INHERITANCE AND LINKAGE RELATIONS OF TWO NEW ENDOSPERM TYPES IN DENT CORN

BY

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I. ACKNOWLEDGMENT

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II. INTRODUCTION

Seed characters of maize have always interested geneticists because of their wide variety and ease of classification, together with the fact that crosses are easily made and large populations can be produced on a single ear under a uniform environment. A very large number of genes affecting the seed have been described, their mode of inheritance determined and their linkage relations established. Pericarp color, aleurone color, endosperm texture, and color, and other qualities of the scutellum and embryo have been the subject of many investigations already reported in the literature dealing with the genetics of maize.

The present investigation deals with genes affecting endosperm texture. It was started at a time when there was much interest in the relation of endosperm texture to disease resistance of young corn seedlings. A thesis entitled, "A Study of Starchy and Horny Endosperms and Their Relation to Disease Resistance," was submitted in 1928 by the writer in partial fulfillment of the requirements for his M.S. degree. The discovery of two new genes producing soft starch in dent corn during this earlier study led to the present investigation.

III. REVIEW OF LITERATURE

The rediscovery of Mendel's laws of inheritance in 1900 stimulated the study of many characters in plants and animals, especially those characters which segregated distinctly and could be easily classified. In maize, there were many characters of the seed, as color and endosperm texture, that lent themselves well to such studies. The literature dealing with inheritance in plants contains many references to these characters. In 1901, Correns (2) described and reported on a number of seed characters in corn. He found that the factor for sugary, now designated as su_1, was a recessive character that did not appear in the first generation but segregated out as a simple recessive in the second generation with a ratio of 3 starchy : 1 sugary. Eyster (6) has reported two other genes that produce sugary seeds. He has designated them by the symbols su_2 and su_3. They are also recessive genes and give ratios of 3 starchy : 1 sugary in the F_2.

In 1909 Collins (1) reported on a new type of endosperm which he found in Indian corn introduced from China. He called this type waxy, because of its appearance, and designated it by the symbol wx. It segregated as a simple recessive to starchy in a 3:1 ratio in the second generation.

East and Hayes (3, 5) made an extensive study of characters affecting the endosperm of corn. They made a large number of crosses between the six most common subspecies of maize; Zea mays tunicata (the pod corns); Zea mays everta (the pop corns); Zea mays indurata (the flint corns); Zea mays indentata (the dent corns; Zea mays amylacea (the flour corns); and Zea mays saccharata (the sweet corns). They reported a single factor difference between the starchy type of endosperm and the sugary type, the starchy condition being dominant. The physical texture of the starch in starchy kernels was controlled by still other genetic factors. The results of the first cross between floury and horny corn made it appear as if the characters were maternally inherited because the maternal characters were expressed to the practical exclusion of the male characters. A floury female crossed with a corneous male gave seeds that were all floury. A corneous female crossed with a floury male gave seeds that were all corneous. When the F_1 seeds were grown, East and Hayes recovered the grandparental types and also obtained several intermediate types, indicating that there were several multiple factors affecting the floury-horny relationship. These genetic factors did not appear to be dominant to their respective allelomorphs but expressed themselves according to the proportion in which they were present. Lack of maturity occasionally made it difficult to properly classify the segregating types in the F_2 and F_3.

In 1921 Hutchison (7) published results on a study of an endosperm character called shrunken. This character reduced the amount of starch formed in the endosperm and resulted in a decided shrinking of the seed. It behaved as a simple recessive to the normal and was found to be closely linked to a color factor, C.

In 1926 Mangelsdorf (9) reported the results of his studies on a number of genes that retarded seed development in various degrees. He referred to these genes as "defectives" and listed 14 of them. They were all inherited as simple recessives to the normal. At least two other defectives have since been added to the above list. Mangelsdorf also reported another endosperm type which he called "brittle", a simple recessive to normal. Other workers have reported two other genes that produce brittle seeds.

Singleton and Jones (11) have been studying two genes which produce starchy endosperms and which they have called opaque 1 and opaque 2. They have crossed these with h, one of the genes studied in this investigation, and have found that h is not identical with either one of them.

IV. MATERIAL

In 1927 the writer began a study of the susceptibility of endosperms of varying degrees of hardness in dent corn to certain fungus diseases, particularly Rhizopus nigricans. Seeds from a large number of ears were classified for hardness and tested for disease resistance in a germinator. Seeds from each classification were planted and the resulting ears were self-pollinated to determine whether the starchiness was a result of environment and would not breed true or was a result of genetic factors and would remain stable from one generation to another.

One strain was found in the soft starch group that bred true for that character. The strain, designated as Inbred 3141, had been inbred by Doctor Woodworth for nine generations. Except for the one ear used in this study, all the other ears were quite horny. It is presumed that a mutation must have occurred at least two generations before it was noticed, because one segregating generation must have intervened in order that all the kernels on an ear could show the character at the time it was discovered. It was interesting that aside from the change in endosperm composition, no other characteristics of the inbred with respect to vigor or plant and ear type seemed to be affected. The new gene for starchiness was designated by the symbol h because it was recessive to horny, designated as H. It was crossed with horny corn from the same inbred and also with unrelated horny corn. In the first generation, there was no indication of its presence. In the second generation it was demonstrated that h behaved as a simple recessive to horny, giving ratios of 3 horny : 1 starchy (Table 1). In 1930 Mumm and Woodworth (10) reported on the breeding behavior and other characteristics of this gene. It produces an endosperm with a soft interior and cap, surrounded by a thin corneous band around the sides of the seed. Plate 1. In well matured ears, in which the horny seeds are well developed, classification of the F_2 generation is not difficult. Plate 2.

In 1929 a new gene for soft starch spontaneously appeared in an inbred strain designated as 4001. This strain had previously bred true for horny endosperm. One ear showed segregation for endosperm texture. It produced 96 horny seeds and 93 very soft seeds, very nearly a 1:1 ratio. Further studies in succeeding generations confirmed this ratio. (Table 2). At first it was presumed that this gene for soft starch was the same as floury, previously studied and reported by Hayes and East (3, 5), because the segregating ratios agreed with the ratios which they obtained.

