COMPLEMENTARY GENES FOR CHLOROPHYLL DEVELOPMENT IN MAIZE AND THEIR LINKAGE RELATIONS¹

E. W. LINDSTROM
Iowa State College, Ames, Iowa

Received March 22, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION.....	305
Experimental report.....	306
Chlorophyll relations.....	306
Linkage relations.....	311
Linkage of W_1 and Y	311
Linkage of W_2 and R	314
Gene W_3 independent of W_1 , W_2 , C and L_g	318
DISCUSSION.....	319
SUMMARY.....	321
LITERATURE CITED.....	321
APPENDIX—Tables 9 to 14.....	322

Whenever a hereditary character develops as the resultant of several genetic factors it becomes of immediate interest to determine the nature of this factorial interaction. Whether this action is "supplementary" or "complementary" is an important consideration. The former bespeaks a different type of chemical (or physiological) behavior, and perhaps evolutionary origin of the chromosomes than does the latter. For example, an F_2 ratio of 15 : 1 or 63 : 1 indicates at least a different chemical relationship between the factors than does a 9 : 7 or 27 : 37 ratio. The chlorophyll characteristics of maize provide a fertile field of research that sheds much light on such problems.

This report is concerned with an intensive study of one phase of the chlorophyll situation in maize, that involving the well known albino seedlings. Technically, albino seedlings are those devoid of the green components of chlorophyll as well as of the yellow xanthophyll or carotenoid pigments. They are distinguished from the virescent-white seedlings, which they simulate in the early stages of growth, by the fact that the virescent-whites ordinarily assume an unmistakable green color soon after emerging. The albino seedlings never become green, although

¹ Paper No. 3, Department of Genetics, IOWA STATE COLLEGE, Ames, Iowa. Presented before the Joint Genetics Sections of the BOTANICAL SOCIETY OF AMERICA and the AMERICAN SOCIETY OF ZOÖLOGISTS meeting at Cincinnati, Ohio, December 27, 1923.

some types may show very faint traces of pale green on the coleoptile. Virescent-whites may grow to full maturity; albinos never do.

Earlier reports (LINDSTROM 1918, 1921) have described the mode of inheritance of the albino seedlings that were due to a single recessive gene. Evidence has been at hand for several years that such white seedlings from different sources were genetically different, although phenotypically similar. Three such types are reported herein. Their factorial interactions and their linkage relations are satisfactorily explained.

EXPERIMENTAL REPORT

Chlorophyll relations

Crosses were made between two inbred stocks of corn, both carrying the recessive albino seedlings. One pedigree culture involved a single factor for white seedlings that had been carried for ten generations of controlled breeding. The other had its origin in some commercial yellow flint corn received from Doctor W. E. CASTLE in 1918, since which time it has been inbred.

Several successful crosses were made in 1920 between green plants heterozygous for the single known albino gene (W_1w_1) and others from the yellow flint cultures which had been giving 9 : 7 ratios of green to albino seedlings. The progenies of three such crosses have been followed through the F_1 , F_2 and F_3 generations.²

In every case the F_1 plants were normal green (table 1). This at once indicated that the albinos from the two sources were genetically different.

TABLE 1

F₁ data from crossing heterozygous green plants involving two and three pairs of chlorophyll factors.

	PEDIGREE NUMBER	PARENTAL GENOTYPES	F ₁ SEEDLINGS
Cross No. 1	4103-20×4265-17	$W_1w_1 yy R^e r^e AA CC P_r P_r ii L_g l_g$ × $W_2w_2 YY r^e r^e AA cc P_r P_r ii L_g L_g$	118 green
Cross No. 2	4103-21×4265-24	$W_1w_1 yy R^e r^e AA CC P_r P_r ii L_g l_g$ × $W_3w_3 YY r^e r^e AA cc P_r P_r ii L_g L_g$	180 green
Cross No. 3	4266-4×4103-14	$W_2w_2 W_3w_3 YY r^e r^e AA CC P_r P_r ii bb p_1 p_1$ × $W_1w_1 yy R^e r^e AA CC P_r P_r ii Bb p_1 p_1$	106 green

² A portion of the F_1 ears were self-pollinated by Prof. J. B. WENTZ, IOWA STATE COLLEGE, in connection with some other problems in genetics. Mr. W. A. CARVER, graduate assistant in genetics, also aided in some of the self-pollination work.

A relatively large number of these fully green F_1 plants were self-fertilized. Being crosses of flint and dent varieties, they were very vigorous. Their seedling progenies were grown in the greenhouse. A large F_3 generation was also grown.

Crosses No. 1 and No. 2 were each found to involve only two different albino genes. The F_2 data as well as the linkage relations prove this fact, conclusively. The parental genetic formulae involved might be given as follows:

Cross No. 1, $W_1w_1 W_2W_2 \times W_1W_1 W_2w_2$

Cross No. 2, $W_1w_1 W_3W_3 \times W_1W_1 W_3w_3$

Under such circumstances the green F_1 plants would be of four types in equal numbers as follows:

Cross No. 1

$W_1W_1 W_2W_2$

$W_1w_1 W_2W_2$

$W_1W_1 W_2w_2$

$W_1w_1 W_2w_2$

Cross No. 2

$W_1W_1 W_3W_3$

$W_1w_1 W_3W_3$

$W_1W_1 W_3w_3$

$W_1w_1 W_3w_3$

When such F_1 plants are selfed, three distinct F_2 seedling progenies should be apparent. One-fourth of the progenies in either cross would consist entirely of green seedlings; one-half of 3 : 1 ratios of green to white seedlings; and the remaining one-fourth should segregate into 9 greens : 7 whites if the factorial interaction of the two genes was complementary in nature, and if they were also inherited independently of each other. Such proved to be the case.

A total of 119 F_2 seedling progenies was tested on a relatively large scale. The complete data are listed in the appendix (tables 9 and 10). A summary of the progenies has been arranged in table 2.

