CONCERNING THE INHERITANCE OF GREEN AND YELLOW PIGMENTS IN MAIZE SEEDLINGS¹

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INTRODUCTION

The inheritance of green and yellow pigments in maize, as demonstrated by the hereditary behavior of certain abnormal chlorophyll-types of seedlings, has been resolved into a fairly simple hypothesis that suggests some interesting relations between these two types of pigments. Green plastid color in the leaves seems to be governed by two pairs of genetic factors and yellow (presumably xanthophyll and carotin) pigment by one. These three pairs of factors appear to be fundamental in the development of these pigments, although there is some evidence of one or two additional factors.

Three general types of abnormal seedlings are common in fields of maize, namely the albino or pure white, the virescent or greenish-white, and the yellow. The first two possess no visible signs of a definite yellow color upon germinating, while the third exhibits a striking lemon-yellow pigment which probably is a mixture of xanthophyll and carotin. The distinction between these three types is remarkably sharp, especially when they are

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grown under uniform conditions in the greenhouse during the winter months. When grown in the field, the distinction between virescent-white and certain types of yellow seedlings is less marked because the virescent type becomes green or yellowish-green very rapidly under field conditions.

All three seedling types are inherited as simple Mendelian recessives to normal green. The interrelations of the three factor-pairs that govern their inheritance have been definitely determined and form the basis for this publication. Their genetic relation to aleurone color is also discussed. Their group relations are verified by two sets of experiments which prove the independent inheritance of these three pairs of factors.

SUMMARY OF PREVIOUS WORK WITH SEEDLING CHLOROPHYLL-FACTORS

White and virescent-white relation ($WwVv$)

The first experiments which established the interrelations between the two seedling factors concerned in chlorophyll development of maize, were reported by MILES (1915). These were the w and v factors, originally named a and b but later changed to fit into the factorial system that is now being followed in the genetical studies with maize (LINDSTROM 1918).

Factor w , when homozygous, entirely prevents the development of normal green chlorophyll, the result being a pure white plant. Such white or albino seedlings are inherited as simple Mendelian recessives to normal green (EAST 1911, EMERSON 1912). Factor v (virescent), when homozygous, has the ability to repress partially or to delay the formation of green pigment. Virescent-white seedlings possess very little green color upon germinating, being almost white, but under optimum conditions of light and temperature they may develop sufficient chlorophyll to mature and set seed. In this respect they differ from the albino seedlings, which die when the food materials in the endosperm are exhausted. This furnishes an unusually distinct means of classifying the two types. When self-pollinated, the virescent-white type produces entirely virescent seedlings.

When a green plant, heterozygous for both factor pairs ($VvWw$), is self-pollinated, a 9:3:4 ratio is obtained as follows:

- 9 VW , green
- 3 vW , virescent-white
- 3 Vw , white
- 1 vw , white

Sufficient data have been accumulated to establish the above interaction of factors and to show that these factors are inherited independently of each other. A summary of 9:3:4 ratios is presented in table 1.

From such a relation we can state that *w* is able to inhibit any green pigment which normally might be produced by the *V* factor or the *v* factor. Put in other words, both *V* and *W* are essential for the development of normal green chlorophyll.

TABLE 1

Summary of data showing 9:3:4 ratio derived from self-pollinated green plants heterozygous for white and virescent-white seedlings (*Ww Vv*).

	GREEN	VIRESCENT-WHITE	WHITE
MILES (1915)	132	36	56
LINDSTROM (1918)	640	190	268
2205- 4	44	11	26
2217-47	109	29	35
-50	102	32	54
-55	86	22	35
-58	78	23	26
-69	89	32	32
2218- 1	80	27	36
-13	54	21	36
-14	73	23	28
2219- 4	65	22	27
-28	59	29	40
2220-12	70	28	29
2670- 1	88	22	13
-11	38	19	22
-22	49	11	30
-25	28	6	11
Total.....	1884	583	804
Theoretical.....	1840	613	818

$$P = 0.26$$

Yellow and virescent-white relation (LlVv)

The next step in the analysis was the determination of the interrelation of the *Vv* and *Ll* factor pairs. Factor *l* (from *luteus*, yellow) is considered as the determiner for a distinct yellow (xanthophyll and carotin) pigment observed in certain maize seedlings (LINDSTROM 1918, plate 1).

When a green plant heterozygous for both virescent-white and yellow seedlings is self-pollinated, a 12:3:1 ratio results as follows:

- 9 *L V*, green
- 3 *l V*, green
- 3 *L v*, virescent-white
- 1 *l v*, yellow (slightly virescent)

A summary of the data to substantiate such a ratio is given in table 2.

