THE EXPRESSION OF MENDELIAN FACTORS IN THE GAMETOPHYTE OF MAIZE

P. C. MANGELSDORF AND D. F. JONES
Connecticut Agricultural Experiment Station, New Haven, Conn.[1]

Received March 1, 1925

TABLE OF CONTENTS

DISTORTED RATIOS IN DEFECTIVE SEEDS

In 1918 a number of plants of a large-seeded, yellow, variety of flint corn, known as Gold Nugget, were self-pollinated. Nineteen selfed ears were secured and among these were three which segregated for a type of endosperm abnormality to which has been applied (JONES 1920) the term "defective seeds". Defective seeds may be described as lethal or semi-lethal characters in which the endosperm and embryo are greatly reduced in size or almost completely lacking. These characters appear very frequently when maize is inbred and it has been shown (MANGELSDORF 1923, 1926) that there are many genetically distinct types of this abnormality.

The defective which appeared in the Gold Nugget variety and to which the factor symbol de_1 has been given, is a semi-lethal type. The recessive seeds are characterized by a smaller size and aborted, shrivelled appearance. Their development is about half that of normal seeds on the same ears and they can, in most cases, be readily and accurately separated from the latter.

---

[1] Also contribution from the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

In 1920 the strain carrying the de_1 factor was crossed with a defective from another variety. In the F_2 endosperm generation of this cross, three ears segregating for defective seeds of the de_1 type were obtained. These ears bore 567 normal and 194 defective seeds, almost a perfect 3:1 ratio. The natural conclusion was that this character is inherited as a simple Mendelian recessive.

In 1922 a de_1 strain of slightly different pedigree from the one used in 1920, but closely related to the latter, served as a pollen parent in crosses with five different unrelated stocks. In the F_2 generation of these crosses fourteen progenies segregating for the parental de_1 type were recovered.

TABLE 1

Progenies segregating for de_1 in the F_2 endosperm generation of crosses made in 1922.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
22	160	49	−3	.7	23.4
24	154	63	9	2.1	29.0
25	183	90	22	4.6	33.0
42	184	55	−5	1.2	23.0
126	428	126	−15	2.2	22.7
128	265	90	1	.2	25.4
129	369	180	43	6.3	32.8
173	200	93	20	4.0	31.7
388	498	197	23	3.0	28.3
389	424	155	6	.9	26.8
391	127	61	14	3.5	32.4
392	328	82	−21	3.6	20.0
393	284	97	2	.3	25.5
1520	346	175	45	6.8	33.6
Total	3950	1513	147	6.8	27.7

When the counts of normal and defective seeds on these ears were combined (table 1) it was found that the defectives occurred in excess of the theoretical expectation. The average percentage of defectives in this group was 27.7. This represents a deviation from a 3:1 ratio of 6.8 times the probable error and is undoubtedly significant. A deviation of this size should occur, by chance alone, only once in several hundred thousand trials. The excess of recessives is especially noteworthy because defectives of other types frequently show small deficiencies (MANGELSDORF 1926).

The reason for the difference between the percentage of recessives in the 1920 and 1922 crosses apparently lies in the different strains of de_1 which were used as parents. Table 2 shows three successive generations of segregating ears of the original Gold Nugget strain. The proportion of normal to defective seeds in this strain had always approximated a 3:1 ratio and the average percentage of recessives for the entire group is 25.5. It is noted, however, that the two of the ten ears showed an excess of defectives of three or more times the probable error. It so happens that one of these excess ears, 105-9-8-2, was used as the pollen parent in the crosses made in 1922, before the significant deviation from a normal ratio had been noted. The 1920 cross, however, which gave normal segregation in F_2 was made with plants grown from ear 105-9-7 which itself segregated normally. Apparently the excess of recessives in the second generation of the 1922 crosses traces back to some condition which caused an excess in the parental ear.

TABLE 2

Segregating progenies of three successive generations of inbreeding of a "defective" strain of Gold Nugget.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	$\frac{\text{DEVIATION}}{\text{P.E.}}$	PERCENT DEFECTIVE
105-9	250	73	−8	1.5	22.6
105-9-3	117	49	8	2.2	29.5
105-9-4	159	46	−5	1.2	22.4
105-9-5	146	44	−3	.7	23.2
105-9-6	185	49	−9	2.0	20.9
105-9-7	243	75	−5	1.0	23.6
105-9-8	180	78	14	3.0	30.2
105-9-9	213	66	−4	.8	23.7
105-9-8-1	202	61	−5	1.1	23.2
105-9-8-2	134	84	30	7.0	38.5
Total and Average	1829	625	11	.8	25.5

The average percentage of defective seeds, 27.7 percent, in the fourteen ears shown in table 1 represents a significant excess but is not as great a deviation as occurred in the parental ear. When the ears in this group are considered separately, however, it is noted that seven of them show no significant departures from a 3 : 1 ratio; one shows a deficiency and the remaining six an excess greater than three times the probable error. Deviations of three times the error are expected, by chance alone, fairly frequently but in this case half of the ears show marked departures. Clearly the group is not a homogeneous one.

When the six ears which show significant plus deviations are combined, the average for the group is 31.9 percent recessives as compared to 25.1 percent for the non-deviating ears and 20.0 percent for the single ear with a significant minus deviation. In other words this lot of fourteen ears can be divided, on the basis of the deviations, into three distinct groups with *high*, *normal* and *low* defective ratios. A deviation of three times the probable error has been arbitrarily chosen as the dividing point.

If the *high* and *low* defective ears are merely the result of chance deviations, then progenies grown from such ears should give approximately normal ratios in F_3. If, however, these deviations have a genetic basis, distorted ratios might again be expected in F_3.

TABLE 3

Segregating F_3 progenies from "high defective" F_2 ear No. 391.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
813	174	85	20	4.3	32.8
822	227	99	18	3.4	30.4
Total	401	184	38	5.4	31.5
Progenies with significant deficiency					
810	108	15	−16	4.9	12.2
811	319	63	−33	5.8	16.5
815	235	56	−17	3.4	19.2
Total	662	134	−65	7.9	16.8
Progenies with no significant deviation					
817	275	83	−7	1.3	23.2
819	130	55	9	2.3	29.7
820	273	89	−2	.4	24.6
Total	678	227	1	.1	25.1
Grand Total	1741	545	−27	1.9	23.8

Third generation progenies of two of the *high* defective ears, 391 and 1520, have been grown and the results are presented in tables 3 and 4.

Twelve progenies were obtained from ear 391 (32.4 percent recessives in F_2), of which eight were segregating for defective seeds. Although the average percentage of recessives for this group is approximately normal, 23.8 percent, it is noted that five of the eight ears deviate from a normal ratio by amounts greater than three times the probable error. Two ears are *high* with an average of 31.5 percent defectives, three are *low* with an average of 16.8 percent recessives and the remaining three are *normal* with an average of 25.1 percent.

In connection with a possible explanation of the distorted ratios in waxy endosperm, presented later, particular attention is called to the difference between the percentage of defectives in the parental F_2 ear (32.4), and the average percentage of defectives in its progenies (23.8). The average of the F_3 progenies approached closely the normal expectation and it is only when the ears are considered separately that irregularities are noted.

Seventeen F_3 progenies were obtained from ear 1520 (33.6 percent recessives in F_2), of which ten were segregating. Four of the segregating ears were *high* with an average of 32.8 percent defectives, one *low*, with 16.8 percent, and five were *normal* with 25.3 percent defectives.

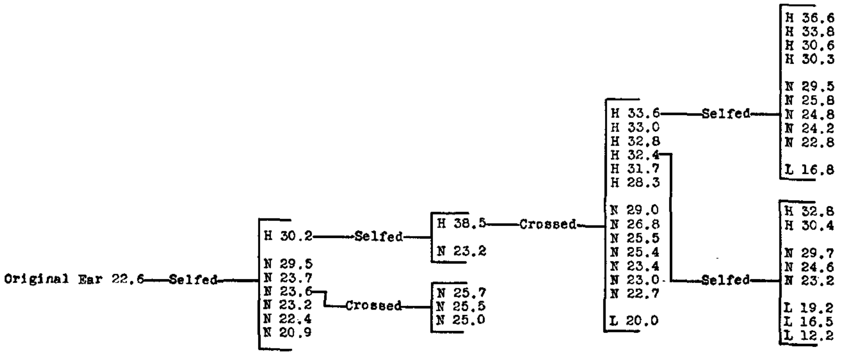

FIGURE 1. Pedigree record of the de_1 stock showing the inheritance of deviations from normal ratios. The letters H and L represent ears with plus or minus deviations greater than three times the probable error. N represents ears in which the deviation is less than three times the error.

Combining the 18 segregating progenies from these two high defective ears we find that six are *high* with an average of 32.4 percent recessives, four are *low* with 16.8 percent recessives and eight are *normal* with 25.2 percent recessives. The average for the entire F_3 group is 26.4 percent as compared to 33.3 percent for the two parental F_2 ears, a difference of practically seven percent between F_2 and F_3.