Table 1.--F_2 Ratios of Starchy and Horny Endosperms Resulting From a Cross Between Line 3141 (hh) and Normal Corn (HH)

Ear number	Horny (HH)	Starchy (hh)	Total	Dev.	P. E.	D/P. E.
1-18	314	123	437	13.8	6.10	2.26
1-19	474	136	610	16.5	7.21	2.29
1-21	501	156	657	8.3	7.49	1.11
1-21	345	109	454	4.5	6.23	0.72
1-23	466	176	642	15.5	7.40	2.09
1-24	487	182	669	15.7	7.55	2.08
1-25	499	181	680	11.0	7.62	1.44
1-27	422	129	551	8.8	6.85	1.28
1-28	602	201	803	0.2	8.28	.02
1-29	464	111	575	32.8	7.00	4.69
1-29	404	150	554	11.5	6.87	1.67
1-30	314	124	438	14.5	6.11	2.37
1-32	445	142	587	4.8	7.08	0.68
1-33	376	127	503	1.2	6.55	0.18
1-35	464	153	617	1.3	7.26	0.18
1-35	384	124	508	3.0	6.58	0.46
2-20	407	147	554	8.5	6.87	1.24
2-21	291	102	393	3.7	5.79	0.64
2-24	381	125	506	1.5	6.57	0.23
2-30	394	152	546	15.5	6.83	2.27
2-33	459	161	620	6.0	7.27	0.83
2-34	499	152	651	10.8	7.45	1.45
2-35	344	114	458	0.5	6.25	0.08
12-17	358	102	460	13.0	6.27	2.07
12-17	421	135	556	4.0	6.89	0.58
12-6	240	96	336	12.0	5.36	2.24
Total	10 755	3 610	14 365	18.7	35.00	.534

Expected ratio = 3 horny : 1 starchy

Table 2.--F_2 Ratios of Starchy and Horny Endosperms Resulting From a Cross Between Floury 2 (fl_2fl_2) and Horny Corn (Fl_2Fl_2)

Ear number	Horny	Starchy	Total	Dev.	P. E.	D/P. E.
5722-1	204	215	419	4.5	6.90	.652
5722-2	98	118	216	10.0	4.96	2.016
5731-1	211	203	414	4.0	6.86	.583
5731-2	169	162	331	3.5	6.14	.570
5731-3	123	133	256	5.0	5.40	.926
5731-4	86	73	159	6.5	4.25	1.529
5731-5	78	91	169	6.5	4.38	1.484
5731-6	14	13	27	0.5	1.75	.285
309-1	88	84	172	2.0	4.42	.452
106-1	164	186	350	11.0	6.31	1.743
106-2	156	175	331	9.5	6.14	1.547
106-3	155	165	320	5.0	6.03	.829
106-4	29	36	65	3.5	2.72	1.287
110-1	95	114	209	9.5	4.88	1.947
Total	1 670	1 768	3 438	49.0	19.77	2.479

Expected ratio = 1 horny : 1 starchy

It was also similar in other respects. In the first generation it behaved as though it was maternally inherited; because when a starchy plant was pollinated with horny pollen, the progeny remained starchy. When a horny plant was pollinated with starchy pollen, the progeny remained horny.

When this character for soft starch was crossed with floury, in the first generation the progeny were all floury and this would be expected whether the condition was due to a single gene or to two different ones, because of their peculiar behavior in crosses. In the F_2, however, the ears were not all starchy but segregated in a ratio of 1 horny : 3 starchy. (Table 3). This would be the expected ratio if the two genes were different. Because of the fact that the new gene, in crosses with horny corn, behaved in the same manner as floury, it was called floury 2 and designated by the symbol fl_2. It became necessary to refer to the original floury gene as floury 1 and to designate it as fl_1.

A careful check on the original material led to the assumption that the new gene originated as a mutation in a pollen grain in the 1928 season. If it had occurred in the egg cell, the mutation would have shown up immediately as a starchy seed. All the breeding material was watched so closely for starchy seeds that the presence of a mutant of this type would not easily have been overlooked. Because of the nature of the inheritance of fl_2, the resulting ear from this seed would have segregated whether it was selfed or not. Fortunately, it was self-pollinated and thus was immediately available for further inheritance studies.

Table 3.--F_2 Ratios of Starchy and Horny Endosperms Resulting From a Cross Between Floury 1 ($fl_1fl_1Fl_2Fl_2$) And Floury 2 ($Fl_1Fl_1fl_2fl_2$)

Ear number	Horny	Starchy	Total	Dev.	P. E.	D/P. E.
154-1	56	228	284	15.0	4.92	3.049
154-2	95	251	346	8.5	5.43	1.565
154-3	40	170	210	12.5	4.23	2.955
154-4	64	201	265	2.25	4.75	.474
154-5	36	157	193	12.25	4.06	3.017
5679-1	168	523	691	4.75	7.68	.618
5679-2	131	414	545	5.25	6.82	.770
5679-3	163	396	559	23.25	6.91	3.365
151-1	34	93	127	2.25	3.29	.684
151-2	115	246	361	24.75	5.55	4.459
151-3	193	480	673	24.75	7.58	3.265
152-1	51	134	185	4.75	3.97	1.196
152-2	97	237	334	13.5	5.34	2.528
152-3	115	309	424	9.0	6.01	1.498
152-4	75	163	238	15.5	4.51	3.437
152-5	58	161	219	3.25	4.32	.752
152-6	15	42	57	.75	2.20	.341
152-7	77	225	302	1.5	5.08	.295
152-8	23	82	105	3.25	2.99	1.087
Total	1 606	4 512	6 118	76.5	22.84	3.349

Expected ratio = 1 horny : 3 starchy

Expected genotypic constitution of F_2 endosperms in a cross between floury 1 ($fl_1fl_1Fl_2Fl_2$) and floury 2 ($Fl_1Fl_1fl_2fl_2$):