The two types of green seedlings *LV* and *IV* have been demonstrated and thoroughly tested genetically. There is no marked phenotypic distinction between them, although there are certainly slight variations among the greens in shade of color.

This genetic hypothesis presupposes that *L* is not absolutely essential for the development of green pigment, as is *V*. What effect this dominant allelomorph *L* really produces is uncertain. A bit of indirect evidence is offered by the color of the striped leaves of the *japonica* type of maize. There are two sorts of *japonica* plants, one with green-and-white-striped leaves, the other with green-and-yellow-striped leaves (see LINDSTROM 1918, plates IV and V). It has been shown that the yellow pigment in the yellow stripes of the latter type is caused by the *l* factor, so that these

TABLE 2

Summary of data showing 12:3:1 ratios derived from self-pollinated green plants heterozygous for virescent-white and yellow seedlings (*LIVv*).

	GREEN	VIRESCENT-WHITE	YELLOW
Table 6 (LINDSTROM 1918)	3583	853	260
Table 8 (LINDSTROM 1918)	3058	767	256
Table 9 (LINDSTROM 1918)	676	190	54
Table 10 (LINDSTROM 1918)	608	155	65
Table 11 (LINDSTROM 1918)	380	88	33
2206-36	169	42	12
2347	637	171	56
2348	204	50	19
Total.....	9315	2316	755
Theoretical.....	9290	2322	774

P = very close fit; $\chi^2 = 0.549$

plants are factorially *jjll*. The *japonica*-white-striped plants, which possess colorless stripes, contain the *L* factor; hence, their genotypic formula is *jjLL*. In this case then, the dominant *L* factor produces no pigment whatsoever. The same might be true in the seedling types *LV* or *Lv*.

The influence of *l* in the *IV* type of green is unknown. If it produces the characteristic yellow pigment there, it would be hidden by the green. Attempts are now being made to differentiate the two types of green (*LV* and *IV*) by spectroscopic tests.

The effect of the virescent factor *v* is seen in both the *Lv* and *lv* genotypes. In the former, the virescent or greening effect is produced on white leaves; while in the latter this effect is seen on yellow leaves, so that they are really virescent-yellow seedlings.

As additional evidence of the interrelations of L and V , data from crosses between two green plants, one $LlVv$ and the other $llVv$ (which when self-pollinated produced 3 greens : 1 yellow), and a reciprocal cross, are presented in table 3.

The close approximation to the unusual 6:1:1 theoretical ratio not only checks the factorial hypothesis but also serves to show that L and V are inherited independently of each other both in the pistillate and in the staminate mechanism for the segregation of factors.

The preceding evidence has shown that W and V are both necessary for normal green color, and that w can inhibit the development of any green pigment. It has also proven that L is not necessary for green color; and that l can produce yellow pigment in the presence of v .

What relation does w bear to l ? If w can inhibit any green pigment produced by v , can it react similarly with l ? The answer is to be found in the subsequent section.

TABLE 3
F₁ seedlings from the cross $LlVv \times llVv$ and its reciprocal.

PEDIGREE NUMBER	GREEN	VIRESCENT-WHITE	YELLOW
2343-22 $\times$ 2206-76	168	25	25
-36 $\times$ 2259-16	126	25	24
2259- 2 $\times$ 2343-22	178	27	32
Total.....	472	77	81
Theoretical (6:1:1)...	472.50	78.75	78.75

TRIHYBRID RELATIONS

P₁, F₁, F₂ and F₃ data of cross $LlVvWW \times LLVvWw$

When the three factor pairs concerned in the development of green chlorophyll and yellow pigment are brought together by hybridization, some interesting relations become apparent. The experimental studies bearing on this situation arose from a cross of two green plants, one producing green, virescent-white, and yellow seedlings when self-pollinated, the other only green and white seedlings. Factorially this cross would be represented as $LlVv \times VVWw$.

All the F_1 plants of this cross were completely green in the seedling and mature-plant stages. Unfortunately only 10 of the 72 green F_1 plants were self-pollinated. Their F_2 seedling progenies have been arranged in table 4.

Since one parent produces four kinds of gametes and the other only two, there should be eight different F_1 genotypes. All the eight types are not

represented among the ten progenies in table 4, which is scarcely to be expected because of the small numbers concerned. Only five of the eight appeared, namely, two kinds of greens (as shown by certain crosses); a type segregating into 3 greens to 1 white (1764-22); another into 9 greens to 3 virescent-whites to 4 whites (1764-30); and finally a new type segregating into all the four seedling types but giving a peculiar ratio of these characters. There were three seedling progenies of this latter sort (1764-4,

TABLE 4
*F₂ seedling progenies from green F₁ plants of the cross LLV_vWW × LLVVWw.
937-14 × 812-1.*

PEDIGREE NUMBER	GREEN	VIRESCENT-WHITE	YELLOW	WHITE
1764- 4	26	4	5	4
- 5	50			
-12	58			
-16	47			
-20	59			
-22	197			61
-24	48			
-30	136	24		46
-40	59	21	15	36
-43	22	4	2	5

TABLE 5
Three F₂ seedling progenies from table 4, which produced all four types of seedlings.

