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The Segregation of Carbohydrates in Crosses between Waxy and Starchy Types of Maize

T. A. Kiesselbach
University of Nebraska-Lincoln

N. F. Petersen
University of Nebraska-Lincoln

THE SEGREGATION OF CARBOHYDRATES IN CROSSES BETWEEN WAXY AND STARCHY TYPES OF MAIZE[1]

T. A. KIESSELBACH AND N. F. PETERSEN
University of Nebraska, Lincoln, Nebraska

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The purpose of this paper is to report the Mendelian segregation of waxy and starchy carbohydrates in both the sporophytic and gametophytic generations of maize. This is of especial genetic interest because the counts of segregate waxy kernels have very commonly been short of the expected numbers, although this character appears to be a simple Mendelian recessive. COLLINS (1909) was the first to point out the physical difference in the endosperm of waxy and ordinary starchy varieties.

While the exact difference in their chemical nature has not been established, WEATHERWAX (1922) considered that the waxy carbohydrate was erythrodextrin because of its reddish color reaction to iodine as compared with the bluish staining of the endosperms of ordinary starchy varieties. DEMEREC (1924), BRINK AND MACGILLIVRAY (1924), AND LONGLEY (1924) have each established a corresponding differential staining of the pollen grains of the two maize sorts. BRINK (1925) has more recently reported finding the same chemical distinction for the carbohydrates of the embryo sacs. In a publication of earlier results the writers (1925) failed, through faulty technique, to observe the differential staining of the pollen, but later results conform with those of the above investigators. Our earlier

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observations as to the wide distribution of starch throughout the sporophytic vegetative tissues have been confirmed.

Taking into consideration the observations of all investigators, it appears that the waxy carbohydrate is restricted to haploid or 1x tissue including pollen, embryo sac, and possibly the tissue developed from the antipodals, and to the 3x tissue of the endosperm. Only ordinary starch has been found in the diploid or 2x tissues which make up the rest of the plant.

The results of this investigation as well as those of DEMEREC, BRINK AND MACGILLIVRAY, AND LONGLEY indicate that those segregated plants following hybridization which come true to endosperm type also come true to the same sort of carbohydrate in the pollen. This is evidence that a single chromosome factor controls both or else that they are very closely linked characters with no observed crossing over.

DISTINGUISHING WAXY AND STARCHY CARBOHYDRATES

Pollen

Repeated attempts to distinguish the pollen of Chinese waxy maize and its hybrids from ordinary starchy maize pollen by the iodine test during the summer of 1924, following receipt of the articles by DEMEREC, and BRINK and MACGILLIVRAY, resulted in failure so that we concluded that such a difference did not exist. Further tests during 1925 showed that with proper staining it is easy to distinguish the two types of pollen and show the segregation in hybrids.

The following method was used succesfully: The pollen was placed on a slide with a few drops of stock solution of iodine in potassium iodide[1] diluted with 3 parts of distilled water. The slide was then left uncovered, more iodine solution being added if necessary, until the pollen appeared sufficiently stained. A small crystal of chloral hydrate was then added as a clearing agent. When this was dissolved the cover glass was applied. The pollen should all be moistened at the same time in order to stain each type of pollen uniformly.

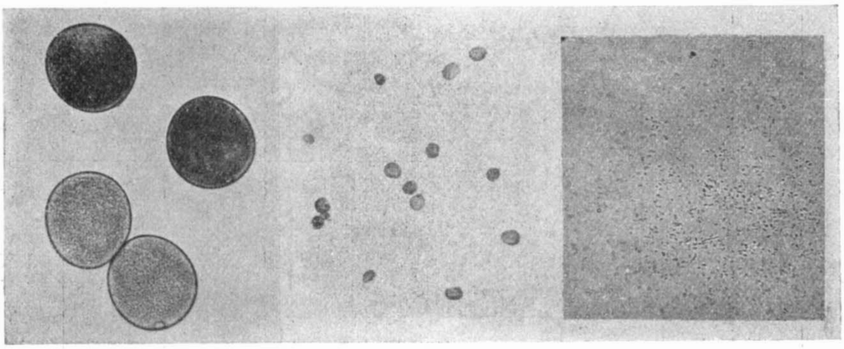
By this method the carbohydrate grains of the pollen of ordinary starchy maize are stained a deep blue, while those in waxy maize are stained red, leaving other parts of the pollen grains unstained and cleared. When pollen is stained in this manner, any imperfect pollen grains which lack carbohydrate reserves remain unstained and may be distinguished from the normal pollen. These methods apply to pollen stored dry as well as in the fresh condition.