TABLE 2

Summary of F_2 seedling progenies involving two complementary genes. Details in tables 9 and 10.

	PROGENIES OF ALL GREEN	PROGENIES OF 3 GREEN : 1 ALBINO	PROGENIES OF 9 GREEN : 7 ALBINO	PROBABILITY P
Cross No. 1	12	27	14	
Cross No. 2	23	30	13	
Total	35	57	27	
Expected	30	59	30	0.56

This summary exhibits a close approximation to the expected 1 : 2 : 1 ratio of the three types of seedling progenies. The deviations can be

reasonably due to errors of random sampling since P has a value of 0.56. Accordingly it becomes evident that the two genes for albino seedlings involved in each of crosses No. 1 and No. 2 are genetically different. It is also apparent that the factorial interaction of their dominant allelomorphs is complementary.

A glance at the detailed data in tables 9 and 10 gives further verification for the hypothesis, since all the individual ratios are surprisingly close to expectation. A total of all the 9 : 7 ratios in these tables gives 2036 greens : 1542 albinos, where 2013 : 1565 are expected. This exhibits a deviation of 23, which is only 1.2 times its probable error. From such data one can be reasonably certain that W_1 and W_2 , or W_1 and W_3 are independently inherited. However, their linkage relations, discussed later, serve as a real verification of this fact.

The most interesting developments arose in cross No. 3, which was a cross between green plants segregating for 3 : 1 and 9 : 7 ratios. The parental genotypes in this case may be written as follows:

$$W_1W_1 W_2w_2 W_3w_3 \times W_1w_1 W_2W_2 W_3W_3$$

Such a situation calls for an F_1 generation of green plants comprising eight different genotypes in equal proportions as follows:

$W_1W_1 W_2W_2 W_3W_3$	All green
$W_1w_1 W_2W_2 W_3W_3$	3 green : 1 white
$W_1W_1 W_2w_2 W_3W_3$	3 green : 1 white
$W_1W_1 W_2W_2 W_3w_3$	3 green : 1 white
$W_1w_1 W_2w_2 W_3W_3$	9 green : 7 white
$W_1w_1 W_2W_2 W_3w_3$	9 green : 7 white
$W_1W_1 W_2w_2 W_3w_3$	9 green : 7 white
$W_1w_1 W_2W_2 W_3w_3$	27 green : 37 white

If the third gene is acting in a complementary fashion like the others, a triheterozygous green F_1 plant should produce an F_2 seedling progeny of 27 green : 37 white or albino seedlings. This is a new sort of chlorophyll ratio, one in which there is a preponderance of the recessive, white seedlings. Eleven such progenies were found among the 72 F_2 progenies that were tested (see table 3).

The eleven progenies gave a total of 1094 green and 1422 albino seedlings. The theoretical expectancy, based on a 27 : 37 ratio, typical of three pairs of independent, complementary genes, is 1061 : 1455, a deviation of 33, which is only twice as large as the probable error. In this case we have the usual slight excess of the green class, a phenomenon which is characteristic of large counts involving recessive defects in maize. A slightly lower germination or viability of the recessive class, coupled

TABLE 3

The 72 F₂ seedling progenies of cross 3 arranged in four classes according to chlorophyll behavior.

F ₁ EAR NUMBER	ALL GREEN	F ₁ EAR NUMBER	3 GREEN : 1 ALBINO		F ₁ EAR NUMBER	9 GREEN : 7 ALBINO		F ₁ EAR NUMBER	27 GREEN : 37 ALBINO	
201	86	205	168	53	202	121	75	209	90	135
217	138	207	159	52	203	104	67	213	74	123
225	147	210	261	81	204	116	91	215	67	80
227	126	211	127	28	206	136	126	216	66	79
234	148	218	100	43	208	159	113	219	127	184
249	66	223	120	35	212	148	103	230	133	163
257	133	224	201	61	214	123	76	237	179	191
264	150	226	126	32	220	99	63	248	42	44
		228	186	62	221	129	83	261	112	156
		229	116	32	222*	162	174	268	87	110
		232	267	89	231	174	113	273	117	157
		239	265	83	233	114	93			
		240	171	65	235	173	134			
		241	174	60	236	216	165			
		253	297	119	242	179	132			
		254	156	38	243	123	118			
		258	101	33	244	185	139			
		259	137	42	245	148	111			
		260	131	35	246	75	67			
		262	164	55	247	96	77			
		266	111	41	250	93	64			
		267	88	29	251	140	110			
		269	114	38	252	84	74			
		271	102	34	255	79	53			
		272	95	26	256	91	44			
					263	179	134			
					265	229	166			
					270	102	64			
Total	994		3937	1266		3777	2829		1094	1422
Expected	994		3902	1301		3716	2890		1061	1455
Dev.										
P.E.	0		1.7			2.2			2.0	

* Determined by linkage tests to be a 9 : 7 ratio.

with the inevitable small percentage of contamination by stray pollen (which invariably adds to the normal class) could easily account for the slight excess of the dominant class, were this necessary. Obviously the deviation of 33 individuals in this case falls within reasonable limits of error due to random sampling. However, the progeny test in cross No. 3 gives a much safer and surer criterion of the factorial relations (table 4).

Before leaving table 3, it is worth noting that the individual 3 : 1 and 9 : 7 ratios therein are based on sufficiently large numbers so as to cause no difficulty in differentiating them from one another or from the 27 : 37 progenies. It was not thought necessary to calculate the individual probable errors, since mere inspection affords a reasonable certainty of their correct classification. Accordingly only the totals for each type of ratio were tested for their agreement with the expected results. In no case was the deviation beyond reasonable limits of divergence.

A summary of the F_2 seedling progenies of cross No. 3 is afforded in table 4.

