Kohn, H. I., "On the Mode of Action of Sulfonamides. II. The Specific Antagonism Between Methionine and the Sulfonamides in *Escherichia coli*," *Ibid.*, **73**, 383–400 (1941).

DIRECTED FERTILIZATION IN MAIZE

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Ordinarily the two gametes in the maize pollen grain are genetically identical. However, a plant carrying an A-B interchange produces a pollen grain in which the gametes are not alike.[1] This paper deals with the rôle of the dissimilar gametes in fertilization.

An A-B interchange is one between the supernumerary B-type chromosome[2] and a member of the basic complement (A-type chromosome). The behavior of the interchange chromosomes during the development of the pollen grain may be illustrated by the case of TB-4a. In this interchange, the distal seven-eighths or so of the short arm of chromosome 4 was transferred to a segment of a B-type bearing the centromere of the latter. The resulting chromosome is designated the B^4 chromosome. The other interchange chromosome (4^B) contains the rest of chromosome 4 and most or all of the distal heterochromatic segment of the B-type.

The 4^B chromosome is orthodox in its behavior and is found regularly in each gamete of the pollen grain. The B^4 chromosome also follows the normal pattern until the division of the generative nucleus is reached. In this division the B^4 chromosome frequently undergoes non-disjunction so that one of the gametes receives two B^4 chromosomes and the other receives none. A second type of pollen grain is the result of normal disjunction; both gametes of the pollen grain are identical, each containing one B^4 chromosome.

Three classes of seed are expected when a normal seed parent is crossed with a pollen parent homozygous for TB-4a. Two of these are obtained from fertilization involving the first type of pollen grain. If the gamete that is deficient for the B^4 chromosome fertilizes the egg and its partner unites with the polar nuclei, a seed with a deficient embryo and a hyperploid endosperm will result (Class I). If the gametes exchange their respective rôles in fertilization, the seed will have an embryo hyperploid for the B^4 chromosome and a deficient endosperm (Class II). A third kind of seed is produced when the second pollen type is involved; in this case, the embryo is heterozygous for the interchange and the endosperm also carries a single B^4 chromosome (Class III).

The three classes of seed may be readily identified through the use of the sugary endosperm gene, *su*. The B^4 chromosome carries the dominant allele *Su*. If the seed parent is homozygous for *su*, the three kinds of seed would have, respectively:

Class I: A non-sugary, hyperploid endosperm and a deficient embryo, hemizygous for *su;*

Class II: A sugary, deficient endosperm and an embryo of composition *su Su Su;* and

Class III: A non-sugary, euploid endosperm with a euploid embryo of composition *su Su.*

If fertilization involving the first type of pollen grain occurs in either direction with equal frequency (i.e., random fertilization), seed classes I

TABLE 1

RESULTS OF CROSSES BETWEEN NORMAL SEED PARENTS HOMOZYGOUS FOR *su* AND . POLLEN PARENTS HOMOZYGOUS FOR TB-4a AND *Su*

CROSS	SUGARY SEEDS	TOTAL SEEDS	% SUGARY	±S.E.
GC-1 × 25-2	162	271	59.8	3.0
2 × 25-2	175	267	65.5	2.9
3 × 25-2	141	217	65.0	3.2
4 × 25-2	158	229	69.0	3.1
5 × 25-2	106	218	48.6	3.4
107-3 × 25-2	164	274	59.9	3.0
107-4 × 25-2	136	262	51.9	3.1
2268-3 × 25-2	130	210	61.9	3.4
2269-2 × 25-2	226	410	55.1	2.5
2269-7 × 25-2	314	504	62.3	2.2
108-28 × 50-13	76	132	57.6	4.3
273-2 × 50-13	124	216	57.4	3.4
263-10 × 50-14	169	303	55.8	2.9
277-4 × 50-14	74	118	62.7	4.5
Totals	2155	3631	59.4	0.8

and II should be equal in number assuming they are equally viable. The number of seeds with a sugary endosperm (Class II) would not be expected, on this basis, to exceed the number of non-sugary seeds. Actually, the sugary seeds should be in the minority since the non-sugary seeds are expected to include not only those of Class I but also those of Class III, the latter derived from fertilization involving the second type of pollen grain. The results of crosses published previously[1] gave an average value of approximately 50% for the proportion of sugary seeds and this was interpreted, assuming random fertilization, as an indication of a very low rate of normal disjunction. There were two crosses, however, in this first

group that gave what appeared to be a significantly higher proportion of sugary seeds. Subsequent crosses involving three different but closely related pollen parents, cytologically identified as homozygous for TB-4a, have given a segregation of sugary seeds clearly in excess of 50% (table 1). It should be noted that these data are not selected but represent the total harvest of crosses involving these parents.