[1] The stock solution was made by dissolving 1 gram of potassium iodide and 0.3 gram iodine in 100 cc. distilled water.

Endosperm

Satisfactory differential staining of the carbohydrates in the endosperm is more easily secured than in the case of the pollen, due, perhaps, to the much larger starch grains in the endosperm. (Figures 1b and 1c). If too strong iodine solution is used, there may be a tendency to produce such an intense stain that the endosperms all appear black rather than red or blue. The ordinary starchy endosperm seems to have a greater affinity for the iodine and will stain in a weaker solution than the waxy.

For extensive observations, rapid results may be secured by dipping the ears into iodine solution after the endosperms have been exposed by cut-



a b c

Figure 1a. Representative pollen grains from a heterozygous plant showing segregation into equal numbers of waxy and starchy grains which stain red and blue respectively, with iodine. Those which stain red and carry the factor for waxy are shown light in this photograph.

Figure 1b. Typical carbohydrate grains from the endosperm of maize, to be compared for size with those from pollen, shown in figure 1c.

Figure 1c. Typical carbohydrate grains from pollen to be compared for size with those from the endosperm of maize, shown in figure 1b.

ting off the crowns from the kernels. This may be done easily before the grain has hardened. The ears stain more promptly and evenly when first dipped momentarily in boiling water. After staining, the ears may be rinsed with tap water to remove loose starch which might cause confusion. Should the color fade before completing the counts, the ear may be restained.

Prior to the discovery of the difference in staining with iodine in 1922, all separations of starchy and waxy kernels were necessarily based on their difference in physical appearance. This can be done fairly accurately when maize is thoroughly cured. A dark-colored aleurone layer which is frequently present in crosses with Chinese maize tends to obscure the difference and makes such separation more difficult. If the starchy segregates are of a "soft flour" type, the distinction is far less certain than in the case of more horny texture.

TABLE 1

Pollen segregation in hybrids of waxy×starchy maize, as determined by the reaction to iodine.

TASSEL NO.	NUMBER STAINING			RATIO RED TO BLUE	TASSEL NO.	NUMBER STAINING			RATIO RED TO BLUE
	Blue	Red	No stain			Blue	Red	No stain	
				F_1 Reid Yellow Dent×Chinese					
1	125	124	2	1.00	6	193	152	3	0.79
2	94	92	..	0.99	7	118	112	..	0.95
3	131	156	5	1.19	8	210	217	2	1.03
4	102	137	2	1.34	9	289	271	1	0.95
5	175	189	4	1.08	10	294	314	..	1.06
					Total	1731	1764	19	1.02
				F_1 Hogue Yellow Dent×Chinese					
11	314	354	2	1.13	14	302	296	4	0.93
12	356	335	7	0.94	15	324	358	2	1.10
13	355	369	3	1.04	16	334	296	1	0.89
					Total	1985	2008	19	1.01
				F_2 Chinese×Yellow Flint					
17	295	292	..	0.99	37	167	184	6	1.10
18	128	157	..	1.23	38	308	310	8	1.01
19	340	284	..	0.84	39	341	346	2	1.01
20	321	279	..	0.87	40	73	94	..	1.29
21	190	198	..	1.04	41	264	269	..	1.02
22	424	493	..	1.16	42	109	109	..	1.00
23	191	180	6	0.94	43	186	170	..	0.91
24	139	122	5	0.88	44	143	129	5	0.90
25	336	315	2	0.94	45	75	57	..	0.76
26	225	229	8	1.02	46	56	55	..	0.98
27	242	327	21	1.35	47	196	215	6	1.10
28	201	252	..	1.25	48	267	323	1	1.21
29	424	428	..	1.01	49	299	301	9	1.01
30	207	191	7	0.92	50	748	734	..	0.98
31	112	116	..	1.04	51	236	257	..	1.09
32	201	206	..	1.02	52	296	308	..	1.04
33	35	43	63	1.23	53	384	378	14	0.98
34	28	25	..	0.89	54	290	261	3	0.90
35	347	392	21	1.13	55	41	45	2	1.10
36	118	85	36	0.72	56	315	298	..	0.95
					Total	9298	9457	225	1.02

SEGREGATION OF POLLEN

Counts were made for the pollen from 88 segregating plants, resulting in 13014 pollen grains stained blue and 13229 stained red. This is 49.59 percent blue and 50.41 percent red. The imperfect grains which often appear have not been included in calculating the ratios. The results are given in table 1. The variations from the 1:1 ratio are doubtless due mainly to the use of samples which were not exactly representative rather than to actual differences in number of the two types of pollen. This statement is confirmed by the lack of correlation between pollen and kernel ratios as shown in table 2.