TABLE 4
*Summary of F_2 seedling progenies involving three complementary genes.
Details in table 3.*

	PROGENIES OF ALL GREEN	PROGENIES OF 3 GREEN : 1 ALBINO	PROGENIES OF 9 GREEN : 7 ALBINO	PROGENIES OF 27 GREEN : 37 ALBINO	PROBABILITY
Cross No. 3..	8	25	28	11	
Expected.....	9	27	27	9	
Deviation...	-1	-2	+1	+2	$\chi^2=0.740$

If the factorial hypothesis is correct there should be among these 72 progenies one-eighth of the total showing all green, three-eighths giving 3 : 1 ratios, three-eighths giving 9 : 7 ratios and one-eighth showing 27 : 37 ratios. The actual data show very little deviation from this expectancy. The closeness of fit gives a value for χ^2 of 0.740 which means a value for P of over 0.80. Such an agreement most certainly substantiates the theory that in this material the inheritance of the green pigment, chlorophyll, as evidenced by the action of the recessive, albino character, can be explained on the basis of three complementary genes.

Accordingly, normal green pigment can be said to be the product of the dominant genes, $W_1W_1W_2W_2W_3W_3$ acting in a complementary fashion. The homozygous recessive condition of any one gene, or two, or three together, is sufficient to inhibit chlorophyll development. It might be noted at this time that the action of each of the three recessive genes in this case is slightly different, although these differences are so minute that they are easily overlooked. Gene w_2w_2 produces the purest type of white seedling. The color is a paper white. With optimum conditions of growth genes w_3w_3 and w_1w_1 apparently permit a very slight development of chlorophyll, especially on the coleoptile. In no case, however, does real

green pigment develop in the leaves, so that these so-called albinos might be phenotypically confused with extreme types of virescent-white seedlings. Incidentally it might be added that these three types of albinos have been crossed with pedigree virescent-white material and the resulting seedlings were entirely green.

Linkage relations

That the three complementary genes W_1 , W_2 and W_3 are inherited independently of each other is practically proven by the close approximation to the theoretical 27 : 37 ratio of green to albino plants produced by a triheterozygous green plant. In addition a large amount of data has been accumulated to prove that W_1 , W_2 and W_3 belong in separate linkage groups. Fortunately these linkage groups are involved in cross No. 3 whose parental genotypes were as follows: $W_1W_1 W_2w_2 W_3w_3 YY r^gr^g AA CC bb p_1p_1 \times W_1w_1 W_2W_2W_3W_3 yy R^gr^r AA CC Bb p_1p_1$.

Linkage of W_1 and Y

The yellow endosperm factor Y in this cross is one that comes from a pure, inbred yellow flint variety. Fortunately, this type of yellow endosperm when crossed with white endosperm gives a single-factor relationship permitting a reasonably clean-cut F_2 distribution of yellow and white grains. Even here, however, some slight difficulty is occasionally experienced in separating a small percentage of the kernels. Most of the F_2 ears nevertheless show an unmistakable 3 : 1 ratio of yellow to white grains. These afford a critical test of the linkage between W_1 and Y .

This linkage may be the same as that mentioned by KEMPTON (1917). It is identical with that reported by STROMAN (1924), although the data presented herewith do not give exactly the same intensity of linkage as was found by STROMAN.

Of the 72 F_2 ears in cross No. 3, there were 37 that possessed aleurone color (3 purple : 1 colorless), and 35 that were entirely colorless as regards aleurone. The latter afford a good means of testing the endosperm-chlorophyll linkage, since all of them had approximately 75 percent yellow and 25 percent white grains.

Among the 35 ears devoid of aleurone color there were 14 that segregated for 3 green : 1 albino; 14 for 9 green : 7 albino; and 4 for 27 green : 37 albino. The remaining 3 ears gave only green seedlings. Linkage between W_1 and Y was observed in 8 of the 3 : 1 ears, 7 of the 9 : 7 ears, and in all of the 4 ears giving the 27 : 37 chlorophyll ratio. The individual counts of these 16 ears are arranged in table 11. The data on the 13 ears

that did not show any linkage between Y and albino genes are recorded in table 12.

A summary of the linkage progenies that appear in table 11 is arranged in table 5, as follows:

TABLE 5

*Summary of F_2 data showing linkage between W_1 and Y , giving 35 percent crossing over.
Cross No. 3. Details in table 11.*

GENOTYPE OF F_1 MOTHER PLANT	PARENTAL TYPES Yw AND yw		NON-PARENTAL TYPES Yw AND yW		Dev. P. E.
	Observed	Expected	Observed	Expected	
$YW_1 \cdot yw_1 \dots \dots \dots$	1211	1214	496	493	0.2
$YW_1w_2 \cdot yw_1W_2$ OR $YW_1w_3 \cdot yw_1w_3$ } ...	789	796	546	539	0.6
$YW_1w_2w_3 \cdot yw_1W_2W_3$	435	420	390	405	1.5

In this table, as in others to follow, the method of grouping the parental against the non-parental classes has been followed. There is an obvious advantage in such a grouping whenever a defective character is concerned, one that may cause a lower viability or germination of the individuals possessing it. Under such circumstances it is not only legitimate but essential to group a normal and a defective character into one class. In this particular case both the parental and non-parental classes comprise a green and an albino type.

Thus, in calculating the deviations from a dihybrid distribution the 9- and 1-classes of the F_2 phenotypes are grouped against the two 3-classes, giving, with independent inheritance, a 10 : 6 ratio. With various degrees of linkage this 10 : 6 ratio is modified. The probable error is that calculated on such a modified basis.

The same procedure is followed for a trihybrid and for a tetrahybrid. With complementary action of two chlorophyll genes plus an endosperm gene, the trihybrid situation becomes a modified form of the 27 : 9 : 21 : 7 ratio. When the parental and non-parental classes of phenotypes are grouped this ratio becomes a modified 34 : 30, the modification being dependent on the degree of linkage. The tetrahybrid situation (in this case due to $Yy W_1w_1 W_2w_2 W_3w_3$) becomes an 81 : 27 : 111 : 37 phenotypic ratio which, when grouped according to parental and non-parental types, becomes a modified 118 : 138 ratio.