Polar Nuclei	Pollen	Phenotype
$Fl_1Fl_2 \cdot Fl_1Fl_2$	(Fl_1Fl_2	Horny endosperm
	(Fl_1fl_2	" "
	(fl_1Fl_2	" "
	(fl_1fl_2	" "
$Fl_1fl_2 \cdot Fl_1fl_2$	(Fl_1Fl_2	Starchy endosperm
	(Fl_1fl_2	" "
	(fl_1Fl_2	" "
	(fl_1fl_2	" "
$fl_1Fl_2 \cdot fl_1Fl_2$	(Fl_1Fl_2	Starchy endosperm
	(Fl_1fl_2	" "
	(fl_1Fl_2	" "
	(fl_1fl_2	" "
$fl_1fl_2 \cdot fl_1fl_2$	(Fl_1Fl_2	Starchy endosperm
	(Fl_1fl_2	" "
	(fl_1Fl_2	" "
	(fl_1fl_2	" "

Phenotypic ratio = 1 horny : 3 starchy

The gene, fl_2, produces a very soft endosperm that is easy to distinguish on segregating ears when they are fully mature. Plates 3, 4. The seeds are as soft as those produced by the gene, fl_1, and are softer than those produced by the gene, h, though starchy seeds on segregating ears when both fl_2 and h are involved cannot be separated for the two characters with any degree of certainty.

In addition to the new genes that have just been described, other breeding material included homozygous strains of flour corn (fl_1), sweet corn (su_1), and waxy corn (wx), besides a number of strains of known genetic constitution used in the study of linkage.

V. RESULTS AND CONCLUSIONS

1. Crosses Between h and Other Endosperm Characters

When the gene h was crossed with floury 1, the F_1 was horny when the ear parent was hh and starchy when the ear parent was fl_1fl_1. In the first instance, the pollen parent (fl_1fl_1HH) brought in the gene H which was dominant over h and produced a horny endosperm. In the second instance, while the pollen parent ($hhFl_1Fl_1$) brought in the gene Fl_1, the seeds were still starchy because they had the composition $fl_1fl_1Fl_1$. Table 4 gives the F_2 ratios for four segregating ears. On the basis of independence a ratio of 3 horny : 5 starchy would be expected. Three of the ears have deviations no larger than would be expected on the basis of chance. Ear 80B-2 had a deviation 3.928 times the probable error. This was a case where classification was difficult because the horny seeds were somewhat starchy. The deviation of the total was 35.5, which was 2.791 times the probable error, but was not too large to be expected on the basis of chance.

When the gene h was crossed with waxy (wx) the F_1 was horny regardless of which way the cross was made. Table 5 gives the F_2 ratios for two segregating ears. A ratio of 9 horny : 3 starchy : 4 waxy was indicated by the data and when the X^2 test was applied the value of P was .13 which indicates that the deviations from the above ratio are not larger than would be expected on a chance basis.

Table 4.--F_2 Ratios of Starchy and Horny Endosperms Resulting From a Cross Between Floury 1 (fl_1fl_1HH) and Starchy (Fl_1Fl_1hh)

Ear number	Horny	Starchy	Total	Dev.	P. E.	D/P. E.
77B-1	172	300	472	5	7.09	.705
77B-2	120	187	307	5	5.72	.874
80B-1	157	230	387	12	6.42	1.869
80B-2	155	195	350	24	6.11	3.928
Total	604	912	1 516	35.5	12.72	2.791

Expected ratio = 3 horny : 5 starchy

Genotypic constitution of the F_2 generation of a cross between floury 1 (fl_1fl_1HH) and starchy (Fl_1Fl_1hh):

Polar nuclei	Pollen	Phenotype
Fl_1 H·Fl_1 H	(Fl_1 H	Horny endosperm
	(Fl_1 h	" "
	(fl_1 H	" "
	(fl_1 h	" "
Fl_1 h·Fl_1 h	(Fl_1 H	Horny endosperm
	(Fl_1 h	Starchy "
	(fl_1 H	Horny "
	(fl_1 h	Starchy "
fl_1 H·fl_1 H	(Fl_1 H	Starchy endosperm
	(Fl_1 h	" "
	(fl_1 H	" "
	(fl_1 h	" "
fl_1 h·fl_1 h	(Fl_1 H	Starchy endosperm
	(Fl_1 h	" "
	(fl_1 H	" "
	(fl_1 h	" "

Phenotypic ratio = 3 horny : 5 starchy

Table 5.--F_2 Ratios of Crosses Between Starchy ($WxWxhh$) and Waxy ($HHwxwx$)

Ear number	Number of seeds			Total
	Horny	Starchy	Waxy	
227B-1	372	98	165	635
225B-1	313	97	140	550
Total	685	195	305	1 185
Calculated (9:3:4)	666.6	222.2	296.2	1 185
Difference	+ 18.4	− 27.2	+ 8.8	

$X^2 = 4.0989 \quad P = .13$

Crosses between the gene h and sugary (su_1) gave horny seeds in the F_1 regardless of which way the cross was made. Table 6 gives the F_2 ratios for two segregating ears. A ratio of 9 horny : 3 starchy : 4 sugary was indicated by the data. The deviations from such a ratio were quite small. When the X^2 test was applied, the value of P was .4, indicating that the deviations from the above ratio are not larger than would be expected on the basis of chance.

The above crosses between h and sugary and between h and waxy indicate that h is hypostatic to su_1 and wx.

The results of crosses between h and fl_2 are discussed in the section in which the linkage data are presented.