Obviously the marked departures from normal ratios in ten of the eighteen F_3 ears cannot be attributed to chance alone. Nor can the results be explained by faulty classification since errors of this sort are usually consistently in one direction. That the marked deviations have a genetic basis is indicated by the fact that they appear in four separate generations as is shown in figure 1.

Apparently the original stock contained a factor or factors which in some way disturbed the normal segregation. The action of this factor first became apparent in the second generation of inbreeding. It has since been transmitted to later generations both in selfed lines of the original stock and in crosses.

TABLE 4

Segregating F_3 progenies from "high defective" F_2 ear No. 1520.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
1788	224	99	18	3.5	30.6
1790	156	90	28	6.1	36.6
1792	365	186	48	7.1	33.8
1806	205	89	15	3.0	30.3
Total	950	464	109	10.0	32.8
Progenies with significant deficiency					
1794	188	38	−18	4.1	16.8
Progenies with no significant deviation					
1795	219	70	−2	.4	24.2
1796	167	58	2	.5	25.8
1797	85	28	0	. .	24.8
1802	170	71	11	2.4	29.5
1803	227	67	−7	1.4	22.8
Total	868	294	4	.4	25.3
Grand Total	2006	796	96	6.2	28.4

INTERPRETATION OF DEFECTIVE RATIOS

Whenever marked deviations from expected ratios are encountered the possibility of lethal factors at once suggests itself. In maize, however, where a large number of seeds are arranged in a very regular fashion on a single inflorescence, lethal factors, or any sort of differential mortality of the zygotes are readily noted by the occurrence of aborted kernels scattered over the ear. The defective seed in question is, in fact, itself a semi-lethal character.

Lethal factors, moreover, should cause an excess of recessives only when they are linked with the dominant allelomorph of the recessive in question. The marked plus deviations in six of the F_2 ears shown in table 1 could be explained on the basis of lethal factors, only by assuming that each of the five separate seed parents involved in these crosses was heterozygous for a lethal factor linked with the dominant allelomorph of the de_1 gene. The possibility of lethal factors can, therefore, be safely ruled out.

The next hypothesis to be considered is that of differential pollen tube growth. The fact that defectives of other types had frequently given slight deficiencies suggested that these characters, which have such a profound deleterious influence on the sporophyte, might also have some effect in the gametophyte generation, particularly on the rate of pollen tube growth. To test this hypothesis a large number of ears segregating for various types of defectives were arbitrarily divided into upper and lower halves and the proportion of defectives in each half determined separately. If the two types of pollen tubes differ in their rate of growth it might be expected that the greater distance which the tubes were required to travel in reaching the ovules at the base of the ear, would react in favor of the faster growing tubes; that the longer the race the more certain the success of the faster individuals.

TABLE 5

Segregation in upper and lower halves of nine "high defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	Upper	Lower	
25	75	35	108	55	31.8	33.7	1.9
129	179	83	190	97	31.7	33.8	2.1
173	101	49	99	44	32.7	30.8	−1.9
388	246	91	252	106	27.0	29.6	2.6
391	77	32	50	29	29.4	36.7	7.3
1788	108	45	116	54	29.4	31.8	2.4
1790	77	43	79	47	35.8	37.3	1.5
1792	189	92	176	94	32.7	34.8	2.1
1806	106	40	99	49	27.4	33.1	5.7
Total	1158	510	1169	575	30.6	33.0	2.4

The results of these counts indicated that, with most of the defective types, there was no appreciable difference between the upper and lower halves of the ear in proportion of defectives, and that the defective seed factors do not *per se* reduce the rate of pollen tube growth.

It happened, however, that several of the *high* and *normal* ears of the de_1 stock were included in this particular study, without realizing at the time that some of these ears were exceptional. The counts on these ears and several additional ones which have since been examined are shown in tables 5 and 6.

In the *high* ears the average percentage of defectives in the upper halves was 30.6 as compared to 33.0 in the lower halves of the same ears. This difference, though not great, appears to be significant, the odds against its chance occurrence being about 70 to 1 (calculated by Student's formula). The fact that the difference is consistent, all the ears in table 5 with one exception showing a greater proportion of recessives in the lower halves, is regarded as particularly important.

The *normal* ears on the other hand (table 6) show differences in favor of the upper halves as frequently as the lower and the averages of the two halves are very closely alike, 24.6 and 25.5 percent. These results, so far as they go, point to differential rate of pollen tube growth in the *high* ears and equal rate of pollen tube growth in the *normal* ears.

TABLE 6

Segregation in upper and lower halves of seven "normal defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	UPPER	LOWER	
22	84	27	76	22	24.3	22.4	−1.9
24	75	32	79	31	29.9	28.2	−1.7
42	91	27	93	28	22.9	23.1	.2
126	211	54	217	72	20.4	24.9	4.5
128	142	52	123	38	26.8	23.6	−3.2
389	235	75	189	80	24.2	29.7	5.5
393	160	59	124	38	26.9	23.5	−3.4
Total	998	326	901	309	24.6	25.5	.9

Apparently the differential rate of growth is not due to the action of the de_1 factor itself. If pollen tubes carrying this gene were regularly faster or slower in rate of growth, the deviations should regularly occur in the same direction in *all* of the ears. This is not the case. Some ears have marked minus deviations, some marked plus deviations and the remainder segregate normally.

These peculiar results may, however, be explained by assuming that the chromosome pair which carries the De_1 and de_1 factors also bears a gene which influences the rate of pollen tube growth. Since this hypothetical factor has its expression in the gametophyte generation we may give it the factor symbol *Ga*. This factor speeds up the rate of growth in the pollen tubes which carry it and, in the original stock, was linked with de_1, thus enabling a larger proportion of de_1 gametes to accomplish fertilization than would occur by chance alone.

On this assumption the *high* defective plant which served as pollen parent for the 1922 crosses was of the genetic composition *Gade*$_1$, *gaDe*$_1$. Such a plant would produce four kinds of gametes, *Gade*, *gaDe*, *gade*, and *GaDe*. The seed parents of these crosses contributed only one kind of gamete *gaDe*. Thus four distinct classes of F$_1$ plants would be produced as follows:

1. *gaDe GaDe*
2. *gaDe gaDe*
3. *gaDe Gade*
4. *gaDe gade*

These four types would not be expected to occur in equal numbers because their proportion depends upon the amount of crossing over between *Ga* and *de* in the original stock.

Classes 1 and 2 are homozygous for the dominant *De*$_1$ factor and need not be considered further since they do not segregate for defective seeds. Plants in class 4 give segregating ears but the ratios should be normal because the plants are homozygous for *ga* and no differential pollen tube growth can occur. Plants of class 3, however, should, when selfed, give only *high* defective ears because both factors are in the heterozygous condition and the *Ga* factor which speeds up the rate of pollen tube growth is linked with the *de*$_1$ factor which causes defective seeds, thus enabling a larger proportion of the *de*$_1$ gametes to accomplish fertilization than would occur by chance alone.

A glance at table 1 shows that this is exactly the situation in the fourteen F$_2$ ears. Seven *normal* and six *high* ears resulted from these crosses. In addition, however, there is one *low* ear. *Low* ears are not expected from these particular crosses and it is not certain whether ear 392 with 20.0 percent recessives is an exception to theory or merely a chance variation from a *normal* ratio.

It is obvious that in dividing the ears into *high*, *low* and *normal* on the basis of the size of their deviations, some ears which have plus or minus deviations due to chance alone and not to genetic factors, will be included in the two extreme groups. Also some ears which are genetically *high* or *low* will be classed as *normal* because the number of seeds counted is not sufficient to establish the significance of the deviations.

What should happen in F$_3$ when the F$_2$ seeds from a *high* defective ear are grown? The *high* ear, we assume, was borne on a plant of the composition *Gade gaDe*. Such a plant would form four kinds of gametes in the pollen as well as in the ovules and self-pollination should give ten classes of seeds. The genetic constitution of these ten classes and their theoretical behavior when grown and self-pollinated is shown below:

GENOTYPE	BEHAVIOR
1. *GaDe GaDe*	Does not segregate
2. *GaDe gaDe*	Does not segregate
3. *gaDe gaDe*	Does not segregate
4. *GaDe Gade*	Segregates; 3 : 1 ratio
5. *gaDe gade*	Segregates; 3 : 1 ratio
6. *Gade gaDe*	Segregates; *high* defective ratio
7. *GaDe gade*	Segregates; *low* defective ratio.
8. *Gade Gade*	Defective seed
9. *Gade gade*	Defective seed
10. *gade gade*	Defective seed

Genotypes 1, 2, and 3 are homozygous for De_1 and do not segregate. Genotypes 8, 9, 10 are defective seeds and will not grow. Genotypes 4 and 5 segregate but both give normal ratios because the plants are homozygous for *Ga* or *ga* and differential pollen tube growth cannot occur. Plants in genotype 6 are heterozygous for both factors and, since *Ga* and de_1 are linked, should give *high* defective ears. Genotype 7 represents a new combination in which the linkage relations have been reversed. Here the *Ga* factor is linked with the dominant allelomorph and instead of an excess of defectives we should expect a deficiency.