PEDIGREE NUMBER	GREEN	VIRESCENT-WHITE	YELLOW	WHITE
1764- 4	26	4	5	4
-40	59	21	15	36
-43	22	4	2	5
Total.	107	29	22	45
Theoretical (36:9:7:12)...	114	29	22	38

$$P = 0.64$$

-40, -43), and it is with them and their progeny that we shall be concerned in establishing an hypothesis to interpret the interaction of the three factors and their allelomorphs. The F₂ progenies in table 4 do not offer sufficient evidence in themselves simply because their numbers are too small, but they do agree with the hypothesis to be developed, as will be seen later.

From the three lots of the new types of seedling progenies mentioned above, which have been grouped in table 5, has come the critical evidence.

Many of the green plants from each of these three progenies (1764-4, -40, -43) were self-pollinated and the F₃ seedling progenies tested. They are arranged in table 6.

TABLE 6

Showing F_2 seedling progenies from self-pollinated green F_2 plants of table 5.

F_2 GENOTYPES	PEDIGREE NUMBER	F_2 SEEDLING TYPES				CLOSENESS OF FIT		
		Green	Virescent-white	White	Yellow	Dev. P. E.	χ^2	P
1 $LLVVWW$	2205- 1	64						
	-16	71						
2 $LIVVWW$	2206-21	91						
	-54	100						
1 $llVVWW$	2207-10	96						
Total		422						
2 $LLVvWW$	2206-37	74	22					
Theoretical . . .		72	24			0.7		
2 $LLVVWw$	2205-10	224		56				
Theoretical . . .		210		70		2.9		
2 $llVVWW$	2205-11	91			31			
2 $llVVWw$	2207-12	73			24			
Total		164			56			
Theoretical . . .		165			55	0.2		
4 $llVvWw$	2205-14	47			35			
	2206-23	45			29			
	-29	70			60			
	-31	66			42			
	-75	131			78			
Total		359			244			
Theoretical . . .		339			264	2.7		
4 $LIVvWW$	2206-36	169	42		12			
Theoretical . . .		167	42		14		0.31	
4 $LLVvWw$	2205- 4	44	11	26				
Theoretical . . .		46	15	20				0.23
4 $LIVVWw$	2205-12	54		12	4			
	2206-73	150		34	11			
	-84	56		13	12			
Total		260		59	27			
Theoretical . . .		259		65	22			0.44
8 $LIVvWw$	2206- 2	63	18	26	15			
	-15	172	27	42	32			
	-46	166	28	63	31			
	-57	176	50	63	41			
	-71	78	16	21	10			
	2207-23	26	11	13	6			
Total		681	150	228	135			
Theoretical . . .		672	168	224	131			0.53

Hypothesis for factorial interactions of the three factors

From the data in tables 5 and 6 the following hypothesis has been derived. This hypothesis has four general points, namely:

1. That three pairs of factors (Ll , Vv , Ww) are concerned in the development of the green and yellow pigments in the maize seedlings of this experiment.
2. That these three factor-pairs undergo ordinary Mendelian segregation and recombination.
3. That they are inherited independently of each other, not linked.
4. That they exhibit certain constant interrelations.

It is only the fourth point that needs explanation. Assuming three pairs of factors with complete dominance, there are only eight possible combinations which might give different phenotypic characters, namely, LVW , LVw , LvW , Lvw , lVW , lVw , lvW , and lvw . If a green plant heterozygous for all three is self-pollinated, they would occur in the ordinary 27:9:9:3:9:3:3:1 proportion, respectively.

It will be noted that only four different phenotypes are recorded in table 5, namely green, virescent-white, yellow, and white. The distinction between these four classes of seedlings was remarkably clear. Practically all of the data were recorded before the present hypothesis was evolved, and no difficulty in classifying the material was experienced.

To account for the unusual ratio observed in the progenies of tables 5 and 6, which were segregating for the four seedling characters, the following interactions between the three pairs of factors was assumed:

27 LVW , green seedlings	3 Lvw , white seedlings
9 lVW , green seedlings	3 lVw , yellow seedlings
9 LvW , virescent-white seedlings	3 lvW , yellow seedlings
9 LVw , white seedlings	1 lvw , yellow seedling

This arrangement results in a 36:9:7:12 ratio of green : virescent-white : yellow : white. Among the F_1 genotypes of the original cross ($LlVvWW \times LLVVWw$) there should be one of the factorial composition $LlVvWw$. According to the hypothesis this should produce a seedling progeny segregating into the 36:9:7:12 ratio. Three such progenies appear in table 5, while in the last section of table 6 there are six progenies (2206-2, -15, -46, -57, -71 and 2207-23) of the same sort. The actual data in both tables are well in accord with the hypothesis, the value for P in table 5 being 0.64 and in table 6, 0.53.