Table 6.--F_2 Ratios of Crosses Between Starchy (SuSuhh) and Sugary (HHsusu)

Ear number	Number of seeds			Total
	Horny	Starchy	Sugary	
227F-1	268	78	103	449
222B-1	211	87	114	412
Total	479	165	217	861
Calculated (9:3:4)	484.3	161.4	215.3	861
Difference	- 5.3	+ 3.6	+ 1.7	

X^2 = .1517 P = .4

2. Crosses Between fl_2 and Other Endosperm Characters

The results of crosses between fl_2 and fl_1 have been discussed in the section dealing with "Material". The F_1 seeds were starchy regardless of which strain was used as the ear parent. Floury 1 ($fl_1fl_1Fl_2Fl_2$) brought in Fl_2 when it was used as the pollen parent and floury 2 ($Fl_1Fl_1fl_2fl_2$) brought in Fl_1 when it was used as the pollen parent but fl_1 fl_1 Fl_1 and fl_2 fl_2 Fl_2 are both starchy. The F_2 segregated in a ratio of 1 horny : 3 starchy. See Table 3. Nineteen ears gave a total of 6118 seeds, of which 1606 were horny and 4512 starchy. The deviation was 76.5 and the probable error was 22.84, giving a ratio of 3.349. The deviation was somewhat larger than would be expected (Odds = 41:1) but the individual ear counts showed that most of them came rather close to the expected ratio. The differences were not consistently in one direction. It has been previously pointed out that in some ears the horny seeds were not easy to distinguish if the proper maturity of the ears was somehow prevented. In such cases the horny seeds were also somewhat starchy, and separation was not always certain.

A cross was made between fl_2 and sugary (su_1) with the sugary type used as the ear parent. In the F_1 the progeny were all horny.

The results obtained in the F_2 generation are given in Table 7.

Table 7.--F_2 Ratios of Crosses Between
Floury 2 (fl_2fl_2SuSu) and
Sugary (Fl_2Fl_2susu)

	Phenotypic classes			
	Fl_2Su	fl_2Su	Fl_2su fl_2su	Total
Observed	209	194	143	546
Calculated 3:3:2	205	205	136	546
Difference	+ 4	- 11	+ 7	

$X^2 = 1.0285$ $P = .60$

A ratio of 3 horny : 3 starchy : 2 sugary was indicated by the data. This would be the expected ratio if fl_2 was hypostatic to sugary. The differences from this ratio were quite small. When the X^2 test was applied, the value of P was .60. This indicates that the deviations from the above ratio are most probably chance differences, and that it is the correct ratio. It also indicates that fl_2 must be hypostatic to sugary.

No cross was made with waxy.

The results of crosses between h and fl_2 are discussed in the section in which linkage data are presented.

3. Linkage Relations

The investigation of the linkage relations of h and fl_2 with the ten established linkage groups in maize has extended over a period of ten years. Certain difficulties were encountered in finding suitable testers in each group. Several of the genes most commonly used in linkage tests are concerned with pericarp and aleurone colors. In the present study, these interfered too much with classification to be useful because they concealed the nature of the endosperm. Shrunken, waxy, and sugary endosperm have been used in many linkage tests but they were not so suitable in this investigation since it also deals with endosperm characters. However, it was possible to test the data to determine whether the ratios obtained indicated independence by applying the probable error or the X^2 test for goodness of fit. Plant characters, especially seedling characters, were used wherever possible.

Another difficulty developed because of the fact that most of the linkage testers were obtained from Minnesota, Wisconsin, and New York; consequently, they were considerably earlier than the Illinois strains carrying h and fl_2. Many times crosses were not obtained because the pollen was all gone before the shoots were developed far enough to be pollinated.

It was necessary to use F_2 populations because the investigation had not been continued long enough to recover the double recessives. All combinations tested were of the repulsion type, with one recessive and one dominant coming in from each side of the cross. A number of double recessives have been obtained from the F_2 segregates and they will be used in the future as an additional check on the data reported here.

In all of the linkage tests except one, only one gene was tested in each group. It would still be possible to have linkage, even though there was no evidence of it in these tests. It is possible to have genes so far apart in the same group that they give 50 percent of crossing over, indicating independence. However, it is believed that the data reported in the following pages indicate definite linkages.

a. Linkage Tests With h

Table 4 indicates that h is probably independent of Group 2, because fl_1 is in this group. The totals approximate a ratio of 3 horny : 5 starchy, the expected ratio if the two genes are independent. The deviation is 2.79 times the probable error and is, therefore, not significant. Only one ear, 80B-2, gave a ratio large enough to suggest more than a chance difference. The difference was 3.93 times the probable error. This was a case where there was difficulty in making a positive separation. Whenever something interfered with complete maturity, the horny seeds remained somewhat starchy. A prematurely broken stalk or broken shank was sufficient to produce such a condition. In this particular instance the horny seed count was too high by 24 seeds, but this was partly attributed to uncertain classification.

Table 6 shows the relation between h and sugary, which is in Group 4. A ratio of 9 horny : 3 starchy : 4 sugary would be expected on the basis of independence. The value of P is .4, which indicates that the differences are very likely due to chance and that h is not in Group 4.

The gene Y for yellow endosperm was used as a tester in Group 6. A count was made on one ear segregating for white and yellow endosperm with the results, as given in Table 8.

Table 8.--F_2 Ratios of Crosses Between
h (hhYY) and White Endosperm (HHyy)

	Phenotypic classes				
	HY	Hy	hY	hy	Total
Observed	303	114	110	39	566
Calculated 9:3:3:1	318	106	106	36	566
Difference	- 15	+ 8	+ 4	+ 3	

$X^2 = 1.7122 \quad P = .64$

These results indicate that h is probably independent of Group 6.

The genes v_5 and gl_1 were used as testers for Group 7. The results are given in Tables 9 and 10.

Table 9.--Progeny of the F_1 of the Cross hhV_5V_5 x HHv_5v_5

Pedigree number	Number of seedlings				
	H		h		
	V_5	v_5	V_5	v_5	Total
2049-1	157	33	51	13	254
2049-2	125	54	45	15	239
Total observed	282	87	96	28	493
Calculated 9:3:3:1	277	92.5	92.5	31	
Difference	+ 5	- 5.5	+ 3.5	- 3	

$X^2 = .8400 \quad P = .833$

Table 10.--Progeny of the F_1 of the Cross
$hhGl_1Gl_1$ x $HHgl_1gl_1$

Pedigree number	Number of seedlings				
	H		h		
	Gl_1	gl_1	Gl_1	gl_1	Total
2049-1	149	42	49	14	254
2049-2	129	50	46	14	239
Total observed	278	92	95	28	493
Calculated 9:3:3:1	277	92.5	92.5	31	
Difference	+ 1	- 0.5	+ 2.5	- 3	

X^2 = .3642 P = .928

The results obtained with crosses between h and v_5, and between h and gl_1, are so near the expected 9:3:3:1 ratio that h is probably not in Chromosome 7.