Briefly summarized, then, the seeds from a *high* F_2 ear should, when grown and selfed, give *high, low*, and *normal* F_3 progenies. The 18 segregating ears in tables 3 and 4 meet these requirements very satisfactorily. Six are *high*, eight are *normal* and four are *low*. The ratio in which these three types of segregating progenies appear depends not only on the amount of crossing over between *Ga* and de_1 but on the ratio in which the *Ga* and *ga* gametes are presented at fertilization.

It should be emphasized that the validity of this interpretation does not necessarily depend upon the assumption of differential pollen tube growth. Any factor which affects the proportion in which the gametes are presented at fertilization would act in the same manner although its effect might be produced in a number of ways, as for example, by causing differences in the duration of viability or resistance to unfavorable moisture and temperature extremes.

The important fact is that this stock apparently *does* carry a factor which disturbs normal segregation and that this factor is transmitted from generation to generation as is any other Mendelian character. *How* the factor produces its disturbances is another question and one which seems to be partially answered by the data which point to differential pollen tube growth.

DISTORTED RATIOS IN SUGARY ENDOSPERM

The hypothesis of an accessory factor causing differential rate of pollen tube growth has been adopted because the situation is almost identical to that encountered when sugary endosperm is crossed with certain small seeded corneous varieties of the *Zea maize everta* type, known as Squirrel Tooth or Rice Pop.

Although sugary is usually inherited as a simple recessive and gives ratios closely approximating expectation, the Rice Pop × sugary crosses are exceptional in that they always produce a marked deficiency of sugary seeds in the F_2 endosperm generation. This deficiency was first noted by CORRENS (1902) who reported 16 percent sugary seeds in the second generation. EAST and HAYES (1911) also noted the same peculiarity and suggested that the disturbance might be due to faulty classification or to unknown characters contributed by the parents. JONES (1924) and EMERSON (1925) have both made detailed studies of this cross and both have come to the conclusion that the aberrant ratios may be attributed to differential pollen tube growth. EMERSON, however, suggested that the differential rate of growth is not due to the *Su* gene itself but to an accessory factor on the same chromosome. The details of EMERSON'S interpretation of this cross have not yet been published and it is not known whether his explanation agrees with the one given here.

The results of the genetic studies of the sugary × pop cross at the CONNECTICUT STATION are briefly summarized in the following paragraphs.

An inbred strain of pointed pop corn was crossed with a first generation hybrid of two inbred strains of sweet corn. One of the sweet strains was a small yellow-seeded variety known as Golden Bantam, the other a large white-seeded variety known as Evergreen. This material was used because it had shown a high degree of selective action in pollen mixtures where pollen from two different types of plants, acting in competition, fertilized more of the ovules of its own type than of the diverse types as previously reported by JONES. (1920, 1922).

In a total of 3681 seeds from eight self-pollinated F_1 plants of this cross shown in table 7, there are only 16.2 percent of sugary seeds. This represents a departure from the normal ratio of 18.3 times the probable error. The percentages of recessives in the individual ears range from 10.5 to 21.4 and the deficiency is greater than three times the probable error in all but one of the ears. In this ear the deviation is 2.6 times the probable error.

F_1 plants of similar cross, though of somewhat different ancestry were backcrossed both ways with the recessive parent. In the backcross of the heterozygote with pollen from the recessive parent (*Susu* × *susu*), 1374 starchy and 1397 sugary seeds were produced. In the reciprocal backcross (*susu* × *Susu*) 885 starchy and 939 sugary seeds occurred. The deviation from a 1 : 1 ratio is not significant in either case. In this respect the results differ from observations with waxy corn which are discussed later. In all this material the segregation of smooth, corneous seeds and wrinkled, glassy seeds was very clear-cut. Occasionally a recessive seed was found that was not deeply wrinkled and at first glance might be classed as a dominant. However, the opportunity for faulty classification was just as great in the F_1 plants backcrossed with the recessive as in the F_1 plants self-pollinated. In the one case a marked deviation was found; in the other, not.

TABLE 7

F_2 progenies of a cross between sugary endosperm and Squirrel Tooth Pop.

EAR NO.	STARCHY	SUGARY	DEVIATION	DEVIATION / P.E.	PERCENT SUGARY
(*Su*×*su*) −1	366	43	−59	10.0	10.5
2	346	94	−16	2.6	21.4
3	376	65	−45	7.3	14.7
(*su*×*Su*) −1	322	56	−39	6.9	14.8
2	378	77	−37	5.9	16.9
3	421	98	−32	4.8	18.9
4	391	47	−63	10.3	10.7
5	485	116	−34	4.8	19.3
Total	3085	596	−324	18.3	16.2

The next step was to backcross the F_1 plants with the dominant parent (*Susu* × *SuSu* and *SuSu* × *Susu*). Since the result of fertilization by a recessive or dominant carrying gamete would be obscured, because all the seeds would have the dominant endosperm condition, it was necessary to grow the plants and determine which were segregating and which were not. This was done by planting the backcrosses, made both ways, and allowing all of the plants to interpollinate naturally. Any plants that resulted from *Su* × *Su* fertilization would show no recessives, while most of the plants of the composition *Su* × *su* would be expected to show some recessive seeds. With no selective action one-half of the plants would be segregating so that one-fourth of the pollen in the field would be carrying the recessive factor. Segregating plants would be expected to have half of their ovules with the recessive factor so that segregating ears should have, on the average, one-eighth of their seeds recessive. Selective pollination working against the recessive gametes would reduce this proportion. Only one ear was taken from each plant and most of these contained several hundred kernels. All ears which had any sugary seeds were classed as segregating. The number of sugary seeds varied on different ears but the number of plants which had no recessive seeds and for that reason would be wrongly classified was probably less than seven percent and was not likely to be enough to disturb the results seriously in view of the wide departure from the normal 1 : 1 ratio obtained. The backcross of pollen from the homozygous dominant parent on the heterozygous F_1 plants (*Susu* × *SuSu*) gave 207 segregating and 213 non-segregating individuals. These numbers differ from a 1 : 1 ratio less than the probable error. But the pollen from the heterozygous F_1 plants backcrossed on the homozygous dominant parent (*SuSu* × *Susu*) gave 88 segregating and 353 non-segregating individuals, a deviation from a 1 : 1 ratio of 18.7 times the probable error.

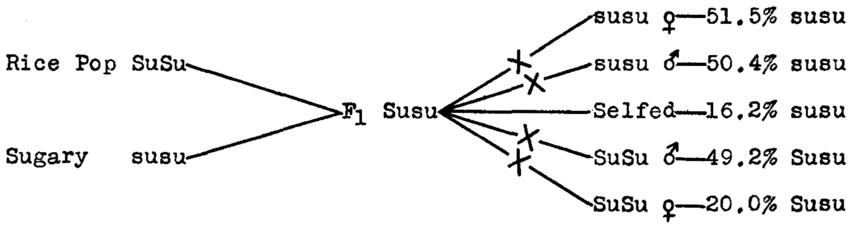

Figure 2. Diagram showing the behavior of the Rice Pop×Sugary cross when selfed and backcrossed reciprocally with both parents.

To sum up, the F_1 plants self-fertilized showed a selective action such that more of the gametes carrying the dominant factor united than would be the case in random mating. The heterozygous F_1 plants backcrossed with the recessive parent showed no selective action either way the pollinations were made. Backcrossed with the dominant parent there was no selective action when the pollen was alike but the pollen from the heterozygous F_1 plants showed a markedly greater pollinating ability on the part of the gametes carrying the dominant factor. These results are shown graphically in figure 2.

This series of facts shows that the selective action is not due to differences in the functioning of the pollen alone, although the differential action is exhibited only when the pollen is diverse, otherwise unequal numbers would be obtained in the backcross on the recessive parent as well as on the dominant.

As a result of these experiments Jones (1924) concluded that: "There is apparently an interaction between the pollen tube and the tissues in which it grows, such that pollen carrying the dominant factor is better able to accomplish fertilization than the pollen carrying its recessive allelomorph, only in a sporophyte which also has the dominant factor either in the haploid or diploid state."

At that time it was thought that the *Su* factor itself was responsible for the speeding up of the pollen tubes in certain combinations. It now seems more probable that the results are due to linkage of the *Su* factor with a gametophyte factor (*Ga*). If the linkage relations between *Su* and *Ga* were reversed the results would be entirely different but the above statement as applied to the *Ga* factor would still hold.

The factor symbol for the gametophyte factor has been written in capital letters as though it were a true dominant. It may be questioned whether this is legitimate since the *Ga* factor has its expression in the gametophyte where it necessarily occurs in a haploid condition and is not required to compete with its allelomorph. The *Ga* pollen tubes are given an advantage, however, only when the sporophytic tissue in which they grow also has this factor, and the fact that in the sporophyte it is apparently as effective when heterozygous as when homozygous indicates that it is a dominant factor.