These relationships may be stated for this problem in general terms, involving the gametic ratio ($r : s$), as follows:

	<i>Parental types</i>	<i>Non-parental types</i>
Dihybrid	$4r^2 + 2(2rs + s^2)$	$2(2rs + s^2)$
Trihybrid	$13r^2 + 7(2rs + s^2)$	$3r^2 + 9(2rs + s^2)$
Tetrahybrid	$43r^2 + 25(2rs + s^2)$	$21r^2 + 39(2rs + s^2)$

Accordingly, with any degree of linkage (percentage of crossing over) it merely becomes necessary to determine the gametic ratio, in other words, the values of r and s . In this way the theoretical or expected frequencies may be found and the deviations therefrom calculated.

In regard to the probable errors of such a method of expressing linkage intensities it seems legitimate to use an extension of the ordinary method of calculating probable errors, as is done for 9 : 7 and 27 : 37 ratios that involve complementary interaction of factors. Such probable errors are based on the general formula, $.6745\sqrt{p \times q \times n}$, in which p and q are the elements of the ratio whose sum totals 1.000. With this particular formula for the probable error the actual deviations are calculated from the expected frequency expressed in terms of absolute numbers.

Probable-error formulae under such circumstances may be written as follows:

$$\begin{aligned} P. E._{10:6} &= .6745\sqrt{[4r^2 + 2(2rs + s^2)] \times [2(2rs + s^2)] \times n} \\ P. E._{34:30} &= .6745\sqrt{[13r^2 + 7(2rs + s^2)] \times [3r^2 + 9(2rs + s^2)] \times n} \\ P. E._{118:138} &= .6745\sqrt{[43r^2 + 25(2rs + s^2)] \times [21r^2 + 39(2rs + s^2)] \times n} \end{aligned}$$

In case of the W_1Y linkage, for example, the data fit very well the hypothesis of 35 percent crossing over between YW_1 and yw_1 (see table 5). With this amount of crossing over, the gametic ratio ($r : s$) is 1.8571 : 1. In this cross we are dealing with the 'coupling' phase of the linkage, since Y and W_1 came from one parent.

The probable errors for the three sorts of distributions in table 5 with 35 percent crossing over have been calculated as follows:

$$\begin{aligned} P. E. \text{ of dihybrid} &= .6745 \sqrt{.711 \times .289 \times n} = \sqrt{.0935n} \\ P. E. \text{ of trihybrid} &= .6745 \sqrt{.596 \times .404 \times n} = \sqrt{.1095n} \\ P. E. \text{ of tetrahybrid} &= .6745 \sqrt{.509 \times .491 \times n} = \sqrt{.1137n} \end{aligned}$$

An inspection of the data in table 5 shows clearly that the three distributions therein agree remarkably well with the expected results based on 35 percent crossing over between W_1 and Y . The largest deviation is

only 1.5 times as large as its probable error. If the data are compared with the expected results with independent inheritance, the deviations show beyond doubt that independent inheritance of W_1 and Y is impossible. The deviation in the case of the dihybrid involving these two factors proves, for example, to be eleven times larger than its probable error.

A glance at the individual progenies from which the summary in table 5 has been arranged, leaves no uncertainty as to their similarity (see table 11). Accordingly, there should be no hesitation in stating that W_1 and Y belong in the same linkage group, a group which already has three other factors, making a total of five factors, $Y-P_l-S_m-F_i-W_1$.³ Incidentally, STROMAN (1924) has reported that W_1 and P_l show linkage, but the data are very limited.

In order to present a complete record of this experiment it is necessary to give the data for those progenies showing no linkage between Y and the albino genes (in this case w_2 and w_3). Such a record is presented in table 12. Here there are 13 progenies segregating for albino seedlings, 6 on a 3 : 1 basis and 7 on a 9 : 7 basis. Obviously there would be no 27 : 37 ratios in this table. The data in table 12 agree very well with a theoretical distribution based on independent inheritance between Y and either W_2 or W_3 .

Linkage of W_2 and R

This is a new linkage whose details have not as yet been reported. Cross No. 3 carried the R factor for aleurone development as follows:

F_1 genotype $RW_2w_1W_3.rw_2W_1w_3$ (AA CC P_rP_r ii)

In this cross there were 37 selfed F_1 ears that came from purple F_1 grains of the crossed ear. In other words there were 37 F_2 distributions of 3 purple : 1 colorless. Of these, 5 did not segregate for albino seedlings; 11 gave 3 : 1 ratios; 14 gave 9 : 7 ratios; and 7 gave 27 : 37 ratios of green to albino seedlings. Accordingly there will be dihybrid, trihybrid and tetrahybrid F_2 generations involving aleurone color (Rr) and the various chlorophyll factors.

The detailed progenies of these three sorts are listed in two tables. In table 13 are those progenies showing a linkage between the aleurone

³ Since this paper was written an article by DEMEREC (1923) has appeared, adding two other albino genes, W_5 and W_6 . Each of these shows about 25 percent crossing over with Y , and apparently are located on either side of this endosperm factor. Thus, this group has in reality seven different genes, since it is not likely that W_1 is identical with either W_5 or W_6 , their linkages with Y rendering this impossible in all likelihood.

and chlorophyll factors. In table 14 are the progenies that showed no linkage, obviously because W_2 was not concerned.

In table 13 there are 3 progenies segregating for 3 greens : 1 albino that show an unmistakable linkage. In addition there are 10 progenies with 9 greens : 7 albinos, and 7 that give 27 : 37 ratios on chlorophyll. A summary of these 20 progenies has been arranged in table 6.

TABLE 6

Summary of F_2 data showing linkage between W_2 and R giving 15.4 percent crossing over. Cross No. 3. Details in table 13.