Tables 9 and 10 and also Table 13 have had their frequencies adjusted because of the fact that the number of seedlings observed from the horny and starchy classes were not in the 3:1 ratio in which the horny and starchy seeds segregated on the parent ears. A convenient number of each was chosen for planting, which was done on a sand bench in the greenhouse. It was necessary to save some seeds for field planting, so the entire seed supply could not be used. In any case an adjustment becomes necessary because not all of the seeds germinate.

Table 11.--The Original Data from Which Table 9 Was Calculated

Ear number	Endosperm type	Normal green	V_5	Total
2049-1	HH	132	28	160
	hh	75	19	94
2049-2	HH	100	43	143
	hh	73	23	96
Total	HH	232	71	303
	hh	148	42	190

Table 12.--The Original Data from Which Table 10 Was Calculated

Ear number	Endosperm type	Normal green	gl_1	Total
2049-1	HH	125	35	160
	hh	73	21	94
2049-2	HH	103	40	143
	hh	73	23	96
Total	HH	228	75	303
	hh	146	44	190

The gene wx for waxy endosperm was used as a tester for Group 9. The results are shown in Table 5. A ratio of 9 horny : 3 starchy : 4 waxy would be expected on the basis of independence. The X^2 test gives a value for P of .13, which indicates that the differences are due to chance. Therefore, h is probably independent of Group 9.

No tests were made with Groups 1, 5, 8, and 10.

The results of tests with Group 3 indicate that h is linked with that group. The gene d_1, which produces a decided dwarfing both in the seedling and mature plant, makes a good tester for this group. The results are recorded in Table 13.

Table 13.--Progeny of the F_1 of the Cross
$hhD_1D_1 \times HHd_1d_1$

Pedigree number	Number of seedlings				
	H		h		
	D_1	d_1	D_1	d_1	Total
2043-1	130	61	58	5	254
2043-2	118	62	50	9	239
Total observed	248	123	108	14	493
Calculated on basis of 25% crossing over	254	115.5	115.5	8	

It has already been explained that an adjustment was necessary in the different classes to adjust the number of horny and starchy seeds to a 3:1 ratio.

Table 14.--Original Data From Which
Table 11 Was Calculated

Ear number	Endosperm type	Normal	d_1	Total
2043-1	HH	109	51	160
	hh	87	7	94
2043-2	HH	94	49	143
	hh	82	14	96
Total	HH	203	100	303
	hh	169	21	190

When the percent of crossing over is calculated by Woodworth's modification (unpublished) of Emerson's formula (4) for repulsion $\left[\frac{\sqrt{E-H}}{\sqrt{N}} \times 100\right]$, the result is 25.08 percent. It can, therefore, be assumed that h is in Chromosome III at some distance (25 percent of crossing over) from d_1.

Reference to Immer's tables (8) indicates 32 ± .0269 crossing over. However, the calculated frequencies with 32 percent of crossing over did not fit the data as well as when 25 percent of crossing over was assumed.

A map of Chromosome III shows that h either is located very near cr_1 on one side or between Rg and ts_4 on the other side of d_1.

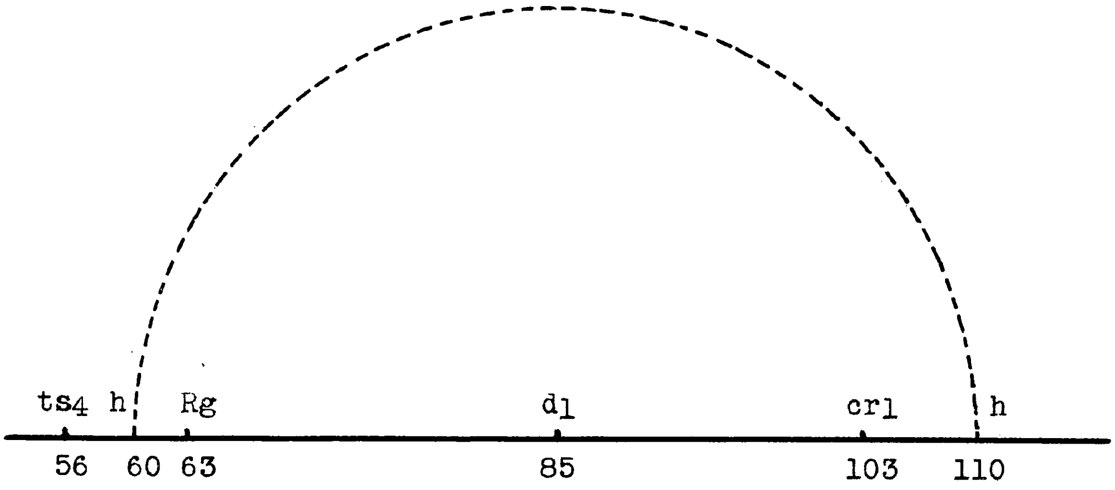

Figure 1.--A map of a portion of Chromosome III showing the possible locations of h, if we assume that it is linked with d_1 with 25 percent of crossing over.

b. Linkage Tests with fl_2

Several of the linkage groups were used to determine the group to which fl_2 belonged. Table 3 indicates that fl_2 is probably independent of Group 2, because fl_1 is in this group. The data have already been discussed. The segregation in the F_2 approximates a 1:3 ratio of horny to starchy, which is the expected ratio if fl_1 and fl_2 are independent.

The gene su_1 is located in Group 4. The results of a cross between fl_2 and su_1 have already been presented. (Table 7). The expected ratio if fl_2 and su_1 are independent is 3 horny : 3 starchy : 2 sugary. The deviations from the expected ratio were so small that it is highly probable the two genes are independent.

The gene Y for yellow endosperm was used as a tester in Group 6. The results obtained in a cross between y and fl_2 are presented in Table 15.