On the basis of a gametophyte factor linked with the *Su* factor, the combinations here discussed may be given the following factorial representation:

TYPE OR COMBINATION	COMPOSITION OF PLANT	MALE GAMETES			
Rice Pop parent	*SuSuGaGa*	*SuGa*			
Sugary parent	*susugaga*	*suga*			
F_1 selfed	*SusuGaga*	*SuGa*	*Suga*	*suGa*	*suga*
F_1×sugary parent	*SusuGaga*	*suga*			
Sugary parent ×F_1	*susugaga*	*SuGa*	*Suga*	*suGa*	*suga*
F_1×starchy parent	*SusuGaga*	*SuGa*			
Starchy parent×F_1	*SuSuGaGa*	*SuGa*	*Suga*	*suGa*	*suga*

With this factorial composition in mind the results reported in the preceding paragraphs are readily understood on the assumption that the *Ga* factor is effective in speeding up pollen tube growth only in a sporophyte which has the *Ga* factor in the homozygous or heterozygous state.

The F_1 selfed shows a selective action because *Ga* and *ga* pollen tubes compete with each other in sporophytic tissue which has the *Ga* factor in the heterozygous condition. The *Ga* tubes are given an advantage under these conditions and since *Ga* and *Su* are linked, more *Su* than *su* gametes, reach the micropyle and accomplish fertilization. F_1 plants pollinated by the sugary parent show no selective action because only one type of male gamete is involved. The backcross of the sugary parent by the F_1 involves four types of male gametes but no selective action is shown because the sporophyte in which the pollen tubes must grow lacks the dominant *Ga* factor. F_1 backcrossed by the starchy parent shows no selective action because only one kind of male gamete is present. But the backcross of the starchy parent by the F_1 again shows a very marked selective effect because *Ga* and *ga* gametes are competing in sporophytic tissue which has the dominant *Ga* factor in the homozygous state.

The F_2 progenies which show such a marked deficiency of sugary seeds, should, when selfed, produce the same three types of segregating progenies as did the *high* defective ears when selfed. Here of course the proportion of the three types would be reversed and (1) the majority should be deficient in sugary seeds, (2) some should segregate normally, and (3) a few should give an excess. EMERSON has grown such progenies and has obtained exactly these results, his *low* ears giving 15 percent recessives, his *high* ears 35 percent. The writers have also grown a few F_3 progenies from this cross and have obtained the expected types, although in the case of the *high* sugary ears the number of seeds counted was not great enough to prove that the deviations were statistically significant.

LINKAGE BETWEEN *Su* AND de_1

The question at once arises whether the deviations found in the de_1 stock are caused by the same accessory factor, always present in Rice Pop, which causes such marked disturbances in the starchy: sugary ratio. To determine this point crosses of *high* defective × pop, *low* defective × pop, *high* defective × sugary, and *low* defective × sugary have been made and the F_2 endosperm generation of these crosses will be available in another season. In the meantime, indirect evidence that the accessory factor in both stocks is probably the same is available in a cross of *normal* defective × sugary, which had been made for another purpose. Four F_2 progenies of this cross are shown in table 8.

It is noted that the two parental classes are in excess, while the two new combinations show a deficiency. When parental classes are combined and compared to the new combinations in the form of a 10 : 6 ratio, the deviation is found to be 4.9 times the probable error. Such a deviation should occur by chance alone only once in 1052 trials but can readily be explained by assuming linkage (repulsion phase) between *su* and de_1. The percentage of crossing over as determined from the normal seeds is 38.5; determined from the defective class it is 39 percent.

RELATIVE EFFECTIVENESS OF *Ga* AND *ga* GAMETES

Knowing that the de_1 and *su* factors are linked and that both are affected to a marked degree by an accessory factor located on the same chromosome, it is almost certain that the same accessory factor is acting in both stocks. On such an assumption it is possible to estimate approximately the position on the chromosome of the *Ga* factor in relation to the *su* and de_1 genes and at the same time to determine roughly the ratio of *Ga* to *ga* gametes which accomplish fertilization.

The presence of gametophyte factors which disturb the equality in which two kinds of gametes are presented at fertilization, brings up entirely new problems in Mendelian segregation. The amount of crossing over between a gametophyte factor and a sporophyte factor may range from 0 to 50 percent. At the same time the ratio in which the *Ga* and *ga* gametes occur at fertilization may range from 1 : 1 to 1 : 0.

TABLE 8

Segregation in F_2 of a cross between sugary endosperm and defective seed.

EAR NO.	NORMAL SEEDS		DEFECTIVE SEEDS	
	Starchy	Sugary	Starchy	Sugary
947	71	36	42	10
951	213	89	75	27
953	219	81	85	18
956	98	32	45	9
Total	601	238	247	64
Ex. 9 : 3 : 3 : 1	647	216	216	72
Deviation	−46	22	31	−8

Grouping into a 10 : 6 ratio
Found 665 485
Ex. 10 : 6 *719* *431*
Deviation 54±11.1
Dev./P. E. 4.9

The amount of crossing over and the relative effectiveness of the accessory factor are, then, the two variables which determine the size of the deviations from normal ratios. Table 9 shows the theoretical percentage of recessives which should occur with different combinations of these two variables, assuming that a gametophyte factor *G* which increases the chances of the gametes, in which it occurs, to accomplish fertilization, is linked with the dominant allelomorph *S* of a recessive sporophyte factor. If the linkage were reversed (*Gs gS*), the deviations would be of the same magnitude but would occur in the opposite direction. The size of the deviations produced by different combinations of the two variables is also illustrated in figure 3.

The average percentage of defective seeds in the *high* ears of tables 3 and 4 is 32.4, a deviation of 7.4 percent from normal. The *low* ears from the same families average 16.8 percent recessives, a deviation from normal of 8.2 percent. The average deviation for both groups is 7.8 percent. In the *low* sugary ears of table 7 the average deviation from a normal ratio is 8.8 percent.

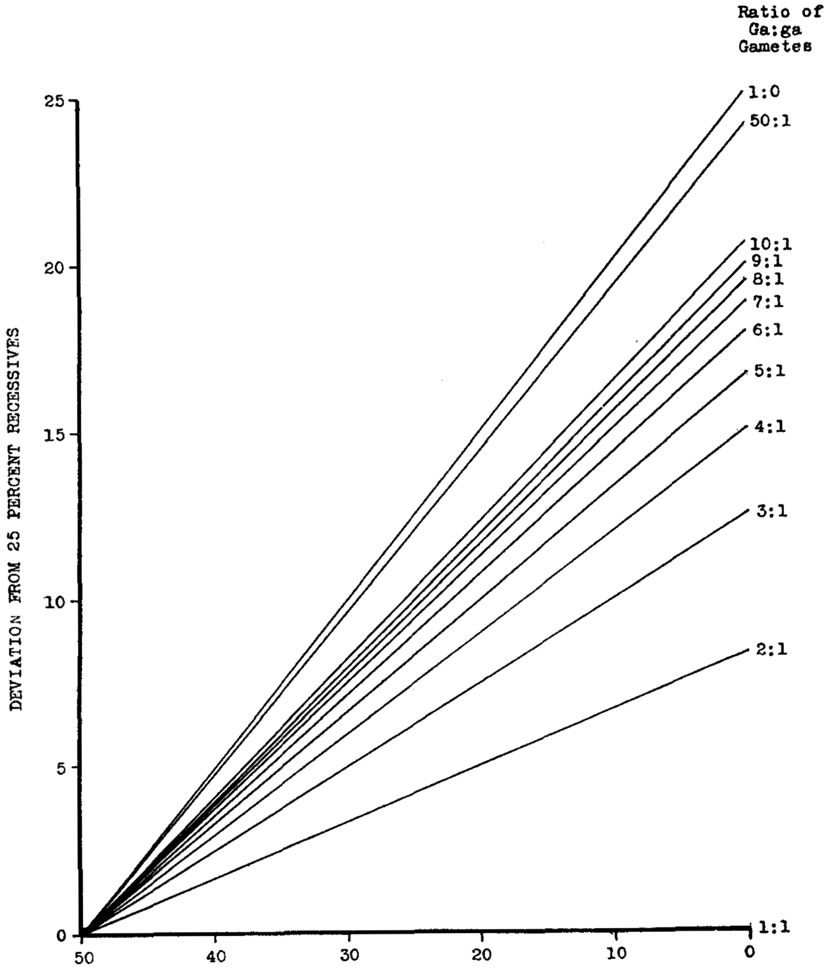

FIGURE 3. Diagrams showing the theoretical deviations from 25 percent recessives resulting from various combinations of crossing over and relative effectiveness of two kinds of gametes in accomplishing fertilization.

A deviation of 7.8 percent in the defective seed ratios and 8.8 percent in the sugary ratios might be caused by any number of combinations of crossing over and differential effectiveness of the *Ga* and *ga* gametes. This is illustrated by the curves in figure 3. Thus if the value of one of these influences were known it would be a fairly simple matter to determine the value of the other. Unfortunately this is not the case.

It is known, however, that the crossing over between *Su* and de_1 is approximately 39 percent. An accessory factor could affect the two almost equally, only if it were approximately the same distance from both. This means that its locus must lie between the *Su* and de_1 loci.