GENOTYPE OF F_1 MOTHER PLANT	PARENTAL TYPES RW AND rw		NON-PARENTAL TYPES Rw AND rW		Dev. P. E
	Observed	Expected	Observed	Expected	
$RW_2 \cdot rw_2$	805	824	155	136	2.6
$RW_2W_1 \cdot rw_2W_1$ or $RW_2W_3 \cdot rw_2w_3$ } ...	1769	1735	689	723	2.2
$RW_2W_1W_3 \cdot rw_2W_1w_3$	1015	1001	676	690	1.0

In table 6 the same procedure has been followed, namely, of grouping the parental against the non-parental combinations. From this grouping the deviations and probable errors have been calculated exactly as was done in the case of the W_1 - Y data.

In this R - W_2 situation there is somewhat more variability in the progenies. Most of this is probably due to the fact that not only is W_2 linked with R , but also with a defective type of endosperm. This linkage between W_2 and D_1 has been reported in a previous paper (LINDSTROM 1923). A situation in which two lethal factors (one for endosperm and the other for chlorophyll) are completely linked is certain to disturb the viability of that class more than of the other. Notwithstanding this difficulty, the data in table 13 and table 6 fit a hypothesis of 15.4 percent crossing over between W_2 and R reasonably well.

In determining the goodness of fit of these data, the probable errors were calculated with a gametic ratio of 5.5 : 1, which gives 15.4 percent breaks in the linkage. These probable errors, which are based on modifications of the 10 : 6, 34 : 30 and 118 : 138 ratios, were determined to be:

$$\text{P. E. of dihybrid} = .6745 \sqrt{.858 \times .142 \times n} = \sqrt{.0554n}$$

$$\text{P. E. of trihybrid} = .6745 \sqrt{.706 \times .294 \times n} = \sqrt{.0944n}$$

$$\text{P. E. of tetrahybrid} = .6745 \sqrt{.592 \times .408 \times n} = \sqrt{.1099n}$$

Using this method on the data in table 6, it will be seen that, while the deviations are not beyond the limits of a reasonable fit, they are somewhat larger than they should be ideally. As a matter of fact the poorest fit occurs in the 3 : 1 progenies ($RW_2.rw_2$) and in this case the divergence from the expected can be attributed to one of the three progenies comprising this total. The F_1 ear No. 210 is the major cause of the poor fit. Whether its deviations are due to pollen contamination (the aleurone ratio is somewhat abnormal) or to errors of random sampling it is impossible to determine. Inasmuch as all the other progenies are within reasonable limits of the hypothesis we may perhaps believe that a deviation 2.6 times as large as its probable error should be expected occasionally.

In order to give the complete data on the relation between aleurone and chlorophyll factors in cross No. 3, the 12 progenies showing no linkage with R have been tabulated in table 14. In this case the progenies involve the factors W_1 and W_3 which are not linked with R . There is no semblance of linkage among these 12 progenies.

Additional data on the W_2 - R linkage were afforded in cross No. 1 which carried the following factors: $w_1W_2RAC.W_1w_2rAc$. Some of the F_2 ears of this cross possessed aleurone ratios of 9 purple : 7 colorless, and in addition some segregated for 3 : 1 and others for 9 : 7 chlorophyll ratios. Thirteen such progenies have been listed in table 7.

There are in this table, 8 progenies from triheterozygous F_1 plants of the composition $W_2RC.w_2rc$, and 5 progenies from F_1 plants of the genotype $w_1W_2RC.W_1w_2rc$. With linkage between W_2 and R on a basis of 15.4 percent crossing over, some rather peculiar ratios are observed. These, however, agree very well with the linkage hypothesis, the deviations being within reasonable limits. For example, a total of the 3 : 1 progenies (on chlorophyll) gives 675 of the parental classes and 276 of the non-parental, where 671 and 280, respectively, are expected. This is a deviation of only 4. The 9 : 7 progenies (on chlorophyll) total 399 of the parental classes and 228 of the non-parental, where 401 and 226 are expected, a deviation of only 2. These small deviations obviously indicate excellent agreement with the hypothesis.

Accordingly, from a total of 6687 individuals grown from two separate crosses (Nos. 1 and 3) involving the linkage between W_2 and R , it can be stated with reasonable certainty that these two factors are fairly closely linked. The data in both crosses give a crossover percentage of 15.4 with which there is good agreement.

It is worth noting that the aleurone factor involved in this linkage has been identified as R mainly because of the seedling stem-color determina-

TABLE 7

Individual F₂ seedling progenies showing linkage between R aleurone and W₂ chlorophyll factors when aleurone ratio is 9 : 7 (AA Cc Rr). Cross No. 1.

F ₁ EAR NUMBER	FROM PURPLE SEEDS		FROM COLORLESS SEEDS		CHLOROPHYLL RATIO	DEV. P. E.
	Green	Albino	Green	Albino		
2	84	3	35	34	3 : 1	
6	69	3	26	14	3 : 1	
14	52	2	27	14	3 : 1	
16	59	3	30	18	3 : 1	
21	49	5	33	14	3 : 1	
26	65	3	34	29	3 : 1	
27	72	11	32	27	3 : 1	
28	54	5	24	21	3 : 1	
Total	504	35	241	171		0.4*
Expected	484	51	229	187		
8	51	21	24	24	9 : 7	
9	51	32	25	41	9 : 7	
13	43	24	21	25	9 : 7	
23	54	19	24	33	9 : 7	
24	50	13	25	27	9 : 7	
Total	249	109	119	150		0.3†
Expected	240	113	113	161		

* Calculated on 27 : 9 : 21 : 7 or 34 : 30 basis, modified by linkage.

† Calculated on modified 81 : 63 : 63 : 49 ratio, or 130 : 126 basis.

tions that are characteristic of the multiple allelomorphous situation R^r , R^g , r^r , r^g (EMERSON 1921). All of the data show linkage with seedling stem-color (red and non-red) whenever w_2 is present. In cross No. 3 there was little or no mottling of aleurone when R was carried over from the male parent, probably because of the S factor (KEMPTON 1919).