Table 15.--F_2 Ratio Obtained in a Cross Between Horny White (Fl_2Fl_2yy) and Floury Yellow (fl_2fl_2YY)

	Phenotypic classes			
	Fl_2Y	Fl_2y	fl_2Y	fl_2y
Observed	166	56	163	53
Expected	164	55	164	55
Difference	+ 2	+ 1	- 1	- 2

X_2 = .0560 P = .989

The results indicate that fl_2 is probably independent of Group 6.

The gene j for japonica plants was used as a tester in Group 8. Plants from 80 fl_2 seeds produced 58 normal and 22 japonica plants. Eighty-nine Fl_2 seeds from the same ear produced 63 normal and 20 japonica plants. A ratio of 3 normal : 1 japonica would be expected on the basis of independence. The ratios are so close to the expected that no linkage is suggested.

The gene li for lineate plants was used as a tester in Group 10. The results, for some unknown reason, gave lower counts of lineate plants for both fl_2 and Fl_2 seeds than were expected. (Table 16).

Table 16.--F_2 Ratios of Crosses
Between fl_2fl_2LiLi
and Fl_2Fl_2lili

	Normal	Lineate	Dev. from 3:1	P. E.	D/P. E.
Ear 1, fl_2 seeds	74	15	7	2.76	2.536
Fl_2 seeds	62	24	2.5	2.71	.923
Ear 2, fl_2 seeds	66	10	9	2.55	3.529
Fl_2 seeds	84	10	13.5	2.83	4.770
Total, fl_2 seeds	140	25	16	3.75	4.267
Fl_2 seeds	146	34	11	3.92	2.806

A ratio of 3 normal : 1 lineate was expected. While the expected number of lineate plants is lower for the fl_2 seeds than would be expected by chance, there has not been a corresponding increase from the Fl_2 seeds above a 3:1 ratio to indicate linkage.

No tests were made with Groups 1, 5, 7, and 9.

The best evidence of linkage of fl_2 with some other gene was found in the data on crosses between fl_2 and h. The expected ratio in the F_2 on the basis of independence would be 3 horny : 5 starchy, the same as in crosses between h and fl_1. However, the ratio was very nearly 1 horny : 3 starchy. The data are presented in Table 17. Out of a total of 5195 seeds classified, 1353 were horny and 3842 were starchy. The deviations of each ear exceed the probable error by more than four times and the deviation of the total exceeds its probable error by more than 25 times. This indicates that the deviations have not been compensating but have tended in one direction.

If h and fl_2 were linked very closely with no crossing over, they would segregate in a ratio of 1 horny : 3 starchy. The ratio actually is 26 percent horny : 74 percent starchy, which is the ratio that would be obtained with close linkage with four percent of crossing over.

If h is located in Group 3, then fl_2 must also belong to Group 3 only a short distance from h.

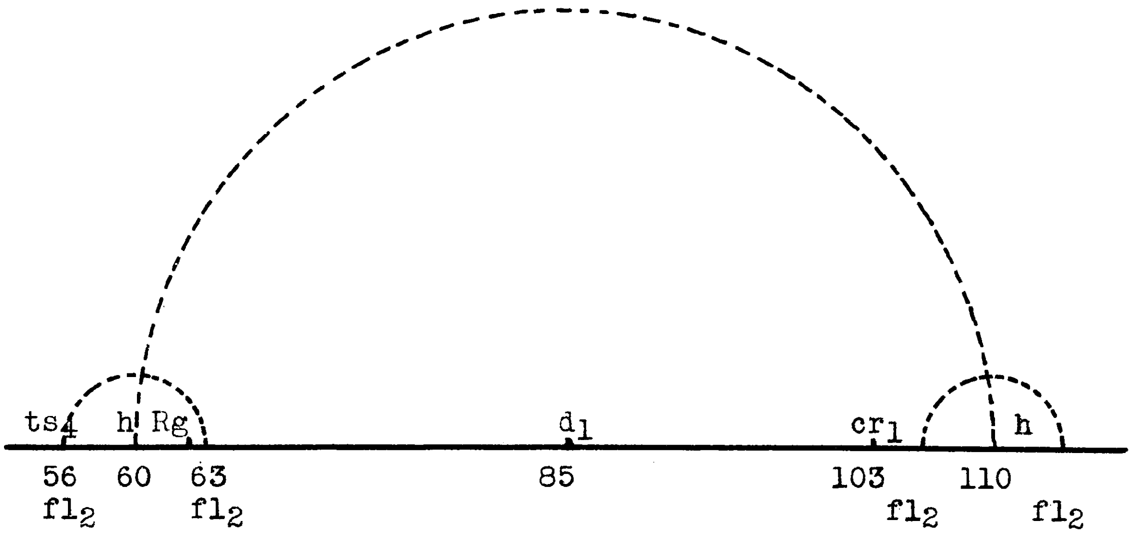

Figure 2.--Map of a portion of Chromosome III, showing the four possible positions of fl_2.

Reference to the map of Chromosome III shows that fl_2 may be near cr_1 on one side of d_1, or it may be near Rg or ts_4 on the other side of d_1, assuming that it is linked with h with four percent of crossing over.

Table 17.--F_2 Segregating Ratios in Crosses Involving fl_2 and h (fl_2fl_2HH x Fl_2Fl_2hh)

Ear number	Horny	Starchy	Total	Based on 3:5 ratio D.	P. E.	D/P. E.
157-1	53	155	208	25	4.71	5.31
157-2	19	74	93	15	3.15	4.76
157-3	202	472	674	50	8.48	5.90
157-4	94	246	340	34	6.02	5.65
158-1	60	170	230	26	4.95	5.25
158-2	160	449	609	68	8.06	8.44
158-3	167	363	530	32	7.52	4.26
158-4	180	479	659	67	8.38	8.00
2074-1	65	280	345	64	6.07	10.54
2074-2	46	217	263	53	5.30	10.00
2075-1	91	230	321	29	5.85	4.96
2094-1	81	266	347	49	6.08	8.06
2096-1	69	211	280	36	5.46	6.59
2100-1	66	230	296	45	5.62	8.01
Total	1 353	3 842	5 195	595	23.54	25.28

Calculations are based on ratio of 5 starchy : 3 horny, which would be the expected ratio if these two characters were independent.