TABLE 9

Percentages of recessives (ss) expected when a factor G which stimulates the rate of pollen tube growth is linked with the dominant allelomorph S of a recessive character s.

RATIO IN WHICH GAMETES ARE PRESENTED	PERCENTAGE OF CROSSING OVER BETWEEN *G* AND *S*									
	0	5	10	15	20	25	30	35	40	45
1 : 1	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2 : 1	16.65	17.50	18.33	19.17	20.00	20.83	21.67	22.50	23.33	24.17
3 : 1	12.50	13.75	15.00	16.25	17.50	18.75	20.00	21.25	22.50	23.75
4 : 1	10.00	11.50	13.00	14.50	16.00	17.50	19.00	20.50	22.00	23.50
5 : 1	8.33	10.00	11.67	13.33	15.00	16.67	18.33	20.00	21.67	23.33
6 : 1	7.14	8.93	10.72	12.50	14.29	16.07	17.86	19.64	21.43	23.22
7 : 1	6.25	8.13	10.00	11.88	13.75	15.63	17.50	19.37	21.25	23.12
8 : 1	5.56	7.50	9.45	11.39	13.33	15.28	17.22	19.17	21.11	23.06
9 : 1	5.00	7.00	9.00	11.00	13.00	15.00	17.00	19.00	21.00	23.00
10 : 1	4.55	6.59	8.64	10.68	12.73	14.77	16.82	18.86	20.90	22.95
20 : 1	2.38	4.64	6.91	9.17	11.43	13.69	15.95	18.21	20.48	22.74
30 : 1	1.61	3.95	6.29	8.63	10.97	13.31	15.64	17.98	20.32	22.66
40 : 1	1.22	3.60	5.98	8.35	10.73	13.11	15.49	17.87	20.24	22.62
50 : 1	.98	3.38	5.78	8.19	10.59	12.99	15.39	17.79	20.20	22.60
1 : 0	.00	2.50	5.00	7.50	10.00	12.50	15.00	17.50	20.00	22.50

TABLE 10

Crossing over values and map distances indicated by ordinates erected at different points on abscissa in figure 4.

POINT ON ABSCISSA	PERCENTAGE CROSSING OVER		MAP DISTANCE		TOTAL MAP DISTANCE
	Ga — Su	*Ga* — de_1	*Ga — Su*	*Ga* — de_1	
3.7	19.3	22.7	20.9	25.0	45.9
3.8	19.8	23.1	21.5	25.5	47.0
3.9	20.3	23.5	22.1	26.0	48.1
4.0	20.8	23.9	22.7	26.5	49.2
4.1	21.2	24.3	23.2	27.0	50.2
4.2	21.5	24.7	23.6	27.5	51.1
4.3	21.8	25.0	23.9	27.9	51.8
4.4	22.1	25.3	24.2	28.3	52.5

The map distance between *Su* and de_1 is 50 units as determined from HALDANE'S table (HALDANE 1919). These tables are, of course, based on the results from the sex chromosome in Drosophila but since they have been found to apply equally well to the other chromosomes of Drosophila and to the linkage groups in Primula, it is probable that they hold for the linkage groups in maize.

The problem in this case is to determine at what point on the abscissa in figure 4 an ordinate can be erected which crosses the two curves at points, whose values converted into map distances and combined, equal 50 units. Table 10 shows the results of erecting ordinates at a number of points along the abscissa.

It is noted that an ordinate erected at 4.1 crosses the two curves at points whose values best fit the facts. In other words, if 4.1 times as many *Ga* as *ga* gametes accomplish fertilization, then the crossing over between *Ga* and *Su* is 21.2 percent and between *Ga* and de_1, 24.3 percent. The map distance between *Ga* and *Su* would then be 23.2 units and between *Ga* and de_1, 27.0 units. These two values combined equal 50.2 units which is approximately the map distance between *Su* and de_1.

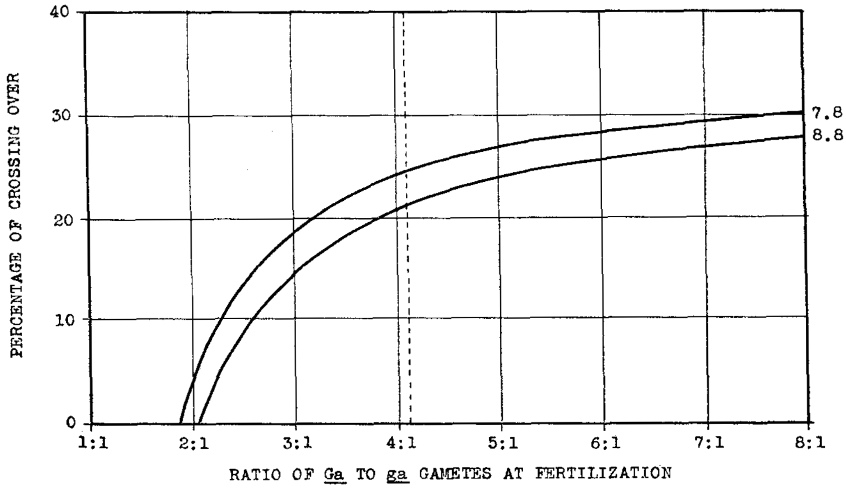

FIGURE 4. The various combinations of crossing over and relative effectiveness of two kinds of gametes which might cause deviations of 7.8 and 8.8 percent from the expected 25 percent recessives. An ordinate erected at 4.1 crosses the two curves at points whose values converted into map distances and added, equal the map distance between de_1 and *Su*.

It is realized, of course, that it is impossible to arrive at more than a crude approximation by this method. It is difficult to determine the true value of either of these influences until one of them is more accurately known. Furthermore, the assumption has been made that the relative proportion of *Ga* and *ga* gametes which accomplish fertilization is always the same. This is probably not strictly true. The data already given, and further data from mixed pollinations presented later, indicate that the selective action is due to differential pollen tube growth. The ratio of the two classes of gametes at fertilization would, therefore, depend to some extent on length of styles, weather conditions, and perhaps many other factors. In the long run these various influences would be expected to balance each other and over a period of years the ratio of the two types of gametes would be relatively constant. In comparing two series of observations, however, a variation in the relative effectiveness of the *Ga* and *ga* gametes must be kept in mind.

PREDICTING THE SIZE OF THE DEVIATIONS

If these approximations have any value it should now be possible to predict the deviations which will occur when other characters on the sugary chromosome are crossed with Rice Pop, providing that the amount of crossing over between sugary and the new character is known. A recent paper by WENTZ (1925) includes data which answer this purpose admirably. WENTZ found that a defective seed type de_{su} is linked with sugary with 3.2 percent crossing over. Since the crossing over between the *Ga* and *Su* loci is approximately 21.2 percent, then the crossing over between *Ga* and de_{su} should be about 23.8 or 18.6 percent depending on the direction of the de_{su} gene from the sugary locus. With the *Ga* and *ga* gametes occurring at fertilization in the ratio of 4 to 1, a cross between Rice Pop (*GaDe GaDe*) and WENTZ'S defective (*gaDe gade*) should produce either 17.1 or 15.6 percent recessives on the segregating ears. Fortunately, WENTZ has crossed his defective seed strain with a stock tracing back to Rice Pop ancestry and apparently heterozygous for the accessory factor of the Pop parent. Eight segregating ears in the F_2 endosperm generation of this cross gave an average of 19.8 percent defective seeds as is shown in table 1 of WENTZ'S paper. This value differs from the value predicted above by 2.7 percent.

Through the kindness of Professor WENTZ the detailed data on the individual ears of this cross, which were not included in his published paper, were made available to us. These data showed that three of the eight ears gave approximately normal ratios averaging 24.7 percent recessives. The other five ears, however, all deviated from a normal ratio by significant amounts and when combined gave a total of 1475 normal and 309 defective seeds, an average of 17.3 percent recessives.

The agreement of this value with the 17.1 percent recessives predicted by the above calculations is surprisingly good and is either a remarkable coincidence or an excellent bit of evidence in substantiation of our hypothesis.

The position of the factors on the sugary chromosome is, then, approximately as shown in one or the other of the two following diagrams.

de_1 *Ga* *Su* de_{su} *Tu*

de_{su} *Su* *Ga* *Tu* de_1

Which of these arrangements is the correct one will be determined when the character *tunicate ear* is crossed with Rice Pop. The crossing over between *su* and *Tu* is approximately 28.6 percent (EYSTER 1921). In the first arrangement *Tu* is so far from *Ga* that its segregation would be affected only very slightly by the differential pollen tube growth. In the second arrangement *Tu* is fairly close to *Ga* and if this is the correct one, marked deviations should occur in the tunicate ratios when this character is crossed with Rice Pop or other stocks carrying the *Ga* factor.

GAMETOPHYTE FACTORS AND MIXED POLLINATIONS

The presence in Rice Pop of a gametophyte factor which influences the rate of pollen tube growth in sporophytic tissue of the same genetic constitution, probably accounts to a large extent for the results obtained in mixed pollination experiments. (JONES 1920, 1922). In these tests where a mixture of the white pop type and a yellow seeded sugary type was applied to the silks of both, there was a marked preference of the plants for their own kind of pollen. The results of five such mixed pollinations are shown in table 11.