Not only are the hypothetical factor-interrelations substantiated but also the independent inheritance of the three factor-pairs is reasonably assured. However, further evidence on this appears later.

Before discussing the various functions of the individual factors, it is necessary to show that the hypothesis is verified in the F_3 generation. For this purpose the reader is again directed to table 6, which has been compiled from the data obtained by self-pollinating twenty-five of the F_2 green

TABLE 7

Showing the theoretical and actual proportions of the F_3 progenies of table 6.

F_2 GENOTYPES	F_3 SEGREGATION OF SEEDLINGS	FOUND	EXPECTED	DEVIATION
1 $LLVVWW$ 2 $LlVVWW$ 1 $llVVWW$	All green	5	3	+2
2 LLV_vWW	3 green 1 virescent-white	1	1	0
2 $LLVVWw$	3 green 1 white	1	1	0
2 llV_vWW 2 $llVVWw$	3 green 1 yellow	2	3	-1
4 llV_vWw	9 green 7 yellow	5	3	+2
4 LlV_vWW	12 green 3 virescent-white 1 yellow	1	3	-2
4 LLV_vWw	9 green 3 virescent-white 4 white	1	3	-2
4 $LlVVWw$	12 green 3 white 1 yellow	3	3	0
8 LlV_vWw	36 green 9 virescent-white 7 yellow 12 white	6	5	+1

$$P = 0.66$$

plants occurring in the 36:9:7:12 distribution listed in table 5. According to the hypothesis there should be nine different types of F_3 segregations from such F_2 plants, and they should occur in definite proportions. These types, their actual proportions, and their theoretical expectancies are arranged in table 7.

It will be noted that every one of the theoretical nine classes is represented in the F_3 generation and that the expected proportions of the classes are unusually well realized, the closeness of fit in this case being very good, since P has a value of 0.66.

A glance at the individual F_3 progenies in table 6 will show that in the great majority of cases, they, themselves, conform very closely to the theoretical proportions. In no case is there any serious deviation from the demands of the hypothesis. Thus the hypothesis is fully verified by the F_3 data.

TABLE 8

Seedling progenies from self-pollinated green plants of a 9:7 ratio of green: yellow seedlings. $UVvWw$.

PEDIGREE NUMBER	ALL GREEN	3:1 RATIO		9:7 RATIO		Dev. P. E.
		Green	Yellow	Green	Yellow	
2455- 1		135	60			2.7
- 6				47	48	1.8
- 7				105	93	1.3
- 8				26	19	0.4
-18				131	89	1.4
-21				96	72	0.3
-23		62	15			1.6
-27		240	93			1.8
-28				83	69	0.6
-30				106	90	0.9
2456- 4	72					
Total.....	72	437	168	594	480	
Theoretical.....	72	454	151	604	470	

Among the F_3 progenies in table 6 are several new types of ratios, notably the 9 green to 7 yellow; the 12 green to 3 white to 1 yellow; and the 36 green to 9 virescent-white to 7 yellow to 12 white. The first and third types have been analyzed, the second has not. A 9:7 ratio of green to yellow seedlings was tested further by self-pollinating eleven of the green plants in such a distribution. In table 8 the seedling progenies of these eleven plants are recorded with the view of substantiating the hypothesis.

Three kinds of progenies appear, one producing nothing but green seedlings, another giving approximately 3 green to 1 yellow, and a third repeating the 9:7 ratio of the parental stock. The proportion of the three sorts is 1:3:7 respectively.

If the hypothesis given above is correct, the 9:7 ratio of the parents was produced by a plant of the composition $UVvWw$. The 9:7 ratio would result from the following interaction of factors:

1 $llVVWW$, green	1 $llVVww$, yellow
2 $llVVWw$, green	2 $llVvww$, yellow
2 $llVvWW$, green	1 $llvvWW$, yellow
4 $llVvWw$, green	2 $llvvWw$, yellow
	1 $llvvww$, yellow

The green plants should produce three kinds of progenies in the ratio of 1:4:4. The actual proportion of 1:3:7 is fairly well in accord with this, and the individual progenies of table 8 are reasonably close to expectation, thus providing another check on the factorial hypothesis.