Expected genotypic constitution of F_2 endosperms in a cross between floury 2 (fl_2fl_2HH) and starchy (Fl_2Fl_2hh):

Polar nucleii	Pollen	Phenotype
$Fl_2H \cdot Fl_2H$	(Fl_2H	Horny endosperm
	(Fl_2h	" "
	(fl_2H	" "
	(fl_2h	" "
$Fl_2h \cdot Fl_2h$	(Fl_2H	Horny endosperm
	(Fl_2h	Starchy "
	(fl_2H	Horny "
	(fl_2h	Starchy "
$fl_2H \cdot fl_2H$	(Fl_2H	Starchy endosperm
	(Fl_2h	" "
	(fl_2H	" "
	(fl_2h	" "
$fl_2h \cdot fl_2h$	(Fl_2H	Starchy endosperm
	(Fl_2h	" "
	(fl_2H	" "
	(fl_2h	" "

Expected ratio = 3 horny : 5 starchy

Expected proportion of horny and starchy seeds in crosses between fl_2fl_2 and hh, if linkage with four percent of crossing over be assumed:

Polar nucleii	Pollen	Phenotypes
1 $Fl_2H \cdot Fl_2H$	(1 Fl_2H	50 horny seeds
	(24 Fl_2h	
	(24 fl_2H	
	(1 fl_2h	
24 $Fl_2h \cdot Fl_2h$	(1 Fl_2H	600 horny seeds
	(24 Fl_2h	
	(24 fl_2H	
	(1 fl_2h	600 (hh) starchy seeds
24 $fl_2H \cdot fl_2H$	(1 Fl_2H	1200 (fl_2fl_2) starchy seeds
	(24 Fl_2h	
	(24 fl_2H	
	(1 fl_2h	
1 $fl_2h \cdot fl_2h$	(1 Fl_2H	50 (fl_2fl_2) starchy seeds
	(24 Fl_2h	
	(24 fl_2H	
	(1 fl_2h	

$\frac{650}{2500}$ = 26% horny seeds

VI. SUMMARY

Two new genes that produce starchy endosperms in dent corn are reported in this investigation. The genes have been designated as h and fl_2, respectively. The gene h is a simple recessive that, when crossed to horny, gives a 3:1 ratio of horny to starchy seeds in the F_2 generation. The gene h is hypostatic to wx and su_1. It gives 25 percent crossing over with d_1, and is therefore assumed to be in Chromosome III.

The gene fl_2 follows the same inheritance as fl_1, which has been reported previously by Hayes and East. Two doses of fl_2 in the endosperm are dominant over one dose of Fl_2 and the reverse is also true. The gene fl_2 is hypostatic to su_1. If the assumption is correct that h is located in Chromosome III, being linked with d_1, then fl_2 is also in Chromosome III, since it is closely linked (four percent of crossing over) with h.

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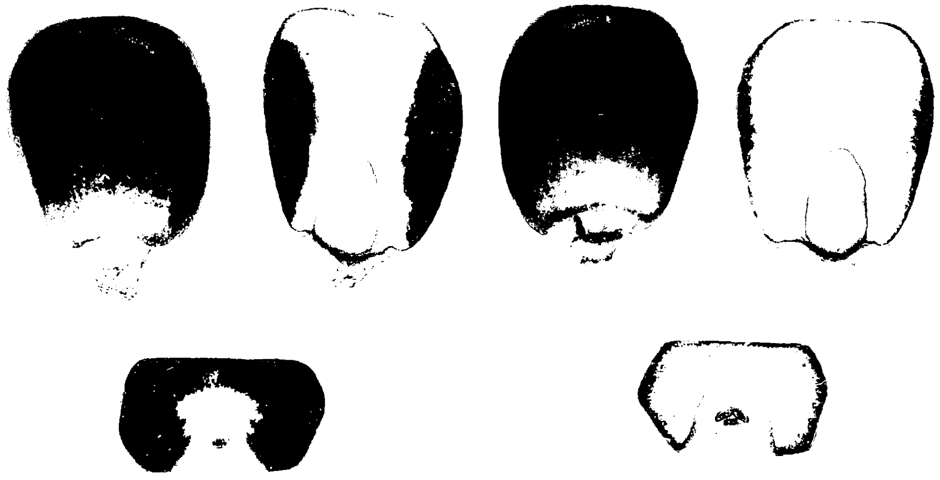
VIII. PLATES



The horny seeds are on the left and the starchy (h) seeds are on the right. Some of the seeds have been sectioned to show the difference in endosperm texture.

Plate 2.--Ears of the inbred line 3141. (A) and (D) are homozygous for HH and produced only horny seeds. (B) and (C) are heterozygous (Hh) and segregated in a ratio of 3 horny : 1 starchy. The starchy seeds have the wrinkled caps.