DEMEREC (1924), and BRINK and MACGILLIVRAY (1924) reported that approximately equal numbers of the two types of pollen grains were found in plants heterozygous for the waxy character. LONGLEY (1924) has also reported such segregation in the pollen of crosses of waxy with starchy maize and teosinte, and in crosses between starchy and waxy Coix. PARNELL had previously reported segregation of the pollen in crosses between glutinous and ordinary rice.

SEGREGATION OF ENDOSPERM

A large number of ears were hand-pollinated with segregating pollen. These belong to 3 groups: (1) pure Chinese ears pollinated with F_1 waxy × starchy pollen; (2) F_1 waxy × starchy plants selfed; and (3) F_2 plants grown from starchy segregated seed, selfed. In the pure Chinese fertilized by F_1 pollen 50 percent waxy kernels would be expected while in the case of F_1 and F_2 ears, the expectation would be 25 percent waxy kernels.

The ear shoots were bagged sufficiently in advance of silking to insure against contamination by stray viable pollen. As an additional precaution the pure Chinese maize was grown in isolation and detasseled because this variety tends to silk while the shoots are small and still enclosed within the leaf sheath.

Segregation where only 1 parent is heterozygous.

In this test pure waxy maize was fertilized by pollen from F_1 starchy × waxy plants. Since the ovules all carried the recessive waxy factor, the ratio of the two types of pollen would determine the kernel segregation unless influenced by differential fertilization.

Sixty-six ears of this combination were obtained as reported in table 2. These ears had been fertilized by pollen from 9 F_1 Reid × Chinese plants whose pollen segregation had been established by the iodine stain. Of

18549 kernels produced 51.5 percent proved to be waxy as determined mainly by the iodine test.

TABLE 2

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

Ear No.	Number of kernels			Percent waxy kernels	Percent deviation of waxy	Dev./P.E.†	Pollen	
	Total	Waxy	Expected waxy				From tassel No. (Table 1)	Percent staining red*
1	343	180	171	53.5	3.5	1.44	1	49.8
2	386	186	193	48.2	−1.8	1.06	1	49.8
3	291	152	146	52.2	2.2	1.04	2	49.5
4	209	115	104	55.0	5.0	2.26	2	49.5
5	301	166	151	55.1	5.1	2.56	2	49.5
6	143	74	71	51.8	1.8	0.74	2	49.5
7	55	21	28	38.2	−11.8	3.11	2	49.5
8	127	69	63	54.3	4.3	1.58	2	49.5
9	222	98	111	44.1	−5.9	2.59	3	54.4
10	432	236	216	54.6	4.6	2.85	3	54.4
11	410	229	205	55.9	5.9	3.51	3	54.4
12	325	151	163	46.5	−3.5	1.97	3	54.4
13	398	229	199	57.5	7.5	4.46	3	54.4
14	348	182	174	52.3	2.3	1.27	3	54.4
15	306	171	153	55.9	5.9	3.05	4	57.3
16	120	67	60	55.8	5.8	1.90	4	57.3
17	273	121	137	44.3	−5.7	2.87	4	57.3
18	345	155	172	44.9	−5.1	2.72	4	57.3
19	420	223	210	53.1	3.1	1.88	4	57.3
20	389	175	195	45.0	−5.0	3.01	4	57.3
21	427	249	213	58.3	8.3	5.16	4	57.3
22	193	98	97	50.8	0.8	....	4	57.3
23	95	52	47	54.7	4.7	1.52	5	51.9
24	317	159	158	50.2	0.2	0.16	5	51.9
25	105	52	53	49.5	−0.5	0.29	5	51.9
26	160	73	80	45.6	−4.4	1.64	5	51.9
27	94	50	47	53.2	3.2	0.92	5	51.9
28	105	56	52	53.3	3.3	1.16	5	51.9
29	276	151	138	54.7	4.7	2.32	5	51.9
30	163	96	82	58.9	8.9	3.25	5	51.9
31	459	241	229	52.5	2.5	1.64	5	51.9
32	332	162	166	48.8	−1.2	0.65	5	51.9
33	272	160	136	58.8	8.8	4.32	5	51.9
34	241	130	121	54.5	4.5	1.71	5	51.9
35	254	143	127	56.3	6.3	2.98	5	51.9
36	346	188	173	54.3	4.3	2.39	5	51.9
37	323	199	162	61.6	11.6	4.46	5	51.9
38	366	182	183	49.7	−0.3	0.15	5	51.9
39	401	192	201	47.9	−2.1	1.33	5	51.9
40	456	196	228	43.0	−7.0	4.44	5	51.9
41	448	208	224	46.4	−3.6	2.24	5	51.9
42	339	165	170	48.7	−1.3	0.81	6	44.1