Incidentally it might be stated that a large amount of data is already at hand verifying this linkage from another angle. In this case W_2 has been found to be linked with the *luteus* factor l (involved in the development of the xanthophyll and carotinoid pigments of maize). The details of this linkage are reserved for a future report.

Thus, the linkage group R - L - G has the proven addition, W_2 . To these four factors must be added another. This is the recessive endosperm defect (LINDSTROM 1923) that is completely (or almost so) linked with W_2 . The genetic symbol for this defective endosperm character may be given as d_f , referring not only to its defective nature but also to the fact that it originated in flint corn.

Two more factors have been suggested as belonging to this group, namely *S* (spotted aleurone) and *L_i* (lineate leaves). The complete group, accordingly, has a total of seven genetic factors, *R-L-W₂-G-D_f-S-L_i*. Gene *W₂* has been located with respect to *R* and *L* but not to *G*.

Gene *W₃* independent of *W₁*, *W₂*, *C* and *L_g*

This third complementary factor for chlorophyll development has not as yet been placed in any of the seven (possibly eight) known linkage groups of maize. Apparently it does not belong in the *Y-P₁-W₁-S_m* group nor in the *R-L-W₂-G-D_f-S-L_i* group, as shown by the preceding experiments.

The following data (table 8) taken from cross No. 2 indicate that it is not related to the *L_g-B-T_e* group. In this case *W₃* has been tested with the liguleless factor *l_g*. In neither the 9 : 3 : 3 : 1 nor the 27 : 9 : 21 : 7

TABLE 8

Showing the independent inheritance of *W₃* and *L_g* in *F₂* progenies in cross No. 2 From *F₁* genotypes *W₃l_g · w₃L_g* and *W₃w₁l_g · w₃W₁L_g*.

PEDIGREE NUMBER	CHLOROPHYLL RATIO	GREEN		ALBINO		DEV. P. E.
		<i>L_g</i>	<i>l_g</i>	<i>L_g</i>	<i>l_g</i>	
123	3 : 1	97	27	26	7	
151	3 : 1	62	22	19	9	
152	3 : 1	76	32	19	11	
158	3 : 1	85	35	20	10	
165	3 : 1	71	26	34	10	
Total		391	142	118	47	.02*
Expected		393	131	131	44	
111	9 : 7	85	32	73	12	
131	9 : 7	60	25	45	10	
134	9 : 7	31	11	24	6	
140	9 : 7	128	47	84	31	
141	9 : 7	91	33	101	30	
146	9 : 7	29	15	30	13	
161	9 : 7	67	16	56	19	
Total		491	179	413	121	2.4†
Expected		508	169	395	132	

* Calculated on 10 : 6 basis.

† Calculated on 34 : 30 basis.

distributions of table 8 is there any real indication that a linkage exists between *W₃* and *L_g*. Both distributions show a reasonably good fit with

those expected when independent inheritance between these two factor pairs is assumed.⁴

Evidence for the independent inheritance of W_3 and C is also afforded in cross No. 2. Six F_1 ears of this cross possessed the genotype W_3w_1ACR . w_3W_1Acr . With this tetrahybrid situation there should be in F_2 a ratio of 81 green seedlings from purple seeds : 63 whites from purple seeds : 63 greens from colorless seed : 49 whites from colorless seed. The 6 F_2 distributions (Nos. 111, 115, 119, 122, 131, and 134) gave a total ratio of 278 : 178 : 208 : 143 of the classes noted above. This results in 421 individuals with the parental combinations (W_3C and w_3c) and in 386 individuals with the recombination or non-parental classes (W_3c and w_3C). With independent inheritance between W_3 and C , the expected frequencies are 410 and 397, respectively. The deviation of 11 proves to be only 0.9 as great as the probable error. Accordingly, genes W_3 and C are not linked.

DISCUSSION

In the discovery of a 27 : 37 ratio with seedling chlorophyll characters in maize, a second case of complementary factorial interaction becomes known. EMERSON (1918) reported the first occurrence of such a ratio with aleurone color. In this situation genes A , C and R interact to give 27 colored : 37 colorless aleurone grains. While it may be premature to speculate on the meaning of various factorial interactions in any species, it is, nevertheless, interesting to note that in maize both complementary and supplementary action of genetic factors are known. The latter type of factorial behavior occurs with the duplicate genes for zigzag stalks and very likely with duplicate genes for yellow endosperm, giving the characteristic 15 : 1 ratio.

Some one has suggested that the 15 : 1 and 63 : 1 ratios, common in the small grains like wheat and oats, are perhaps indicative of an evolutionary origin by chromosome duplication. May it be that 9 : 7 and 27 : 37 ratios, when they become numerous in any species, indicate a different sort of evolutionary origin, at least for the chromosomes thus involved? Not until the genetic analysis of a species becomes better known, however, can we attempt any such generalizations. At the present time it seems more likely that complementary action of genetic factors is merely characteristic of a certain type of chemical activity within the hereditary complex of the species.

⁴ Gene W_3 in this material may be identical with the W_a factor reported by DEMEREC (1923). Until the exact linkage relations are determined this must rest as a pure assumption.

It is gradually being demonstrated that genetic factors for chlorophyll development in maize are to be found in most, if not all, of the known linkage groups. In this report, such a simple phase of the general chlorophyll situation as that of seedling albinism, involved three independent genes. To these must be added at least five other genes for albinism as reported recently by DEMEREC (1923). Accordingly, eight different genetic factors for albinism in maize are known, and before long several more will be added to this list. At least five of these known albino genes are in different chromosomes.

When we consider that a host of other similar factors, as those for virescent-white (several different factors), pale green, yellow, lineate, golden, green-striped, yellow-striped, fine-streaked, fine-striped, *japonica*, white-sheath, zebra and other recessive chlorophyll defects also seem to be located in various chromosomes, it soon becomes evident that all of the maize chromosomes are presumably carrying genes whose most striking expression occurs in the pigment chlorophyll. Accordingly, all of their allelomorphs apparently function together in the genetic development of this pigment. Thus, there are known at present not less than 24 different pairs of factors for chlorophyll development in maize, and the end is not yet in sight.

