in order of effectiveness in causing phenotypic reversion of the plant phenotype, ranging from the slight reversion caused by Cl_2 to the near complete revision of homozygous Cl_3.

These genes could be thought of as only partially duplicate genes which do not duplicate the endosperm activity of Cl_1 at all and only partially duplicate the plant activity. It is simpler, however, to picture the Cl_2-Cl_3 locus as one at which there can occur a closely graded series of suppressor alleles which can vary in their effects from only slight to near complete suppression of the cl_1 seedling phenotype. The fact that reversion to normal is not complete and that not all of the pleiotropic effects of the cl_1 gene are affected by the dominant alleles of the Cl_2-Cl_3 locus lends support to the conclusion that these genes are most likely suppressors. However, this is at best only a tentative decision, and it will be necessary to find out much more about the action of these genes before a definitive answer can be had as to whether we are dealing with duplicate or suppressor genes. Because of this ambiguity, it is perhaps best that these genes be called simply modifiers, a term which does not carry with it any connotation of gene action.

2. STOCKS

Three alleles at the cl_1 locus are discussed in this report, cl_1, cl_p and w_{7716}. The cl_1 mutant was obtained from Dr Everett and the cl_p was found in stocks supplied by the Pioneer Hi-Bred Corn Company. The

w_{7716} allele was found in genetic stocks grown at the California Institute of Technology. Phenotypically, these mutants are indistinguishable.

The modifiers Cl_2 and Cl_3 were obtained from Dr Everett.

The original cl_p stock contained a dominant modifier which was given the symbol Cl_4. As seedlings, plants homozygous for this modifier are green, and mature plants are similar to normals but a little weaker and slower growing. Plants heterozygous for the Cl_4 modifier are green as young seedlings, but under field conditions they turn pale green and soon die.

A modifier of w_{7716} was found in our genetic stocks and was given the symbol Cl_5. Homozygous Cl_5 plants have green seedlings and

TABLE 1

Alleles at the cl$_1$ *locus, their modifiers and phenotypes*

cl_1 Allele	Modifier	Seedling phenotype as affected by modifier		Mature plant phenotype as affected by modifier	
		homozygotes	heterozygotes	homozygotes	heterozygotes
	Cl_2 Cl_3	pale green green	pale green green	lethal green = normal plants	lethal pale green
cl_p	Cl_4	green	green	green little weaker than normals	lethal
w_{7716}	Cl_5	green	pale green	green little weaker than normals	lethal

mature plants which appear somewhat weaker than normals. Heterozygous Cl_5 results in a pale green lethal condition.

The cl_1 alleles and their modifiers are listed and the modified phenotypes are summarised in table 1. The modifiers can be ranked in the following order based on their ability to approximate the normal phenotype: $Cl_3 > Cl_4 > Cl_5 > Cl_2$.

3. GENETIC TESTS

Self-pollination of plants heterozygous for the various albino alleles and modifiers gives 3 modified:1 albino seedlings among the plants coming from white or pale yellow seeds, indicating that, in all cases, the modifiers were independent of the albino alleles.

The symbols chosen for the modifier genes would indicate that they were non-allelic, yet tests for allelism had not been made. During the last two years, allele tests of these four modifier genes have been carried

out. The crosses made in these tests, as shown in table 2, consisted of crossing two stocks which were heterozygous or homozygous for cl_1 or one of its alleles and which were at the same time homozygous for

TABLE 2

F_1 crosses made to determine allelism of cl_1 *modifiers*

$cl_p\ cl_p\ Cl_4\ Cl_4 \times cl_1\ cl_1\ Cl_3\ Cl_3$
$cl_1\ cl_1\ Cl_3\ Cl_3 \times W_{7716}\ w_{7716}\ Cl_5\ Cl_5$
$cl_1\ cl_1\ Cl_3\ Cl_3 \times Cl_1\ cl_1\ Cl_2\ Cl_2$
$Cl_1\ cl_1\ Cl_2\ Cl_2 \times cl_p\ cl_p\ Cl_4\ Cl_4$
$cl_p\ cl_p\ Cl_4\ Cl_4 \times W_{7716}\ w_{7716}\ Cl_5\ Cl_5$
$Cl_1\ cl_1\ Cl_2\ Cl_2 \times W_{7716}\ w_{7716}\ Cl_5\ Cl_5$

different modifier genes. The F_1 offspring from these crosses were grown (both yellow and white seeds where viable) and self-pollinated. White or pale yellow seeds from these self-pollinated ears were then seedling tested. If the modifiers occupy independent loci, then a 15