TABLE 2 (continued)

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

EAR No.	NUMBER OF KERNELS: Total	Waxy	Expected waxy	PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV. / P.E.†	POLLEN: From tassel No. (Table 1)	Percent staining red*
43	348	184	174	52.9	2.9	1.59	6	44.1
44	286	133	143	46.5	−3.5	1.75	6	44.1
45	357	166	178	46.5	−3.5	1.88	6	44.1
46	360	171	180	47.5	−2.5	1.41	6	44.1
47	251	133	125	53.0	3.0	1.27	7	48.7
48	305	142	153	46.6	−3.4	1.87	7	48.7
49	335	172	167	51.3	1.3	0.81	7	48.7
50	428	193	214	45.1	−4.9	3.01	7	48.7
51	190	107	95	56.3	6.3	2.58	9	48.4
52	261	145	131	55.6	5.6	2.57	9	48.4
53	205	113	102	55.1	5.1	2.28	9	48.4
54	412	214	206	51.9	1.9	1.17	9	48.4
55	325	164	163	50.5	0.5	0.16	9	48.4
56	425	250	212	58.8	8.8	5.47	9	48.4
57	273	143	137	52.4	2.4	1.08	10	51.6
58	65	37	32	56.9	6.9	1.84	10	51.6
59	78	43	39	55.1	5.1	1.34	10	51.6
60	232	126	116	54.3	4.3	1.95	10	51.6
61	215	113	108	52.6	2.6	1.01	10	51.6
62	81	39	40	48.2	−1.8	3.29	10	51.6
63	243	130	122	53.5	3.5	1.52	10	51.6
64	171	86	85	50.3	0.3	2.27	10	51.6
65	342	175	171	51.2	1.2	0.64	10	51.6
66	346	166	173	48.0	−2.0	1.11	10	51.6
Total	18549	9547	9275	51.5	1.5	5.92		
Ears grouped according to source of pollen								
1–2	729	368	365	50.5	0.5	0.33	1	49.8
3–8	1126	597	563	53.0	3.0	3.00	2	49.5
9–14	2135	1125	1068	52.7	2.7	3.66	3	54.4
15–22	2473	1259	1236	50.9	0.9	1.37	4	57.3
23–41	5213	2690	2606	51.6	1.6	2.45	5	51.9
42–46	1690	819	845	48.5	−1.5	1.88	6	44.4
47–50	1319	640	660	48.5	−1.5	1.63	7	48.7
51–56	1818	993	909	54.6	4.6	5.85	9	48.4
57–66	2046	1058	1023	51.7	1.7	2.30	10	51.6
1–66	18549	9547	9275	51.5	1.5	5.92		50.5

†Probable errors were calculated by the usual formula: $.6745\sqrt{P \times Q} \times \sqrt{\text{Total number}}$

*Imperfect pollen grains which failed to stain were not included in the percentage calculation.

In comparison with these results (table 3) in which the male parent was segregating, KEMPTON (1919) found an average shortage of 0.8 percent for the waxy kernels although one combination involving 8791 kernels showed no deficiency. Data reported by BREGGER (1918) in connection with linkage studies show a one-percent shortage from the expected number of waxy kernels. With 33020 kernels, BRINK (1925) shows a deficiency of 1.7 percent. Summarizing all of the results, there are 203 ears with 79,381 kernels of which 49.3 percent are waxy.

TABLE 3

Historical summary showing the endosperm segregation in pure waxy maize fertilized by segregating pollen from waxy×starchy hybrids. The expected ratio is 50 percent waxy kernels.