TABLE 11

The amount of selective action shown by maize in five pollen mixtures.

POLLEN MIXTURE NO.	NUMBER OF SEEDS				TOTAL NO. OF SEEDS	DEVIATION FROM PERFECT PROPORTION IN PERCENT
	A×A	A×B	B×A	B×B		
1	811	11	381	2006	3209	41.35
2	4222	27	466	1404	6119	37.22
3	1568	2	319	224	2113	20.56
4	1930	29	73	309	2341	39.71
5	4084	6	963	290	5343	11.50

If there were no differences in pollinating ability, the proportion should be a perfect one within the limits of random sampling. The white smooth-seeded plants are designated as A in the tables and the plants with yellow, sugary seeds are listed under the heading of B. Without selective action the ratio of crossed to selfed seeds should be the same on ears of both types, regardless of the amount or viability of each kind of pollen. The last column gives the deviations from the closest calculated perfect proportion based upon the percentage of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation of 50 percent would indicate complete functioning of each kind of pollen on its own flowers to the exclusion of the foreign type. The deviations actually range from 14 to 41 in favor of self-fertilization. In some tests the deviations have been even higher but only the above five groups are given here because these were also used in determining differences in pollen tube growth.

That the selective action is due, in part at least, to differences in pollen tube growth, is indicated by the data in table 12. The ears were arbitrarily divided into top and bottom halves before shelling. The distance that the pollen tubes had to travel differed considerably in the case of seeds produced at the tip of the spike as compared with those at the base. If the plant's own pollen tubes grew more rapidly than the foreign tubes, we would expect fewer cross-fertilized seeds at the base than at the tip of the spike.

TABLE 12

The ratio of crossed seeds in upper and lower halves of ears resulting from mixed pollinations.

POLLEN MIXTURE NUMBER	PLANT TYPE	TOTAL NO. OF SEEDS	NO. OF CROSSED SEEDS PER 100		RATIO OF CROSSED SEEDS
			TOP	BOTTOM	TOP : BOTTOM
1	Pop	822	1.99	.54	3.7 : 1
1	Sugary	2387	17.23	14.64	1.1 : 1
2	Pop	4249	1.22	.05	24.4 : 1
2	Sugary	1870	26.66	23.01	1.2 : 1
3	Pop	1570	.25	.00	.25 : 0
3	Sugary	543	60.07	57.14	1.1 : 1
4	Pop	1959	2.54	.32	7.9 : 1
4	Sugary	382	24.73	13.78	1.8 : 1
5	Pop	4090	.10	.20	.5 : 1
5	Sugary	1253	78.43	75.35	1.1 : 1
Total and	Pop	12,690	1.22	.22	5.6 : 1
Average	Sugary	6,435	41.42	36.78	1.1 : 1

The last column in table 12 shows the ratio of crossed seeds in the top halves to those in the bottom halves. In all but one mixture there was a greater proportion of crossed seeds in the top halves of the ears and in this one exception there were only six crossed seeds in a total of four thousand. It is also noted that the excess of crossed seeds in the upper part of the inflorescence is much greater on ears of the pop type than those of the sugary type. In the former there were 5.6 times as many crossed seeds in the top halves while in the sugary ears there were only 1.1 times as many crossed individuals in the upper part of the inflorescences. The marked selective action found in these mixtures is apparently due to a condition in the pop parent which permits the plant's own pollen tubes to grow more rapidly in its own styles. That this condition is brought about by a gametophyte factor on the sugary chromosome has already been indicated.

It will be noted that the data also indicate a slight preference of the sugary plants for their own kind of pollen. Probably there are many genetic factors which affect pollen tube growth and any of these which cause increased growth when the sporophytic tissue is of the same genetic constitution, will give the plants' own pollen an advantage over foreign pollen. Almost all pollen mixtures show a slight selective action in favor of the plants' own pollen but only where one of the types possesses a factor which causes a marked increase in the rate of pollen tube growth, such as is found in Rice Pop, is the selective action so decided.

DISTORTED RATIOS IN WAXY ENDOSPERM

The deficiency of waxy seeds originally noted by COLLINS and KEMPTON (1911) in the F_2 endosperm generation of hybrids between waxy and other varieties of maize can also be explained, at least partially, by the action of one or more gametophyte factors linked with the *Wx wx* pair.

New interest in the inheritance of waxy has recently been aroused by the discovery that the carbohydrate reserve in the waxy endosperm is of a different chemical nature than that of other varieties of maize, staining red with iodine, while other types of endosperm stain a deep blue (WEATHERWAX 1922), and that this difference in reaction to staining is also apparent in the pollen grains (BRINK and MACGILLIVRAY 1924, DEMEREC 1924, LONGLEY 1924) and in the embryo sac. (BRINK 1925, KIESSELBACH and PETERSEN 1926)

KIESSELBACH and PETERSEN (1926) have summarized all the available data on the inheritance of waxy endosperm, including considerable new data of their own. When all the results are combined there is a deficiency of 1.1 percent from the expected 25 percent when the heterozygote is selfed and .7 percent from the expected 50 percent when segregating pollen from heterozygous plants is applied to the pure recessives. These writers conclude that "the evidence at hand seems insufficient to definitely establish the causes or significance of the deviations", although the deviation in the first case is 14.8 times the probable error and in the second 5.4 times the error.

The difficulty of interpreting the waxy situation is not due to a deficiency of data but to the fact that data, which are not strictly comparable, have been combined. Different investigators have used different stocks in crossing with waxy, and the waxy stock itself after repeated crossings and extractions, since it was first brought from China, is probably far from homogeneous. If accessory factors are involved in the inheritance of waxy it will be readily seen that the procedure of combining data from a wide variety of sources and especially that of combining different generations, is not a sound one. Merely adding a few thousand individuals to the third of a million already counted will contribute very little toward solving the cause of the deviations.

The fallacy of combining the data from separate generations is well illustrated by the defective seed ratios in table 3. An F_2 ear with 32.4 percent recessives produced F_3 progenies with an average of 23.8 percent recessives. At first glance it appeared that the deviation in F_2 was not inherited. Closer examination, however, shows that although the average percentage of defective seeds in F_3 is almost normal, the individual progenies show marked deviations, some in one direction, some in another.

ANALYSIS OF KEMPTON'S DATA

That a somewhat similar situation exists in the waxy stocks is shown by data from KEMPTON (1919). KEMPTON'S F_2 progenies gave an average of 23.7 percent waxy; his F_3 progenies, an average of 24.6 percent waxy. The difference between these two generations is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials.

The difference between the second and third generations cannot be explained by consistent errors in classification or to regular differences in viability, rate of pollen tube growth, or resistance to unfavorable influences inherent to the waxy factor *itself*, since all of these influences would produce as great a disturbance in one generation as in another. The situation, however, is exactly what would be expected if an accessory factor linked with the waxy gene is involved.

Further evidence of the action of an accessory factor influencing the waxy ratio is found in the data in table 1 of KEMPTON'S paper (1919). In this table are given all the results of selfing and intercrossing F_1 plants. Ordinarily, of course, these two types of pollination should give identical results but if an accessory factor is present in part of the plants, differences might well occur. KEMPTON, in intercrossing a pair of F_1 plants, frequently also self-pollinated one or both members of the pair and the data resulting from selfing are, fortunately, included in the table and can be compared to those obtained from the intercrossing.

A study of the pedigree numbers in this table show that there are 26 intercrosses in which the segregation of the two F_1 plants used in making the cross, has also been determined by selfing. Some of the F_1 plants gave almost normal ratios, others showed a deficiency of waxy seeds. Arbitrarily dividing the selfed plants into *normal* and *low*, using a deviation of twice the probable error as a basis, the intercrosses can be arranged into the following groups; *low* × *low*, *normal* × *low*, *low* × *normal*, and *normal* × *normal*.

Obviously, if the deviations in the selfed plants are merely due to chance, then grouping the intercrosses on the basis of the segregation in the selfed parental plants can have no effect on the average percentage of waxy in each group. If, however, the deviations are due to some condition inherent in the plants, it might be expected that *low* × *low* intercrosses would give different results than *normal* × *normal* pollinations. The results of grouping the intercrosses according to the behavior of both members of the parental pair are shown in table 13.

It is noted that the *low* by *normal* and *normal* by *normal* intercrosses are very closely alike, giving 25.1 and 25.4 percent of waxy seeds respectively. The *normal* by *low* and *low* by *low* intercrosses also resemble each other giving 22.9 and 22.6 percent of waxy seeds respectively. In other words, the groups of intercrosses in which pollen from *normal* plants was used gave *normal* ratios, the average for the two groups being 25.3 percent recessives, a very close approximation to the theoretical ratio. When pollen from *low* waxy plants was used, however, the intercrosses gave *low* waxy ratios, the average for the two groups being 22.7 percent waxy, a deviation from the theoretical ratio of 5.8 times the probable error.