Another very interesting and critical bit of evidence on the factorial interrelations of the seedling chlorophyll-factors was afforded by a detailed study of the yellow seedlings. It was noted that these were actually of two types. One was strikingly pure yellow in color while the other had slight traces of green pigment, especially at the ligule and leaf tips, but not enough to hide the yellow pigment. The two types were not differentiated until most of the material used in these experiments had been grown and destroyed, hence all the yellows are classed together in the preceding tables.

TABLE 9

Classification of yellow seedlings according to presence and absence of virescent pigment.

PEDIGREE NUMBER	PURE YELLOW lVw AND lvw	VIRESCENT-YELLOW lvW
2455- 6	5	4
-18	20	15
2205-14	12	13
2206-15	11	12
-23	10	9
-29	25	21
-31	16	17
-46	10	8
-57	20	17
-71	4	3
-75	26	14
2207-23	4	2
Total.....	163	135
Theoretical (4:3).....	170	128

Later plantings of some of the remaining seed were made, however, and the pure yellows were classified separately from those with traces of green. These data are placed in table 9. The pedigree numbers therein can be traced directly to tables 6 and 8.

A total of twelve progenies which possessed yellow seedlings shows that there are slightly more of the pure yellow type than of the green-yellow or virescent-yellow. In fact they occur in almost a 4:3 ratio, respectively. The explanation of this proportion is as follows.

Factorially there are three, and only three, types of yellow seedlings; lVw , lvw and lvW ; and in an F_2 generation they should occur in a ratio of 3:1:3 respectively. All three types develop yellow pigment because of the l factor. The types lVw and lvw possess no green pigment since w inhibits any green color produced by either V or v . Hence, these two types are pure yellow. The third type lvW possesses the v factor which permits a partial development of green pigment (virescent), and since w is not present this virescent or green pigment is not inhibited. Therefore this third type is really a virescent-yellow seedling, differing from the virescent-white type in that the latter shows the virescent or partial green pigment on whitish leaves, the former on yellow leaves.

The 3:1:3 ratio noted above can then be grouped into a 4:3 proportion of pure yellow to virescent-yellow seedlings. The data in table 9 agree with such an interpretation reasonably well, the deviation being only 1.2 times the probable error.

With such checks as these, the F_2 and F_3 data offer beyond any reasonable doubt a satisfactory confirmation of the hypothesis to account for the inheritance of the green and yellow pigments in maize seedlings. It must not be inferred, however, that the L , V , and W factors are the only ones to be found. Presumably they are only some of the more fundamental ones. The writer has discovered two others in material from other sources but their interrelations with L , V and W are not as yet determined.

GROUP RELATIONS OF THE SEEDLING FACTORS

Independence of L , V and W proven by F_2 and F_3 data

Previous experiments have shown that W and V are not linked. The same is true of the L and V factors. This leaves only the L and W relation open. The trihybrid data in this article afford critical evidence on the independent inheritance of L and W as well as of the others. Since backcrosses cannot be obtained in these seedling studies, one must rely on F_2 and F_3 data to determine the group relations of the factors involved.

Combining the F_2 and F_3 data (tables 5 and 6) which show the trihybrid relationship ($lLVvWw$), we obtain the following totals:

Observed, 788 green: 179 virescent-white: 157 yellow: 273 white.

Calculated, 786 green: 196 virescent-white: 153 yellow: 262 white.

When the closeness of fit, based on independent inheritance of all three factor-pairs, is determined, the value for P becomes 0.56. Hence theory and fact agree sufficiently well to justify the hypothesis of independent inheritance between L , V and W .

Independence of L , V and W proven by relation to the R aleurone-factor

The fact that L , V and W are located in different groups or on separate chromosomes is also checked by the relations of these factors to the R factor concerned in aleurone-color development in the maize kernel.

Data have been published (LINDSTROM, 1920) to show that W and R are inherited independently of each other. Three F_2 seedling progenies from self-pollinated green F_1 plants of the composition $AACCRrWw$ have produced the following relation:

	RW	Rw	rW	rw
Observed.....	482	155	180	52
Calculated.....	489	163	163	54

The deviations from a 9:3:3:1 ratio of independent inheritance between R and W are not marked. In fact when the closeness of fit is determined, P has a value of 0.51 showing that the deviations can very well be accounted for by errors of random sampling. Hence, W and R are not linked but are located on separate chromosomes.

The independent inheritance of R and V is also established by a large F_2 population which was derived from self-pollinating thirty-four F_1 plants of the composition $AACCRrVv$. A total of these 34 progenies is recorded as:

	RV	Rv	rV	rv
Observed.....	2080	730	716	221
Calculated.....	2108	702	702	234

In this case the deviations from a 9:3:3:1 ratio are not wide enough to vitiate the hypothesis of independent inheritance of R and V . The value for P is 0.48 in this distribution.