The same general situation is found in characters like anthocyanin pigment (including aleurone and plant colors), in the various growth characteristics (especially those involving dwarfishness of the plant), and also in the case of endosperm development as is evidenced by the multitude of genes responsible for hereditary defects of the endosperm.

Is it possible that each maize chromosome carries a general determiner for each of the characteristics of the plant noted above, and that these numerous, recessive, mutant genes have merely modified these main centers of activity in the chromosomes? This is not likely when we note that occasionally two recessive chlorophyll defects, for example, show linkage, indicating that they are borne on the same chromosome and probably some distance apart.

It is of course conceivable that the genes which we consider as being responsible for chlorophyll, aleurone or endosperm characters are not all directly nor solely related to these characters, but are rather general chemical determiners whose effect is more or less general. It is difficult to conceive of such a possibility, however, since among the multitude of recessive genes that we already know there are few, if any, that have a second, known, genetic effect. True, there is the occasional complete linkage, so-called, of recessive defects, such as that involving w_2 and d_1 , the endosperm defect. But obviously more of such cases would be apparent

if mutant genes had a very general effect on the genetic development of maize. For this reason we are inclined to believe that the numerous chlorophyll, aleurone, and endosperm genes are perhaps specific in nature, although they may have a general physiological effect on the organism. It is also evident that these genes appear to be scattered throughout all of the chromosomes in maize, sometimes several that affect the same character occurring in one chromosome. Eventually, however, we may be driven to the conclusion that each normal chromosome holds centers of genetic activity for all the hereditary characters involved in Mendelian inheritance.

SUMMARY

1. Three different genes for complete albinism in maize seedlings were found to be genetically distinct. Their normal allelomorphs act in a complementary manner to cause the development of normal chlorophyll.

2. Seedling ratios of 3:1, 9:7 and 27:37 green to albino plants occur when mono-, di- or triheterozygous green plants, respectively, are self-fertilized.

3. Experimental data on a large scale with the 27:37 ratios derived from green plants of the composition $W_1w_1 W_2w_2 W_3w_3$ show such a close approximation to the theoretical expectancy that these three factor pairs must be independently inherited.

4. Verification for this independent inheritance is afforded by certain linkage relations. Linkage tests prove that W_1 belongs in the $Y-P_1-S_m$ group since Y and W_1 show 35 percent crossing over in 3:1, and also in 9:7 and 27:37 combinations with the other albino genes.

5. Gene W_2 falls in the $R-L-G$ linkage group, since a large series of tests prove that R and W_2 are fairly closely linked, giving 15.4 percent crossing over. This linkage was observed in 3:1, 9:7 and 27:37 chlorophyll ratios.

It is also shown that w_2 is the albino gene that is linked with d_f , a factor for defective endosperm. Apparently this is a case of complete linkage.

6. Gene W_3 is independent of the two linkage groups mentioned above. Data are also presented to show that it belongs neither in the $B-L_e$ linkage group nor in the $C-I-S_h-W_x$ group.

7. Probable-error methods for use with F_2 distributions involving linkage are described.

LITERATURE CITED

- DEMEREK, M., 1923 Inheritance of white seedlings in maize. *Genetics* 8: 561-593.
 EMERSON, R. A., 1918 A fifth pair of factors, Aa , for aleurone color in maize, and its relation to the Cc and Rr pairs. *Cornell Univ. Agric. Exp. Sta. Mem.* 16: 225-289.
 1921 The genetic relations of plant colors in maize. *Cornell Univ. Agric. Exp. Sta. Mem.* 39: 1-156.

KEMPTON, J. H., 1917 A correlation between endosperm color and albinism in maize. Jour. Washington Acad. Sci. 7: 146-149.

1919 Inheritance of spotted aleurone color in hybrids of Chinese maize. Genetics 4: 261-274.

LINDSTROM, E. W., 1918 Chlorophyll inheritance in maize. Cornell Univ. Agric. Exp. Sta. Mem. 13: 1-68.

1921 Concerning the inheritance of green and yellow pigments in maize seedlings. Genetics 6: 91-110.

1923 Heritable characters of maize. XIII. Endosperm defects—sweet defective and flint defective. Jour. Heredity 14: 127-135.

STROMAN, G. N., 1924 Genetic relations of chlorophyll and anthocyanin seedling characters in maize. Genetics 9: 91-123.

APPENDIX—Tables 9 to 14

TABLE 9

The 53 F₂ seedling progenies of cross No. 1—W₁w₁ W₂W₂ × W₁W₁ W₂w₂.

F ₁ EAR NUMBER	ALL GREEN	F ₁ EAR NUMBER	3 GREEN : 1 ALBINO		F ₁ EAR NUMBER	9 GREEN : 7 ALBINO	
4	99	1	114	32	8	75	45
7	44	2	119	37	9	76	73
12	39	3	120	44	13	64	49
15	36	5	87	21	23	78	52
19	132	6	95	17	24	75	40
20	113	10	77	33	32	50	34
36	36	11	118	32	33	64	44
38	29	14	79	16	34	84	54
43	38	16	89	21	35	50	45
45	135	17	64	28	39	59	39
46	117	21	82	19	41	49	46
47	121	22	75	32	44	61	43
		25	95	36	49	71	56
		26	99	32	51	73	60
		27	107	38			
		28	76	26			
		30	84	20			
		31	208	72			
		37	102	38			
		40	63	13			
		42	111	33			
		48	109	40			
		50	107	32			
		52	96	32			
		53	80	31			
		54	93	30			
		55	88	32			
Total	939		2637	837		929	680
Expected	939		2605	869		905	704
Dev.							
P. E.	0			1.9			1.8

TABLE 10

The 66 F₂ seedling progenies of cross No. 2—W₁w₁ W₃W₃×W₁W₁ W₃w₃.