It should be mentioned that in addition to the intercrosses shown in table 13 there are two involving plants with *high* waxy ratios. One of these, *normal* × *high*, gave 21.3 percent waxy, the other, *high* × *low*, 28.1 percent waxy. No conclusions regarding the significance or meaning of plus deviations can be drawn from these two ears.

It is apparent that the arbitrary grouping of the F_1 plants into *low* and *normal* on the basis of their deviations has actually divided them into classes which are inherently distinct. Plants giving *low* ratios when selfed give almost exactly the same ratios when their pollen is used on other plants. The remaining plants give ratios closely approximating *normal* whether selfed or intercrossed. In other words, *the random assortment of the male gametes at fertilization is disturbed in about half of the plants, and is relatively normal in the remainder.*

These facts can readily be explained by assuming that one of the parents of KEMPTON'S hybrids was heterozygous for an accessory factor which affected the chances of the two kinds of gametes in accomplishing fertilization. Probably it was the waxy stock which brought in the accessory gene because deviations have been found in almost every case that waxy has been crossed with other stocks.

With the evidence of an accessory factor in mind it should be possible to isolate from such crosses as KEMPTON'S, some heterozygous waxy lines which regularly give normal ratios, some which give a majority of minus deviations and others a majority of plus deviations. We have no doubt that such lines will soon be reported and that crosses with other characters on the waxy chromosome will indicate the approximate location of the accessory factor. BRINK (1925) has, in fact, evidence from one of his crosses which indicates the presence of an accessory factor closely linked with the *I* factor, which is located on the waxy chromosome.

The gametophyte factor in the waxy stock probably disturbs the ratios by causing differential pollen tube growth as does the factor in the sugary and defective seeds stocks. At least this is the simplest explanation and there is some evidence of its correctness from BRINK'S (1925) experiments in which he found that fewer waxy seeds were produced on ears with long styles compared to those in which the styles were cut short.

PECULIAR RATIOS IN COLOR SEGREGATION

It is not improbable that COULTER'S aberrant segregation in a stock heterozygous for the *C* factor (COULTER 1925) is due to the same accessory factor which affects the waxy ratios, since *C* and *wx* are members of the same linkage group.

COULTER'S stock produced some progenies with marked minus deviations of white seeds, some with marked plus deviations, and others with normal ratios. He explains the deviations on the basis of linkage with a zygotic lethal factor and it is true that they can be fairly satisfactorily explained on this basis, although some of his *high* white ratios are higher than would be expected. Zygotic lethals, even with complete linkage, (repulsion phase) should cause a distortion of the ratio only from a 3 : 1 to a 2 : 1. A large proportion of COULTER'S high ears average higher than 33.3 percent recessives and a number of them show 38 to 40 percent of white seeds.

Furthermore, zygotic lethals in maize are usually expressed in the form of defective seeds, and when these are present they can be readily noted and their numbers accurately determined. COULTER asserts that aborted seeds are sometimes found in this stock but apparently not regularly. He writes[1] "Many of my ears have the random distribution of empty pericarps so characteristic of the defectives. On other ears it seems impossible to detect the presence of the abortive grains. Doubtless accessory factors are at play to determine the degree of expression of this character."

From the experience in studying the inheritance of fourteen types of defective seeds, (MANGELSDORF 1926), it may be said that when defective seeds are present at all, their segregation is usually clear-cut and their number can be readily determined. The accessory factor which COULTER postulates is probably not one which so affects the degree of expression of the defectives that they can no longer be distinguished from the normal seeds but rather a gametophyte factor which influences the ratio in which the defective seeds appear, as well as the segregation for color. If this is true it should be possible to isolate from COULTER'S stock, lines which give *high*, *normal* and *low* white progenies but which do not segregate for defective seeds, others which give *high*, *normal*, and *low* defective progenies but do not segregate for color.

HOW GAMETOPHYTE FACTORS OPERATE

If the gametophyte factor on the waxy chromosome is one which affects the rate of pollen tube growth, it differs in two respects from the *Ga* factor on the sugary-defective chromosome. The first difference is that the waxy pollen tube factors reduces the rate of pollen tube growth instead of stimulating it. The second difference is that it operates regardless of the genetic constitution of the sporophytic tissues in which the pollen tubes grow. This is shown by the fact that the *normal* × *low* intercrosses in table 13 gave exactly the same results as the *low* × *low*.

There is, of course, no reason for believing that all gametophyte factors behave in the same way. It is probable that factors operating in the gametophyte generation may have their effect in many different ways, for example; in causing differential pollen tube growth, duration of viability, resistance to drying out, excessive moisture, high and low temperature, etc. Even the factors affecting the rate of pollen tube growth alone may fall into at least four distinct categories, as follows:

1. Factors which reduce the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.
2. Factors which reduce the rate of pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

---

[1] In a letter.

3. Factors which stimulate the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.
4. Factors which stimulate pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

TABLE 13

Kempton's F_1 intercrosses grouped on the basis of the segregation in the selfed parental plants.

TYPE OF INTERCROSS	PEDIGREE NUMBERS			PERCENTAGE WAXY		
	♀	♂	INTERCROSS	♀	♂	INTERCROSS
L×L	1534	1743	1535	21.7	20.5	22.7
L×L	1543	1723	1542	20.9	21.0	23.3
L×L	1723	1543	1722	21.0	20.9	23.9
L×L	1743	1534	1742	20.5	21.7	21.3
Average						22.9
N×L	1117	1118	1116	24.2	21.9	26.0
N×L	1515	1726	1518	25.0	21.6	21.5
N×L	1129	1135	1130	24.1	21.5	21.0
N×L	1133	1118	1134	24.6	21.9	16.4
N×L	1137	1135	1138	26.0	21.5	25.1
N×L	1741	1551	1740	22.7	21.2	25.6
Average						22.6
L×N	1111	1132	1110	22.2	23.3	27.5
L×N	1118	1108	1119	21.9	26.4	25.8
L×N	1121	1117	1120	22.5	24.2	22.1
L×N	1551	1741	1550	21.2	22.7	20.6
L×N	1135	1137	1136	21.5	26.0	28.6
L×N	1726	1519	1725	21.6	23.8	24.2
Average						25.1
N×N	1105	1108	1104	26.4	26.4	23.7
N×N	1527	1745	1526	23.3	23.8	26.8
N×N	1549	1749	1548	24.6	26.3	24.0
N×N	1128	1125	1127	25.0	24.0	24.4
N×N	1132	1117	1131	23.3	24.2	22.9
N×N	1721	1515	1720	23.2	25.0	30.7
N×N	1745	1527	1744	23.8	23.3	26.0
N×N	1749	1549	1748	26.3	24.6	25.8
Average						25.4

Gametophyte factors which fall into the second and third of these categories have apparently already been found in maize in the waxy and sugary-defective pollen tube factors. An example of the first group is found in the inheritance of self-sterile classes in Nicotiana (EAST and A. J. MANGELSDORF 1925, 1926) where certain classes are almost completely eliminated presumably because of the slower rate of pollen tube growth.

CARBOHYDRATE METABOLISM AND POLLEN TUBE GROWTH

It may have been noted that all the characters so far reported *su*, *wx*, de_1 and de_{su} which appear to be associated with differences in pollen tube growth, are endosperm characters which are also associated with differences in the nature or amount of carbohydrate storage.

BRINK (1925) is of the opinion that differential pollen tube growth operates in combination with, or is caused by, differential carbohydrate metabolism. The fact that these four above mentioned characters do affect the nature or amount of carbohydrate storage might be considered as evidence in favor of such a view. We are of the opinion, however, that this is merely a coincidence; that distorted ratios in maize have been found mainly in endosperm characters only because such characters have been studied on a larger scale than any others. We feel certain that similar disturbances in the ratios of seedling and plant characters will also be found when these are studied on a greater scale. In fact, *high*, *low*, and *normal* ratios, apparently due to differential pollen tube growth, are now being obtained in a white seedling stock. (MANGELSDORF Unpublished).

The possibility, however, that differential carbohydrate metabolism may have some effect on pollen tube growth and that certain sporophytic characters may *per se* affect the rate of growth, is not denied. The marked deficiency of waxy seeds which BRINK (1925) obtained in a heterozygous waxy, homozygous sugary stock cannot, perhaps, be explained on the basis of gametophyte factors alone.

DISCUSSION

Selective fertilization has always offered an attractive explanation of peculiar results for which it was difficult to account by other means, and has frequently been called into account for unexpected departures from Mendelian ratios. Cuenot's yellow mice, CORREN'S aberrant ratíos in Melandrium, HERIBERT-NILLSON'S unusual segregation of certain characters in *Oenothera*, are examples[1].

EAST (1922) includes as one of the "Provisional Laws of Heredity" the statement that "there is no selective fertilization between complementary, compatible, functional gametes". This is probably true if the term

---

[1] BRIEGER (1926) has recently summarized the cases in which selective fertilization might be used to interpret the results.