INVESTIGATORS AND THEIR TABLE NUMBERS	NUMBER OF EARS	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	PERCENT OF EARS BELOW 50% WAXY
		Total	Waxy	Expected waxy				
KEMPTON (1919) Table 3	27	11,724	5,802	5,862	49.6	−0.4	1.65	56
KEMPTON (1919) Table 6	20	8,791	4,397	4,396	50.0	0.0	0.00	45
KEMPTON (1919) Table 10	11	6,138	2,925	3,069	47.7	−2.3	5.44	73
Total—KEMPTON	58	26,653	13,124	13,327	49.2	−0.8	3.69	55
BREGGER (1918)	4	1,159	568	580	49.0	−1.0	1.05	50
BRINK (1925) Tables 15–16	75	33,020	15,934	16,510	48.3	−1.7	9.38	67
KIESSELBACH AND PETERSEN (1926) Table 2	66	18,549	9,547	9,275	51.5	1.5	5.92	33
Total	203	79,381	39,173	39,690	49.3	−0.7	5.44	52

In reverse combinations in which the female was the segregating parent, 5 investigators (table 4) report segregations which total a deficiency of 0.02 percent from the expected percentage of waxy kernels. These tests represent a total of 198 ears and 90,944 kernels.

Segregation where both parents are heterozygous.

In these tests both F_1 and F_2 hybrids were included. Since half of the ovules carry the dominant starchy factor, the kernels which result from them will be starchy regardless of the type of pollen. The ratio of the waxy and starchy kernels produced from the other half will be determined by the ratio of the 2 types of functioning pollen grains. Thus approximately a 3:1 ratio is expected in these combinations. The ears of two varietal F_1 crosses, Reid × Chinese and Chinese × Hogue were selfed for an investigation of kernel segregation. A total of 39 selfed ears were obtained, 23 of the former and 16 of the latter cross (table 5). Of a total of 12,376 kernels in the Reid × Chinese cross, 23.2 percent were waxy, while 25.1 percent of the 8,908 Chinese × Hogue kernels were waxy. Twenty-four

TABLE 4

Historical summary showing the endosperm segregation in heterozygous ears from waxy ×starchy hybrids fertilized by pollen from pure waxy maize. The expected ratio is 50 percent waxy kernels.

INVESTIGATORS AND THEIR TABLE NUMBERS	NUMBER OF EARS	NUMBER OF KERNELS			PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	PERCENT OF EARS BELOW 50% WAXY
		Total	Waxy	Expected waxy				
COLLINS and KEMPTON (1911) Table 2	2	1,228	526	614	42.8	−7.2	7.5	100
KEMPTON (1919) Table 4	39	15,305	7,817	7,653	51.0	1.0	3.93	31
KEMPTON (1919) Table 7	26	13,027	6,447	6,514	49.5	−0.5	1.71	62
KEMPTON (1919) Table 11	8	2,866	1,451	1,433	50.7	0.7	1.00	38
Total—COLLINS and KEMPTON, and KEMPTON	75	32,426	16,241	16,213	50.1	0.1	..	80
HUTCHISON Tables 4, 5, 6, and 7	35	19,032	9,583	9,516	50.4	1.4	1.55	46
BREGGER (1918)	6	2,437	1,173	1,219	48.1	−1.9	2.76	67
BRINK (1925) Tables 13, 14	82	37,049	18,457	18,525	49.8	−0.2	1.05	56
Total	198	90,944	45,454	45,472	49.98	−0.02	0.18	50

percent of all the kernels from both combinations were waxy. In another test starchy segregate seed from selfed F_1 ears of a Chinese × Flint cross were planted for a further study of segregation in the F_2 generation. Of the 101 selfed ears produced, 68 were found to be segregating. This corresponds almost exactly with the expected 2:1 ratio. Of the 19,126 kernels (table 6) produced on these segregating ears, 24.2 percent were of the waxy type.

TABLE 5

Endosperm segregation in selfed F_1 waxy×starchy maize hybrids.