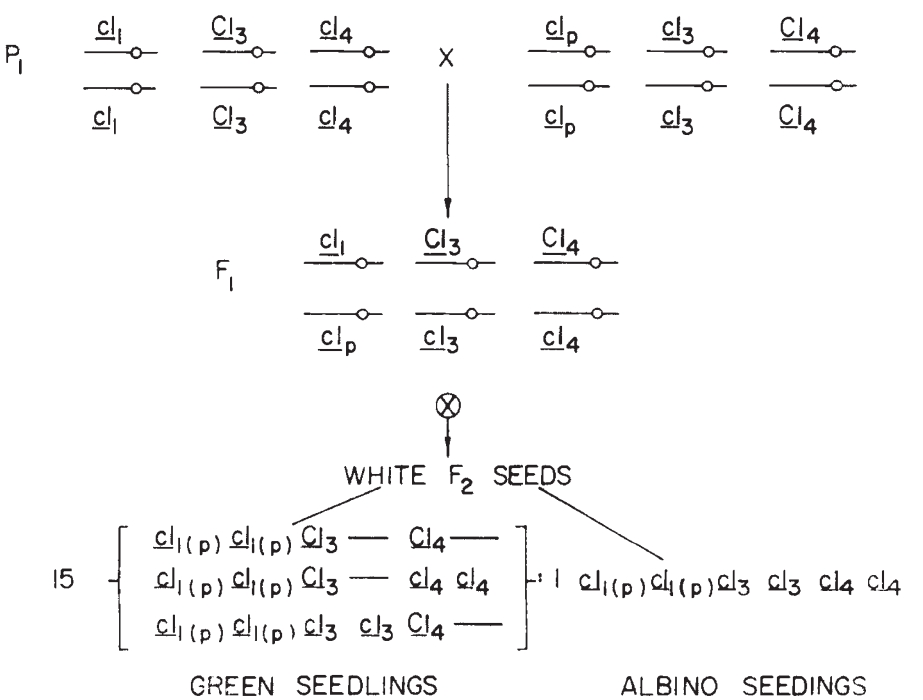

FIG. 1.—Results expected if modifiers are non-allelic (using $cl_1\ cl_1\ Cl_3\ Cl_3 \times cl_p\ cl_p\ Cl_4\ Cl_4$ for illustration).

non-albino:1 albino seedling ratio should be observed among the plants produced by the white or pale yellow seeds (see fig. 1). A lower frequency of albino seedlings would indicate that the modifiers are non-allelic but linked. If the modifiers are alleles, no albino seedlings should be observed in the F_2 seedling test (see fig. 2). The results of these tests are given in table 3 and indicate that these genes are all allelic.

Since these tests indicate that the genes are allelic, the symbols that have been used in the past do not conform to accepted standards for alleles. If further work should indicate that they are acting as suppressors, the symbols Cl^2_S, Cl^3_S, Cl^4_S and Cl^5_S should be used. However,

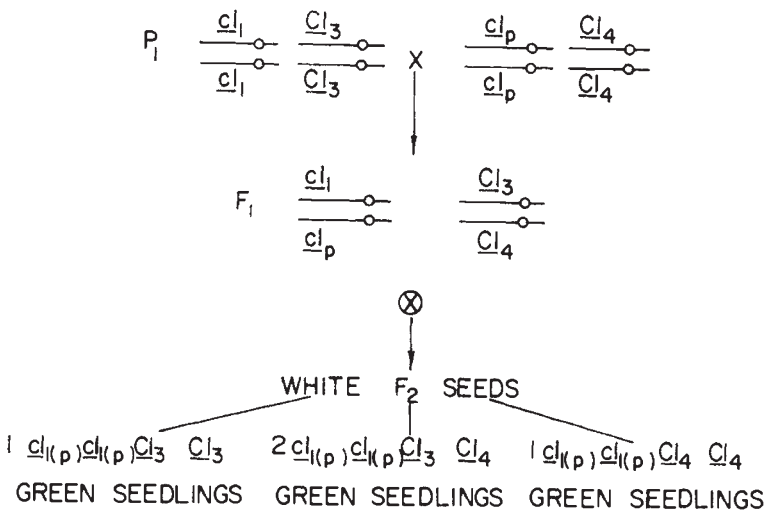

FIG. 2.—Results expected if modifiers are allelic (using cl_1 cl_1 Cl_3 $Cl_3 \times cl_p$ cl_p Cl_4 Cl_4 for illustration).

until these are established as suppressors, it is perhaps best to use the subscript M (for modifier). Hence, for the remainder of this report the symbols Cl^2_M, Cl^3_M, Cl^4_M and Cl^5_M will be used.