Linkage of R and L factors

From such data we are justified in assuming that W , V and R are located on separate chromosomes. In a previous publication (LINDSTROM 1918) evidence was presented to show that the L factor was linked with a certain aleurone factor, which at that time had not been definitely isolated. Since then the material has been tested with appropriate aleurone testers of known constitution obtained from Dr. R. A. EMERSON of CORNELL UNIVERSITY, and it has been definitely determined that it is the R factor which

TABLE 10

Seedling progenies from self-pollinated green plants of the composition RLv.rlv(WWAACCP_rP_r).

PEDIGREE NUMBER	FROM COLORED-ALEURONE SEED			FROM COLORLESS-ALEURONE SEED		
	Green	Virescent-white	Yellow	Green	Virescent-white	Yellow
2343- 7	42	0	0	0	0	0
-15	41	0	0	0	0	0
-62	38	0	0	0	0	0
- 1	28	0	0	20	0	0
-13	27	0	0	22	0	0
-26	28	0	0	13	0	0
-40	28	0	0	24	0	0
-41	18	0	0	19	0	0
-18	129	50	0	0	0	0
-21	103	42	0	0	0	0
-23	141	46	0	0	0	0
-47	26	8	0	0	0	0
- 6	121	27	0	53	0	17
-10	156	50	1	59	1	22
-14	42	12	0	26	1	10
-19	157	53	0	60	1	19
-24	186	52	1	60	1	18
-25	67	23	0	22	1	10
-30	229	75	0	72	0	27
-31	179	50	0	66	1	25
-33	142	51	0	59	3	11
-36	150	51	0	68	3	13
-43	159	57	0	48	0	19
-45	118	41	0	64	11	16
2344- 1	0	0	0	37	0	0
- 6	0	0	0	30	0	0
- 9	0	0	0	34	0	0
-12	0	0	0	29	0	0
-19	0	0	0	33	0	0
-20	0	0	0	31	0	0
-21	0	0	0	30	0	0
- 3	0	0	0	92	0	28
- 5	0	0	0	75	0	30
- 8	0	0	0	110	0	35
-11	0	0	0	53	0	18
-13	0	0	0	104	0	23
-14	0	0	0	97	0	19
-17	0	0	0	93	0	27
-18	0	0	0	74	0	37
-23	0	0	0	81	0	22

is linked with *L*. Additional evidence on this situation will be presented here.

Since back-crosses cannot be used to test the linkage (because the double recessive is not viable), the F_2 and F_3 generations must suffice. By self-pollinating a large number of plants of the factorial composition $RLv.rlV$ ($WWAACC$), it has been possible to demonstrate that *R* and *L* are very closely linked with each other. The results of such self-pollinations are arranged in table 10.

Since only the *Rr* factor-pair for aleurone color is heterozygous in this material, colored-aleurone grains show that the plants developing from them possess the *R* factor, while those plants coming from colorless-aleurone seed possess only the *r* factor. A glance at table 10 shows that among the 24 ears from colored seed (2343), there were 7 that bred true for colored aleurone, while 17 ears showed a segregation into colored and colorless grains (all 3:1 ratios in this material). The expectation here would be 8 and 16 respectively. The progenies from colorless grains bred true for colorless aleurone (2344).

TABLE 11
Summary of seedling progenies of table 10 showing behavior of seedling factors, LLVv.

	ALL GREEN	12 GREEN: 3 VIRESCENT- WHITE: 1 YELLOW	3 GREEN: 1 VIRESCENT- WHITE	3 GREEN: 1 YELLOW
	4 $\begin{cases} 1 LLVV \\ 2 LLVv \\ 1 llvv \end{cases}$	4 <i>LLVv</i>	2 <i>LLVv</i>	2 <i>llVv</i>
Found.....	15	12	4	9
Expected (4:4:2:2)....	13.3	13.3	6.7	6.7
Deviation.....	+1.7	-1.3	-2.7	+2.3

$$P = 0.53$$

As regards the behavior of the seedling-chlorophyll factors themselves, their inheritance obeys the demands of the hypothesis, as can well be seen in the summary that has been arranged in table 11.

With the data agreeing so well in respect to the aleurone and chlorophyll factors separately, we can turn directly to the interrelation between them. It will be noted that in table 10, among those progenies segregating for seedling characters, the normal 12:3:1 ratio does not obtain if one considers those progenies coming from colored seed separately from those developing from colorless grains. In other words very few (only two in this case) yellow seedlings come from colored seed, and only a few virescent-whites appear in the progenies from colorless seed. This means that when-

ever R is present, l is not likely to be, whereas if r is present L is rarely found. Stated in terms of linkage, the parental combinations RL and rl exhibit very close linkage; the crossover classes Rl and rL are correspondingly rare.