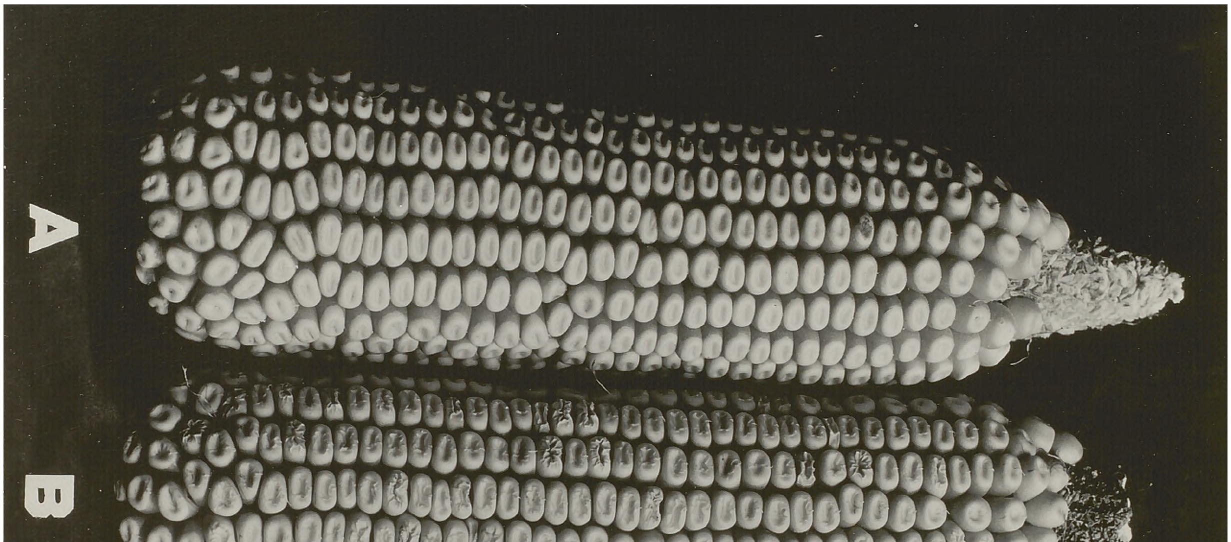
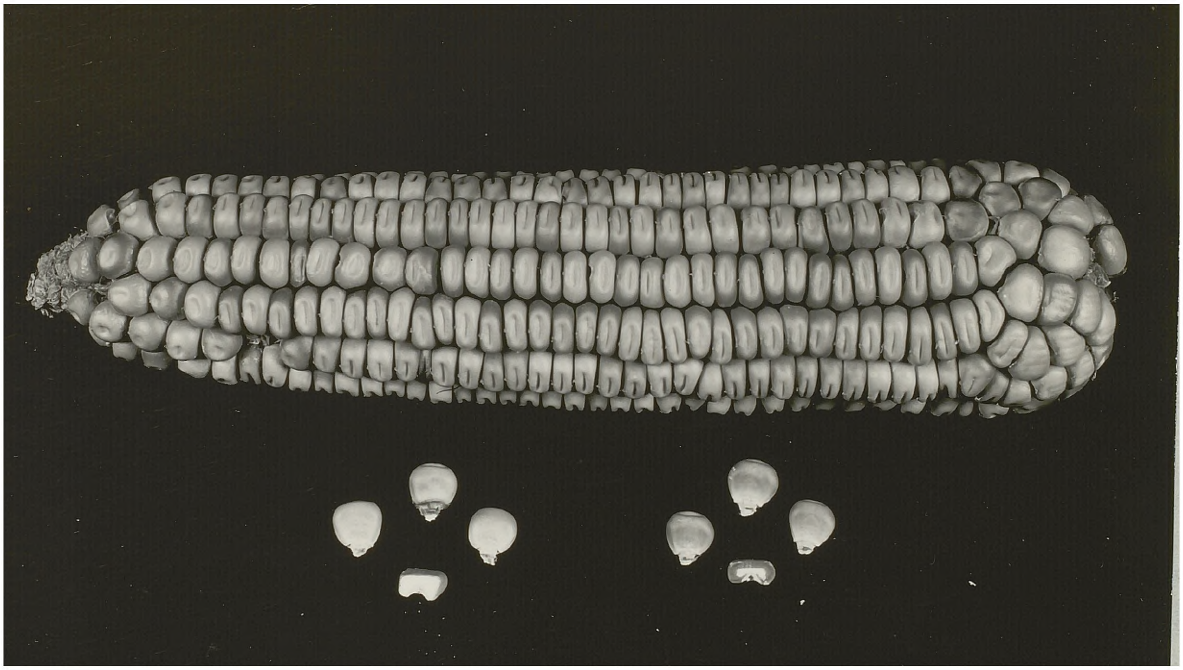

A
B
C
D

Plate 3.--Seeds from an ear segregating for fl_2. The horny seeds are on the left and the floury seeds are on the right. Some of the seeds have been sectioned to show the difference in endosperm texture.



has segregated in a ratio of 1 horny : 1 floury. The horny and floury seeds can be easily distinguished in the photograph.

Plate 5.--The ear on the left is homozygous fl_2 and the ear on the right is homozygous hh. Some of the caps have been removed to show the softness of the endosperm.

Plate 6.--Beginning at the left, Ear 1 is segregating for shrunken (sh), Ear 2 is homozygous fl$_1$, Ear 3 is homozygous h, and Ear 4 is homozygous fl$_2$.

IX. VITA

The writer was born on a farm near Sidney, Illinois, November 20, 1895. His early education was received in the rural school of that vicinity. In 1913 he was graduated from Sidney High School at the head of his class. In 1914 he graduated from Urbana High School where he took additional work necessary to meet the entrance requirements of the University of Illinois. In the fall of 1915 he entered the College of Agriculture at the University of Illinois.

In the spring of 1918 he enlisted in the Coast Artillery Corps of the United States Army. In July, 1918, he entered the Officers' Training School at Fort Monroe, Virginia and was commissioned as a Second Lieutenant in September. He served until his discharge in the Coast Defenses of Boston Harbor with the 61st Ammunition Train and the 28th Regiment, C. A. C. After his discharge, he returned to the University of Illinois. He received his Bachelor of Science degree at the end of Summer School, 1919.

From 1921 to 1926 he was engaged in farming on the home farm at Sidney. In February, 1927, he again entered the University of Illinois to do graduate work in the Department of Agronomy, specializing in Plant Breeding. In September, 1927, he was appointed assistant in the Department of Agronomy and the Experiment Station. In June, 1928, he received the degree of Master of Science in Agronomy. In March, 1929, he was appointed Instructor in the Division of Plant Breeding and First Assistant in the Experiment Station. In September, 1931, he was appointed Associate in Plant

Breeding and in the Experiment Station, serving in this capacity until February, 1938, when he resigned to become Hybrid Corn Specialist with Crow's Hybrid Corn Company at Milford, Illinois.

The writer is affiliated with Phi Sigma, Alpha Zeta, Gamma Sigma Delta, and Sigma Xi.

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