EAR NO.	NUMBER OF KERNELS Total	Waxy	Expected waxy	PERCENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL NO. (Table 1)
			Reid Yellow Dent×Chinese				
1	502	141	126	28.1	3.1	2.29	*
2	609	137	152	22.5	−2.5	2.08	*
3	765	170	191	22.2	−2.8	2.60	*
4	726	177	182	24.4	−0.6	0.64	*
5	638	146	159	22.9	−2.1	1.76	*
6	802	181	201	22.6	−2.4	2.42	1
7	734	172	183	23.4	−1.6	1.39	2
8	22	3	5	13.6	−11.4	1.47	3
9	490	112	122	22.9	−2.1	1.55	*
10	571	133	143	23.3	−1.7	1.43	4
11	879	235	220	26.7	1.7	1.73	5
12	240	51	60	21.3	−3.7	1.99	7
13	140	42	35	30.0	5.0	2.03	8
14	597	143	149	24.0	−1.0	0.84	9
15	304	78	76	25.7	0.7	0.39	*
16	263	68	66	25.9	0.9	0.42	*
{17†	762	172	190	22.6	−2.4	2.23	10
{18	337	75	84	22.3	−2.7	1.68	*
19	464	84	116	18.1	−6.9	5.09	*
20	671	135	168	20.1	−4.9	4.37	*
21	695	157	174	22.6	−2.4	2.21	*
22	602	131	151	21.8	−3.2	2.79	*
23	563	124	141	22.0	−3.0	2.45	*
Total	12376	2868	3094	23.2	−1.8	6.93	
			Chinese×Hogue Yellow Dent				
24	592	147	148	24.8	−0.2	0.14	*
25	596	140	149	23.5	−1.5	1.26	*
26	575	136	144	23.7	−1.3	1.14	*
27	583	152	146	26.2	1.2	0.85	*
28	668	180	167	26.9	1.9	1.72	*
29	789	212	197	26.9	1.9	1.83	*
30	191	47	48	24.6	−0.4	0.25	11
31	579	165	145	28.5	3.5	2.84	*
32	428	102	107	23.8	−1.2	0.83	12
{33	646	180	162	27.9	2.9	2.43	*
{34	362	113	91	31.2	6.2	3.96	*
35	86	49	72	17.1	−7.9	4.65	*
36	605	136	151	22.5	−2.5	2.09	*
37	808	195	202	24.1	−0.9	0.98	*
{38	637	140	159	22.0	−3.0	2.59	*
{39	563	139	141	24.7	−0.1	0.29	*
Total	8908	2233	2227	25.1	0.1	0.22	
Total of both crosses	21284	5101	5321	24.0	−1.0	5.14	

*Where tassel numbers are omitted the pollen had not been examined

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

EAR No.	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL NO. (Table 7)
	Total	Waxy	Expected waxy				
1	312	60	78	19.2	−5.8	3.49	*
{2†	295	67	74	22.7	−2.3	1.39	14
{ 3	288	63	72	21.9	−3.1	1.83	14
4	328	89	82	27.4	2.4	1.32	18
5	313	78	78	24.9	−0.1	0.19	*
6	296	79	74	26.7	1.7	1.00	19
7	127	31	32	24.4	−0.6	0.30	*
8	120	24	30	20.0	−5.0	1.86	*
9	224	56	56	25.0	0.0	....	22
10	328	82	82	25.0	0.0	....	*
{11	270	56	68	20.7	−4.3	2.52	24
{12	251	66	63	26.3	1.3	0.65	24
13	417	89	104	21.3	−3.7	2.68	25
14	118	30	30	25.4	0.4	....	26
15	305	80	76	26.2	1.2	0.78	27
16	158	35	40	22.2	−2.8	1.63	28
17	346	80	86	23.1	−1.9	1.10	29
18	209	58	52	27.8	2.8	1.42	32
19	151	39	38	25.8	0.8	0.28	33
20	463	128	116	27.7	2.7	1.91	34
21	303	79	76	26.1	1.1	0.59	35
22	301	72	75	23.9	−1.1	0.59	37
23	379	105	95	27.7	2.7	1.76	38
24	347	84	87	24.2	−0.8	0.55	39
25	126	26	32	20.6	−4.4	1.83	42
26	74	19	18	25.7	0.7	0.40	47
27	202	56	51	27.7	2.7	1.20	50
28	80	19	20	23.8	−1.2	0.38	55
{29	393	99	98	25.2	0.2	1.72	*
{30	193	49	48	25.4	0.4	0.25	*
{31	345	83	86	24.1	−0.9	0.55	*
{32	219	49	55	22.4	−2.6	1.39	*
33	367	98	92	26.7	1.7	1.07	*
34	243	52	61	21.4	−3.6	1.98	*
35	377	89	94	23.6	−1.4	1.06	*
36	200	49	50	24.3	−0.7	0.24	*
37	337	93	84	27.6	2.6	1.68	*
38	219	58	55	26.5	1.5	0.69	*
39	66	25	17	37.9	12.9	3.38	*
40	337	82	84	24.3	−0.7	0.37	*
41	357	87	89	24.4	−0.6	0.36	*
42	117	35	29	29.9	4.9	1.90	*

*Where tassel numbers are omitted the pollen had not been examined.