The raw data in table 10 are not adapted for a quantitative determination of the percentage of crossing over between R and L . The material in this experiment was segregating for a pericarp color as well as for aleurone color and chlorophyll factors. This pericarp color prevents an accurate classification of aleurone color, a correct classification of which is absolutely essential in determining the presence of the crossover classes. There were certain ears, however, with colorless pericarp which permitted an accurate separation into colored and colorless aleurone. The data from these ears have been gathered into table 12.

TABLE 12
Summary of seedling progenies of table 10 from ears which possessed colorless pericarp.

PEDIGREE NUMBER	FROM COLORED ALEURONE			FROM COLORLESS ALEURONE		
	Green	Virescent-white	Yellow	Green	Virescent-white	Yellow
2343-10	156	50	1	59	1	22
-19	157	53	0	60	1	19
-24	186	52	1	60	1	18
-25	67	23	0	22	1	10
-30	229	75	0	72	0	27
-31	179	50	0	66	1	25
Total.....	974	303	2	339	5	121
Theoretical (independent inheritance)....	959	240	80	349	87	29

If the factors R and L were independently inherited we should expect a 12:3:1 ratio of green to virescent-white to yellow seedlings from both colored and colorless seed in table 12, whereas from the colored aleurone only 2 yellow seedlings actually occurred where 80 would be expected, and from colorless aleurone only 5 virescent-whites appeared where the expectation would be 87. Evidently then the parental gametes RL and rl are produced far in excess of the crossover gametes rL and Rl . In fact, the latter are produced so infrequently that the relationship approaches complete linkage.

The appearance of the crossover classes was very distinct, however, and in each case the aleurone color of the seed producing the crossover seedling was checked by digging up the plant and examining the grain for its aleurone color. Their chlorophyll type was unmistakable as was their aleurone

appearance, although the latter offers a slight source of uncertainty since experience has shown that very rarely a seed which is apparently colorless will produce colored progeny, indicating that it possessed the requisite factors although something acted to prevent their full expression in the mother seed. This would explain a very infrequent occurrence of a virescent-white from apparently colorless seed but would not account for the occurrence of yellow seedlings from colored seeds. Unfortunately it was impossible to mature these seedlings and actually test them. Inasmuch as the aleurone classification was made in an exceedingly careful manner, and the seeds planted so as to prevent accidental mixing, it seems reasonable to suppose that we are dealing with actual crossover types, and that R and L are not completely linked.

As to the quantitative measure of linkage between R and L , this can best be determined by taking from table 12 only the data in which R and L are directly concerned. The green classes are eliminated from consideration since both L and l (LV and lV) are grouped there. Using then only the critical data, the following distribution obtains:

RLv	Rlv	rLv	rlv
303	2	5	121

If there were independent inheritance between R and L , this distribution should resemble a 9:3:3:1 ratio. Obviously the departure therefrom is abnormally great. Instead of a 1:1 gametic ratio, a 60:1 ratio fits the data better, the theoretical expectation being 317:4:4:106 in this case. That is, the non-crossover classes are produced sixty times more often than the crossover classes. The percentage of crossing over between R and L consequently, is about 1.6 percent in this experiment. The numbers upon which this percentage is based are, however, rather small, especially since they are F_2 data; hence, the crossover percentage of 1.6 percent is presumably more or less inaccurate.

The occurrence of the crossover classes Rl and rL , although they have not been proven by tests other than phenotypic appearance, indicates that the factors R and L are not identical, as might have been supposed from preliminary studies reported earlier (LINDSTROM 1918) where the limited data pointed only to complete linkage. In this connection it might be noted that the factors Rr and Gg (the latter factor-pair of which the homozygous recessive condition gg produces a golden plant) have been found to show 23 percent crossing over, while Ll and Gg show 19 percent crossing over. If R and L are not identical but show a crossover percentage of 1.6 percent then the order of the factors must be $R-L-G$, and the difference between 23 percent and 19 percent might possess some significance, although these crossover percentages also are based on relatively small numbers.

DISCUSSION

During the six years which have elapsed since these genetical investigations on chlorophyll in maize seedlings were begun, the inheritance of the three types of abnormal seedlings, white, virescent-white and yellow, has proven itself to be typically Mendelian. The stability of the types has been such that no practical difficulty in classifying them from year to year has arisen, especially when they were grown under optimum conditions. It is true that only the most clearly defined types were chosen to carry on the investigations. This was done to remove any possible modifying factors which would have a tendency to obscure the fundamental relations between the basic factors. That there are such modifying factors is certain. But their influence is to be studied only after the fundamental factors have been isolated and tested genetically. Such a procedure is entirely natural and justifiable if we can prove that these fundamental factors, when so isolated, behave as relatively stable units and follow some definite system of inheritance. This is the true situation with respect to the seedling types of maize. The fundamental factors Ww , Vv and Ll are expressed as stable seedling characters which exhibit strict and typical Mendelian inheritance.