These tests establish not only the allelism of these modifier genes, but also that they all interact with the three albino alleles at the cl_1

TABLE 3

Summary of data from allele tests involving Cl_2, Cl_3, Cl_4 *and* Cl_5

F_1 cross	Number of seedlings tested	Number of albino seedlings	Conclusions
cl_p cl_p Cl_4 $Cl_4 \times cl_1$ cl_1 Cl_3 Cl_3 . . .	6,119	0	Allelic
cl_1 cl_1 Cl_3 $Cl_3 \times W_{7716}$ w_{7716} Cl_5 Cl_5 . .	2,842	0	Allelic
cl_1 cl_1 Cl_3 $Cl_3 \times Cl_1$ cl_1 Cl_2 Cl_2 . . .	13,571	0	Allelic
Cl_1 cl_1 Cl_2 $Cl_2 \times cl_p$ cl_p Cl_4 Cl_4 . . .	9,045	0	Allelic
cl_p cl_p Cl_4 $Cl_4 \times W_{7716}$ w_{7716} Cl_5 Cl_5 . .	1,810	0	Allelic
Cl_1 cl_1 Cl_2 $Cl_2 \times W_{7716}$ w_{7716} Cl_5 Cl_5 . .	1,724	0	Allelic

locus (cl_1, cl_p, w_{7716}). If they did not, white seedlings would be expected in F_2 populations where two different albino alleles were segregating.

The plants grown for these allele tests provided material for observing the effects of combining two different modifiers in the same plant. Table 4 summarises these observations. The following generalisations

can be made: (1) any heterozygote with Cl_M^3 gives plants that approach the normal phenotype, (2) any heterozygote with Cl_M^4 (except that with Cl_M^3) is viable but somewhat less vigorous than normal and (3)

TABLE 4

Phenotypes of plants heterozygous for the various modifiers of the cl_1 *locus*

Genotype	Phenotypes	
	as seedlings	at tasseling
$cl_p\ Cl_M^4/cl_1\ Cl_M^3$	Equal to normals	Average 1 day later. Not quite as vigorous and fewer tillers than normals
$cl_1\ Cl_M^3/w_{7716}\ Cl_M^5$	Equal to normals	Vigour equals normals, average 1 day later
$cl_1\ Cl_M^3/cl_1\ Cl_M^2$	Equal to normals	Vigour equals normals, average 1-2 days later
$cl_1\ Cl_M^2/cl_p\ Cl_M^4$	Equal to normals	2/3 height of normals, average 1 week later
$w_{7716}\ Cl_M^5/cl_p\ Cl_M^4$	Equal to normals	2/3 height of and paler green than normals, average 1 week later
$cl_1\ Cl_M^2/w_{7716}\ Cl_M^5$	Pale green	Died as seedlings

the heterozygote of Cl_M^5 with Cl_M^2 is non-viable. Using the ability of the modifiers to interact in such a way as to approximate the normal phenotype, they can be ranked in the same order that was given before, $Cl_M^3 > Cl_M^4 > Cl_M^5 > Cl_M^2$.

4. DISCUSSION

These genetic tests demonstrate that the series of modifiers of cl_1 that have been studied are all allelic. The variability in the modified phenotype of the different alleles would indicate that this locus is capable of mutating to many different levels of activity. This is in contrast to the cl_1 locus at which three recessive alleles are known, all of which produce the same phenotype. These tests also establish that all modifiers are capable of interacting with the three recessive cl_1 alleles.

These modifiers seem to be very specific for the cl_1 locus. At least 12 other mutants are known in maize that have the basic white (or pale yellow) endosperm-albino seedling phenotype similar to cl_1 (Robertson, 1961). None of these is affected by the Cl_M modifiers. Nor have any locus specific modifiers been demonstrated for these other mutants. There is, however, among these mutants one example of interaction between two loci. This involves the duplicate genes, lw_3 and lw_4. These genes interact in the classical manner expected of duplicate genes, giving 15:1 ratios when both are heterozygous. Unlike the cl_1-Cl_M interaction, Lw_3 and Lw_4 seem to completely duplicate each other's action in both the endosperm and seedling. Thus, they appear to be behaving as true duplicate genes.

Preliminary crosses with the inbred lines M14, W22, OH43 and N25 have revealed modifiers present in the first two, while the latter two are devoid of any modifiers. Everett found the original modifiers in the inbreds T1 and C106 and the Cl^4_M allele was isolated in the inbred CI31A. These observations suggest that the modifiers might be fairly prevalent in our commercial lines of corn.

Biochemical studies of these modified mutants (details will be published in a subsequent paper) indicate that the levels of the three plastid pigments, chlorophyll, carotene and xanthophyll, are all affected by the modifier genes. The evidence suggests that the modifiers primarily affect carotenoid synthesis and that chlorophyll levels are only secondarily involved.

5. SUMMARY

The phenotypes associated with four dominant modifiers of the albino cl_1 mutant are described. These modifiers are shown to be allelic and that they all interact with the three recessive alleles known at the cl_1 locus.

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