"fertilization" is confined to the actual fusion of the nuclei and a distinction is made between selective fertilization and certain other influences which disturb the random assortment of the gametes at the time of fertilization. For example; in plants or animals in which free-swimming, motile sperm come in contact with the egg in large numbers, there may be no discrimination by the female gametes in favor of certain of the male gametes, providing that all are compatible. There is no reason for believing, however, that all the sperm, in all cases, are equally capable of reaching the egg. It is possible that some classes are so handicapped as a result of their chromosomal constitution that though produced in full quota at gametogenesis, they are in minority at the time of fertilization.

In angiosperms an entire generation elapses between the time of gametogenesis and fertilization and the opportunity for differential elimination of certain classes is relatively large.

The pollen tube of angiosperms represents the remnant of the elaborate gametophyte generation in lower plants, in many of which this generation is of equal or greater importance than the sporophyte. In such plants there can be no question that the hereditary factors have almost, if not quite, as great an influence in the gametophyte as in the sporophyte generation. In fact, characters which have their expression in the gametophyte generation have recently been reported by WETTSTEIN (1924) in the mosses and ALLEN (1925) in the liverworts. In angiosperms the gametophyte generation has become greatly reduced and the hereditary factors which influence it have, no doubt, been correspondingly lessened, though not entirely eliminated.

Mendelian factors have been found to affect practically all stages in the ontogeny of the maize plant. The growing embryo, the endosperm, the resting stage of the seed, the young seedling, stature, chlorophyll development and reproductive processes, are all governed by genetic factors which may be unlike for different individuals. *It would be strange indeed if the gametophyte generation, brief though it has become, should be entirely deprived of the governing influence of various Mendelian factors.*

It is not surprising, therefore, to find in maize several Mendelian factors which have their expression in the gametophyte generation. These factors produce a definite physiological effect upon the gametophytes in which they occur, speeding up development in one case, reducing it in the other. They are transmitted from generation to generation in the same manner as any other Mendelian factors, occupy definite loci on certain chromosomes and show characteristic linkage and crossing over phenomena.

In general, the genetic factors which affect the gametophyte probably have little influence on ordinary Mendelian ratios in the sporophyte. In the female gametophyte, at least in angiosperms, they probably seldom cause any disturbance. In the male gametophytes they can have no effect as long as the parental sporophyte remains homozygous for the factors which influence the gemetophyte generation. Even when the gametophytes differ genetically, the favorable and unfavorable factors would, in the long run, tend to balance each other and no classes of gametes would be given a decided advantage. Only in occasional instances where a gametophyte factor produces a marked effect and is at the same time closely linked with a recognizable character in the sporophyte, is there any disturbance in the Mendelian ratios. Even here the total disturbance may not be noticeable when data from several generations are combined because a reversal of the linkage relations will cause deviations to balance each other.

Genetic factors affecting the gametophyte generation, giving certain gametes an advantage or handicap in accomplishing fertilization, need not contradict the Mendelian conception of random assortment. In some plants, however, they must be taken into consideration, as are linkage and lethal factors, in accounting for unexpected results.

SUMMARY

1. A stock segregating for defective seeds, de_1, regularly produces *high* ears with approximately 33 percent recessives, *normal* ears with 25 percent recessives, and *low* ears with 17 percent recessives.

2. These results are explained by assuming a factor, *Ga*, which has its expression in the gametophyte generation, stimulating the rate of pollen tube growth. *Ga* is linked with de_1 and in the coupling phase causes an excess of recessives; in the repulsion phase, a deficiency.

3. The cross of sugary × Rice Pop indicates the presence of a similar gametophyte factor linked with *Su* in the Rice Pop parent. The percentage of sugary seeds in F_2 is 16.2.

4. *Su* and de_1 are linked, with 39 percent crossing over, and it is probable that the gametophyte factor in both stocks is the same.

5. On this assumption it has been calculated that the *Ga* gametes accomplish fertilization 4.1 times as frequently as *ga* gametes and that the crossing over between *Ga* and de_1 is 24.3 percent; between *Ga* and *Su*, 21.2 percent.

6. That these values are approximately correct is indicated by WENTZ' data from a cross between de_{su}, a fourth character in this group, and a

stock with Rice Pop ancestry. This cross resulted in deviations approaching those predicted by the above calculations.

7. Gametophyte factors probably account, to a large extent, for the peculiar results obtained in mixed pollination experiments.

8. An analysis of KEMPTON's data indicates the presence, on the waxy chromosome, of a gametophyte factor causing distortions in the starchy: waxy ratio.

9. The action of the same factor is suggested as explanation of COULTER's peculiar ratios in aleurone color segregation.

10. The various ways in which gametophyte factors may operate and their effects on Mendelian ratios in the sporophyte are discussed in detail.

LITERATURE CITED

ALLEN, C. E., 1924 Gametophytic inheritance in Sphaerocarpos I. Intraclonal variation, and the inheritance of the tufted character. Genetics **9**: 530–587.

ALLEN, C. E., 1924 Gametophytic inheritance in Sphaerocarpos II. The polycladous character. Genetics **10**: 1–16.

BRIEGER, F. G., 1926 Mendelian factors producing selective fertilization. Amer. Nat. **60**: 183–191.

BRINK, R. A., 1925 Mendelian ratios and the gametophyte generation in angiosperms. Genetics **10**: 359–394.

BRINK, R. A. and MACGILLIVRAY, J. H., 1924 Segregation for the waxy character in maize pollen and differential development of the male gametophyte. Amer. Jour. Bot. **11**: 465–469.

COLLINS, G. N. and KEMPTON, J. H., 1911 Inheritance of waxy endosperm in hybrids of Chinese maize. 4th Conf. Inter. Genetique Compt. Rend. et Rapp. 347–357.

CORRENS, C., 1902 Scheinbare Ausnahmen von der Mendelschen Spaltungsregel für Bastarde. Bericht. Deutsch. Bot. Gesell. **20**: 157–159.

CORRENS, C., 1921 Versuche, bei Pflanzen das Geschlecltsverhältnis zu verschieben. Hereditas **2**: 1–24.

COULTER, Merle C., 1925 A distortion of the 3 : 1 ratio. Bot. Gaz. **79**: 28–44.

CUÉNOT, L., 1908 Sur quelques anomalies apparentes des proportions Mendéliennes. Arch. Zool. Exp. et Gen. **9**: 7–15.

DEMEREC, M., 1924 A case of pollen dimorphism in maize. Amer. Jour. Bot. **11**: 461–464.

EAST, E. M., 1922 As Genetics comes of age. Jour. Heredity **13**: 207–214.

EAST, E. M., and HAYES, H. K., 1911 Inheritance in maize. Conn. Agric. Expt. Sta. Bull. **167**: 1–142.

EAST, E. M., and MANGELSDORF, A. J., 1925 A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. **11**: 166–171.

EAST, E. M., and MANGELSDORF, A. J., 1926 Studies on self-sterility VII. Heredity and selective pollen-tube growth. Genetics (in press).

EMERSON, R. A., 1925 A possible case of selective fertilization in maize hybrids. Abstract in Anatomical Record **29**: 136.

EYSTER, W. H., 1921 The linkage relations between the factors for tunicate ear and sugary endosperm in maize. Genetics **6**: 209–240.

HALDANE, J. B. S., 1919 The combination of linkage values and the calculation of distances between the loci of linked factors. Jour. Genetics **8**: 299–309.

HERIBERT-NILSSON, N., 1920 Zuwachsgeschwindigkeit der Pollenschläuche und Gestörte Mendelzahlen bei *Oenothera Lamarckiana*. Hereditas **1**: 41–63.

Jones, D. F., 1920 Heritable characters of maize IV. A lethal factor-defective seeds. Jour. Heredity. **11**: 161–167.

Jones, D. F., 1920 Selective fertilization in pollen mixtures. Biol. Bull. **38**: 251–289.

Jones, D. F., 1922 Selective fertilization and the rate of pollen-tube growth. Biol. Bull. **43**: 167–174.

Jones, D. F., 1924 Selective fertilization among the gametes from the same individuals. Proc. Nat. Acad. Sci. **10**: 218–221.

Kempton, J. H., 1919 Inheritance of waxy endosperm in maize. U. S. Dept. Agric., Bureau of Plant Industry Bull. **754**: 1–99.

Kiesselbach, T. A., and Petersen, N. F., 1926 The segregation of carbohydrates in crosses between waxy and starchy types of maize. Genetics **11**: (in press).

Longley, Albert E., 1924 Chromosomes in maize and maize relatives. Jour. Agric. Res. **28**: 673–682.

Mangelsdorf, P. C., 1923 The inheritance of defective seeds in maize. Jour. Heredity **14**: 119–125.

Mangelsdorf, P. C., 1926 The genetics and morphology of some endosperm characters in maize. Connecticut Agric. Exp. Sta. Bull. 279: 513–614.

Weatherwax, Paul., 1922 A rare carbohydrate in waxy maize. Genetics **7**: 568–572.

Wentz, John B., 1925 Linkage between sweet defective and sugary endosperm in maize. Genetics **10**: 395–401.

Wettstein, F., 1924 Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage. Zeitschr. indukt. Abstamm. u. Vererb. **33**: 1–236.