The genetic analysis of the three factors, w , v and l , the first two of which are related to the development of green pigment and the third to the formation of yellow color, has established a definite interrelation between these factors that should offer some suggestions as to their chemical or physiological behavior. Whether one can draw an analogy between genetic relations and chemical or physiological relations is still in the realm of speculation. It seems reasonable to suppose however, that the two processes are not independent, and that future development will see them intimately related with each other.

Our genetic hypothesis has demonstrated that the various types of maize seedlings possess the following factors:

Green.....	LVW and lVw
Virescent-white.....	LvW
White.....	LVw and Lvw
Virescent-yellow.....	lvW
Pure yellow.....	lVw and lvw

From this series of interrelations we can derive several interesting and critical suggestions as to the relationship between the green and yellow pigments here concerned.

For the complete development of green color it seems that there are at least two factors necessary, W and V . It must not be thought, however,

that these two are the only ones concerned. Suggestions are now at hand of another factor which apparently interacts with *W* to give a 9:7 ratio of green to white seedlings. Green pigment is then dependent on two, possibly three factors. How are these green-producing factors and their allelomorphs related to yellow pigment?

A genetic relationship between green and yellow pigment, as shown by the factorial behavior, is clearly seen in the action of the *w* factor. This factor is able to inhibit or repress the green or virescent color produced by *v* but does not interfere with the development of the yellow pigment produced by *l*.

This is most definitely seen by considering the four genotypes, *LVw*, *Lvw*, *lVw*, and *lvw*, of which the first two are white and the last two yellow. In the first type (*LVw*), *w* inhibits any green pigment which might be produced by *V* or even *L*; and in the second genotype (*Lvw*) it even represses the slight amount of green pigment caused by *v* as well as any pigment that might be produced by *L*. In the third type (*lVw*), however, although *w* inhibits any green-producing effect of the *V* factor, it does permit the yellow pigment caused by *l* to appear, producing a pure yellow seedling. If *w* were replaced by *W* in this type, the result would be a green seedling (*lVW*) in which the yellow pigment caused by *l* would presumably be hidden by the green color. The fourth genotype (*lvw*) is also pure yellow because *w* prevents the virescent or greening effect of *v* from functioning but allows *l* to form its yellow pigment. These reactions of the *w* factor can be resolved into the simple statement that a plant possessing the *w* factor lacks something essential for the development of normal green pigment but not for the development of yellow pigment, which apparently is normally produced whenever *l* is present.

Genetically, then, green and yellow pigment in this respect are independent of each other. Whether such independence of action actually occurs between the formation of the chlorophyll and xanthophyll pigments in leaf tissue is at present unknown. It is entirely possible that the formation of these two pigments is unrelated, at least in some stage of development, and this genetic relation offers some such suggestion.

The fact that all three factor-pairs concerned in the formation of green and yellow pigment are located in different groups or in different chromosomes agrees with the situation in *Drosophila*, namely, that factors which are closely related as to their phenotypic expression are not necessarily grouped together in the germ-plasm. This of course is also true for aleurone color in maize, for which three factors (*A*, *C*, *R*) are responsible, all of them being located in different chromosomes.

SUMMARY

1. The inheritance of three factors, *w* (white), *v* (virescent) and *l* (yellow), and their respective allelomorphs, *W*, *V* and *L*, which govern the formation of green and yellow pigments in maize seedlings, is shown to be typically Mendelian.

2. A summary of previous work, including some new data, showing the interaction between the factors *Ww* and *Vv* and between *Vv* and *Ll* is given.

3. An hypothesis to explain the trihybrid interaction of the factors is proposed. The F_2 generation of the trihybrid *LlVvWw*, based on this hypothesis, results in a 36:9:7:12 ratio of green : virescent-white : yellow : white seedlings, respectively, with which the actual data are well in accord.

4. The F_2 and F_3 data show that the factors *L*, *V* and *W* are inherited independently of each other.

5. The factors *W* and *V*, which are necessary for the formation of green pigment, show no interaction with *l* which is concerned in the formation of the yellow pigment in seedlings. Factor *w* interacts with *v* but not with *l*. In other words, *w* inhibits the virescent-green pigment produced by *v* but allows the yellow pigment produced by *l* to develop independently.

6. The independent inheritance of *Ll*, *Vv* and *Ww* is also shown by their relations to the *Rr* factor-pair for aleurone-color development, both *Ww* and *Vv* being inherited independently of *Rr*.

7. Very close linkage between *Rr* and *Ll* is demonstrated. Crossing over occurs very infrequently.

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