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6 (continued)

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

EAR NO.	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL NO. (Table 7)
	Total	Waxy	Expected waxy				
43	421	97	105	23.0	−2.0	1.82	44
44	312	72	78	23.1	−1.9	1.16	48
45	400	108	100	27.0	2.0	1.37	*
46	188	48	47	25.5	0.5	0.25	*
47	236	48	59	20.3	−4.7	2.84	*
48	357	89	89	24.9	−0.1	..	*
49	354	92	89	26.0	1.0	0.55	*
50	292	69	73	23.6	−1.4	1.60	*
{51	342	66	85	19.3	−5.7	4.26	*
52	315	60	79	19.0	−6.0	3.67	*
{53	479	114	120	23.8	−1.2	1.25	*
54	370	77	92	20.8	−4.2	3.38	*
55	206	38	52	18.4	−6.6	3.34	*
56	172	38	43	22.1	−2.9	0.31	*
57	214	46	53	21.5	−3.5	1.64	*
58	44	12	11	27.3	2.3	0.52	51
59	249	52	62	20.9	−4.1	2.17	*
60	349	86	87	24.6	−0.4	0.37	*
61	389	72	97	18.5	−6.5	4.34	*
62	475	115	119	24.2	−0.8	0.63	*
63	362	94	90	26.0	1.0	0.72	*
64	538	129	135	24.0	−1.0	0.98	*
65	381	130	95	34.1	9.1	6.14	*
66	264	72	66	27.3	2.3	1.26	*
67	208	34	52	16.4	−8.6	4.28	*
68	308	67	77	21.8	−3.2	1.95	*
Total	19126	4623	4781	24.2	−0.8	3.91	

*Where tassel numbers are omitted, the pollen had not been examined.
†The bracket indicates that the two ears were borne on the same plant.

A summary of the results (table 7) obtained by COLLINS and KEMPTON (1911), and KEMPTON (1919) from crosses corresponding with the above in that both parents were heterozygous show that of 102,429 kernels 23.9 percent were waxy. In observations by BRINK (1925) with 10,032 kernels, 22.9 percent were of the waxy type. Summarizing all data where both parents were heterozygous, 356 ears including 152,871 kernels resulted in 23.9 percent waxy kernels.

TABLE 7

Historical summary showing the endosperm segregation where both parents are heterozygous for the waxy character. The expected ratio is 25 percent waxy kernels

Investigators and their table numbers	No. of ears	Number of kernels: Total	Waxy	Expected waxy	Percent waxy	Percent deviation of waxy	Dev./P.E.	Percent of ears below 25 % waxy
Collins and Kempton (1911) Table 1	45	22,339	5,179	5,585	23.1	-1.9	9.24	64
Kempton (1919) Table 1	96	54,759	13,088	13,690	23.9	-1.1	8.8	65
Kempton (1919) Table 4	28	12,474	3,114	3,118	25.0	0.0	0.1	54
Kempton (1919) Table 7	17	8,177	1,981	2,044	24.3	-0.7	2.4	71
Kempton (1919) Table 9	4	1,055	257	264	24.3	-0.7	0.74	50
Kempton (1919) Table 11	8	3,625	881	906	24.3	-0.7	1.42	62
Total, Collins and Kempton, and Kempton	198	102,429	24,500	25,607	23.9	-1.1	11.85	64
Brink* (1925) Table 7	15	1,263	258	316	20.4	-4.6	5.59	80
Brink, Table 8	13	3,569	855	892	24.0	-1.0	2.13	69
Brink, Table 9	6	1,675	384	419	22.9	-2.1	2.93	100
Brink, Table 10	7	1,717	399	429	23.2	-1.8	2.48	86
Brink, Table 11	4	869	209	217	24.1	-0.9	0.92	50
Brink, Table 12	6	939	195	235	20.8	-4.2	4.47	67
Total, Brink	51	10,032	2,300	2,508	22.9	-2.1	7.11	78
Kiesselbach and Petersen (1926) Table 5	39	21,284	5,101	5,321	24.0	-1.0	5.14	67
Same, Table 6	68	19,126	4,623	4,782	24.2	-0.8	3.91	59
Total, Kiesselbach and Petersen	107	40,310	9,723	10,078	24.1	-0.9	6.06	63
Total all investigators	356	152,871	36,523	38,218	23.9	-1.1	14.85	65

*In table 7 the silks were allowed to grow extra long before pollination, while in table 8 normal silks were used for comparison. In table 9, 10, and 11, the pollen had been treated with ultraviolet light, and in table 12, the pollen had been desiccated before use.