F ₁ EAR NUMBER	ALL GREEN	F ₁ EAR NUMBER	3 GREEN : 1 ALBINO		F ₁ EAR NUMBER	9 GREEN : 7 ALBINO	
101	64	102	48	17	111	109	79
104	93	103	106	34	115	69	39
108	78	105	175	63	119	105	64
114	150	106	45	9	122	76	54
116	104	107	111	38	131	85	55
117	129	109	135	56	134	42	30
118	117	110	128	44	140	165	117
120	164	112	94	31	141	124	131
121	146	113	135	42	146	44	43
125	142	123	124	33	157	79	71
129	114	124	123	45	161	82	75
130	130	126	102	28	164	57	47
132	90	127	105	38	166	70	57
133	112	128	81	31			
135	58	138	213	65			
136	75	142	164	48			
137	81	144	96	28			
139	74	145	186	59			
143	65	148	87	26			
147	134	149	105	40			
150	110	151	85	28			
153	131	152	107	30			
163	151	154	109	37			
		155	118	41			
		156	112	39			
		158	121	31			
		159	112	40			
		160	91	33			
		162	91	33			
		165	97	44			
Total	2512		3406	1131		1107	862
Expected	2512		3403	1134		1108	861
Dev.							
P. E.	0		0.2			0.1	

TABLE 11

Individual F_2 seedling distributions with respect to endosperm and chlorophyll factors, showing linkage between factors Y and W_1 . Cross No. 3.

F ₁ EAR NUMBER	CHLOROPHYLL RATIO	FROM YELLOW SEEDS		FROM WHITE SEEDS	
		Green	Albino	Green	Albino
239	3 : 1	208	54	57	29
241	3 : 1	142	28	32	32
253	3 : 1	239	65	58	54
258	3 : 1	79	19	22	14
260	3 : 1	109	15	22	20
269	3 : 1	87	22	27	16
271	3 : 1	79	19	23	15
272	3 : 1	77	15	18	11
Total		1020	237	259	181
242	9 : 7	143	92	36	40
245	9 : 7	112	75	36	36
246	9 : 7	60	45	15	22
247	9 : 7	71	52	25	25
250	9 : 7	77	46	16	18
252	9 : 7	67	48	17	26
256	9 : 7	74	26	17	18
Total		604	384	162	185
248	27 : 37	36	29	6	15
261	27 : 37	92	113	20	43
268	27 : 37	76	70	11	40
273	27 : 37	90	114	27	43
Total		294	326	64	141

TABLE 12

Individual F₂ seedling distributions with respect to Y endosperm and W₂ and W₃ chlorophyll factors showing no linkage. Cross No 3.

F ₁ EAR NUMBER	CHLOROPHYLL RATIO	FROM YELLOW SEEDS		FROM WHITE SEEDS		DEV. P. E.
		Green	Albino	Green	Albino	
240	3 : 1	128	48	43	17	
254	3 : 1	114	26	42	12	
259	3 : 1	103	32	34	10	
262	3 : 1	120	41	44	14	
266	3 : 1	83	31	28	10	
267	3 : 1	59	22	29	7	
Total		607	200	220	70	0.8*
Expected		617	205	205	69	
243	9 : 7	88	90	35	28	
244	9 : 7	126	105	59	34	
251	9 : 7	106	84	34	26	
255	9 : 7	56	42	23	11	
263	9 : 7	151	102	28	32	
265	9 : 7	191	123	38	43	
270	9 : 7	67	42	35	22	
Total		785	588	252	196	1.0†
Expected		768	598	256	199	

* Calculated on basis of 10 : 6 ratio.

† Calculated on basis of 34 : 30 ratio.

TABLE 13

Individual F₂ seedling distributions with respect to aleurone and chlorophyll factors showing linkage between factors R and W₂. Cross No. 3.

F ₁ EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS	
		Green	Albino	Green	Albino
210	3 : 1	221	22	40	59
224	3 : 1	185	24	16	37
232	3 : 1	242	28	25	61
Total		648	74	81	157
202	9 : 7	105	36	16	39
203	9 : 7	93	33	11	29
204	9 : 7	105	43	11	48
206	9 : 7	121	80	15	46
208	9 : 7	144	53	15	60

TABLE 13 (continued)

F ₁ EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS	
		Green	Albino	Green	Albino
212	9 : 7	132	60	16	43
214	9 : 7	110	40	13	36
221	9 : 7	114	39	15	44
235	9 : 7	153	72	20	62
236	9 : 7	202	82	14	83
Total		1279	543	146	490
209	27 : 37	80	80	10	55
213	27 : 37	69	79	5	44
215	27 : 37	61	54	6	26
216	27 : 37	60	46	6	33
219	27 : 37	118	115	9	69
230	27 : 37	112	111	21	52
237	27 : 37	169	124	10	67
Total		669	609	67	346

TABLE 14

Individual F₂ seedling distributions with respect to R aleurone and chlorophyll factors showing no linkage. Cross No. 3.

F ₁ EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS		DEV. P. E.
		Green	Albino	Green	Albino	
205	3 : 1	128	42	40	11	
207	3 : 1	114	37	45	15	
211	3 : 1	98	23	29	5	
218	3 : 1	69	29	31	14	
223	3 : 1	90	27	30	8	
226	3 : 1	95	26	31	6	
228	3 : 1	150	44	36	18	
229	3 : 1	85	23	31	9	
Total		829	251	273	86	1.3*
Expected		809	270	270	90	
220	9 : 7	74	43	25	20	
222	9 : 7	106	138	56	36	
231	9 : 7	115	82	59	31	
233	9 : 7	89	64	25	29	
Total		384	327	165	116	2.5†
Expected		418	325	140	109	

* Calculated from 10 : 6 ratio.

† Calculated from 34 : 30 ratio.