DEVIATION FROM EXPECTED RATIOS

Several explanations have been offered to account for the deviations from expected ratios noted throughout the foregoing investigations. The totals for most groups of ears fertilized by segregating pollen have shown such consistent deficiency of waxy kernels as to appear significant. The suggestions by KEMPTON (1919) to account for the deficiency may be summarized as follows:

(1) The two types of gametes may form in unequal numbers.

(2) There may be a differential death rate of the gametes during their formation so that equal numbers do not function.

(3) Lack of vigor of the pollen carrying the factor for waxy causing slower growth of their pollen tubes.

(4) Differential selection of sperms by female gametes.

(5) Greater death rate of zygotes homozygous for waxy.

(6) Mistakes of kernel classification.

After the discovery of the difference in the chemical nature of the carbohydrate reserves of the pollen, BRINK and MACGILLIVRAY (1924) suggested that the deficiency of waxy kernels may be associated with a difference in the metabolism of the pollen leading to a slower growth of the pollen tubes carrying the waxy factor.

BRINK (1925) has indicated that there may be other factors operating in conjunction with waxy which causes a deviation in the number of waxy kernels by influencing the rate of pollen tube growth. Such a factor resulting in a deviation in the number of sweet kernels has been reported by JONES and MANGELSDORF (1925). WENTZ (1925) has also presented a case which may be accounted for by such factors.

In addition to the above possible explanations the following are offered:

(1) Differential retention of viability by pollen under the artificial conditions frequently accompanying control pollination. BRINK'S experiment in which the deficiency of waxy kernels was increased by desiccating the pollen over calcium chloride suggest differential viability in stored pollen.

(2) Accidental contamination by stray pollen.

(3) Since the starchy outweighed the waxy in paired segregating kernels on a number of ears examined, it would seem that the starchy pollen may also have a greater quantity of carbohydrate reserve, resulting in differential pollen tube growth.

CONCLUSIONS

An examination of all available data leads to the following conclusions:

The endosperm of certain maize has come to be known as *waxy* because of its peculiar appearance which distinguishes it from the endosperms of other types of maize. This is due to the presence of a carbohydrate which stains red with iodine, while ordinary starch stains blue.

This term, *waxy*, has been extended for convenience to this carbohydrate wherever found in other parts of the plants, and to the factor which determines it.

Waxy carbohydrate occurs in the pollen and embryo sacs as well as endosperms of waxy maize and so far as known is limited to these regions. The presence of this carbohydrate in these three regions appears to be determined by the same factor.

In crosses with starchy maize this waxy character behaves as a simple Mendelian recessive. The hybrid kernels have starchy endosperms. When planted no waxy carbohydrate appears in the plant until after the reduction division when equal numbers of gametophytes with the starchy or waxy carbohydrate (pollen and embryo sacs) are found as shown by the iodine test.

Kernel segregation for waxy endosperm occurs on ears produced by (1) homozygous waxy plants fertilized by segregating pollen, (2) plants heterozygous for waxy, fertilized by pure waxy pollen, and (3) plants heterozygous for waxy fertilized by segregating pollen.

In the first two combinations 50 percent waxy kernels would be expected and 25 percent waxy in the last case, if free from differential fertilization. However, the combined results of all investigators for those combinations in which segregating pollen was used show a slight deficiency of waxy kernels.

Of the various explanations that have been offered for this deficiency the most plausible would seem to be, (1) unequal fertilization resulting from differential pollen tube growth dependent upon the waxy factor or a linked factor, and (2) systematic errors connected with either the pollinations or with the kernel classification.

The theory of such differential pollen tube growth is supported primarily by the fact that when all results are combined, there is a deficiency of 0.7 percent waxy kernels from the expected 50 percent ratio and 1.1 percent deficiency from the expected 25 percent ratio when segregating pollen was used, whereas no deviation from expected ratios resulted when pure waxy pollen and segregating ovules were used. The greater deficiency secured by BRINK with extra long silks tends further to substantiate this.

Based on probabilities the deviation from the expected ratio, if due to slower pollen tube growth associated with the waxy factor, should be twice as large when only the male parent is segregating as when both parents segregate. This is contrary to the results stated above and suggests that other causes are involved. This is further indicated by the fact that only 60 percent of the total combined number of 559 ears fertilized by segregating pollen showed a deficiency of waxy kernels, and also by the fact that certain rather large groups of such ears were free from deficiency.

The evidence at hand seems insufficient to definitely establish the causes or the significance of the deviations herein reviewed.

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