The extent to which the sugary seeds outnumber their reciprocal counterparts (Class I) cannot be determined directly from the data in table 1, since the non-sugary seeds that have a hyperploid endosperm are indistinguishable from those with a euploid endosperm (Class III). The two types of non-sugary seeds may, however, be separated on the basis of the difference between their embryos. This may be accomplished, in the plants grown from these seeds, by a direct cytological examination of the microsporocytes, by an examination of the mature pollen for the percentage of aborted grains, or on the basis of sugary ratios in crosses with a *su su* parent. The identification methods employed will be described in detail elsewhere.

TABLE 2

EMBRYO COMPOSITION OF NON-SUGARY AND SUGARY SEEDS OF CROSSES GIVEN IN TABLE 1. SEE TEXT FOR FURTHER EXPLANATION

CROSS	NON-SUGARY SEEDS			SUGARY SEEDS		
	− (CLASS I)	+ (CLASS III)	+ +	−	+	+ + (CLASS II)
GC-4 × 25-2	25	28	1	1	1	131
GC-5 × 25-2	40	32	2	0	0	87
2269-2 × 25-2	44	16	2	0	1	83
2269-7 × 25-2	43	48	0	0	0	81
Totals	152	124	5	1	2	382

Table 2 shows the distribution of embryo types found in non-sugary seeds. Also included for comparison are the types contained in sugary seeds. The symbols −, + and + + refer, respectively, to: (1) an embryo deficient for the B^4 chromosome, (2) a heterozygous embryo, carrying one B^4 chromosome and (3) a hyperploid embryo, with two B^4 chromosomes. Among the non-sugary seeds, only about half contain the deficient embryo indicative of Class I.

The data in table 2 also reaffirm the conclusion reached from previous evidence that a plant homozygous for TB-4a produces two types of functional pollen grains. With only eight exceptions among 681 seeds tested, the seeds fall into the three classes described above. The exceptions would be expected normally, as a consequence of heterofertilization,[3] in which the gametes of two different pollen grains unite with the egg and polar nuclei of the same embryo sac.

If we assume from the data in table 2 that half of the non-sugary seeds belong to Class III and apply this correction to the data in table 1, the

preponderance of Class II seeds over Class I becomes even more evident. The cross 2269-7 $\times$ 25-2 gave 314 sugary seeds and 190 non-sugary seeds; when the correction is applied, the ratio of Class II to Class I is 314 to 95, or roughly 3:1. In the cross GC-5 $\times$ 25-2, which gave a relatively low percentage of sugary seeds, the ratio is 106 to 56, or approximately 2:1. If we take 60% as an average value for the frequency of sugary seeds in all of the crosses of table 1, the proportion of Class I:Class II:Class III becomes, using the same correction, 1:3:1. Thus, four-fifths of the progeny were obtained from fertilizations involving pollen grains in which non-disjunction had occurred and in three-fourths of these, the deficient gamete united with the polar nuclei and the hyperploid gamete with the egg.

It was expected, assuming random fertilization, that the Class I and Class II seeds would occur in equal numbers. The low frequency of Class I seed brings up the question of the relative viability of this type. Are these seeds being lost in development? If this were so, it should result in a detectable amount of sterility on the ear since the loss would comprise two-thirds of this class or two-sevenths of the total population. Actually, several of the ears in table 1, including those which gave the most disproportionate ratios, had full sets of seed. It seems likely, therefore, that the low proportion of Class I seeds is evidence that fertilization is not random; that the union of the egg with the hyperploid gamete and the concomitant fusion of the polar nuclei with the deficient gamete is preferred to the reverse order of fertilization, in which the deficient gamete unites with the egg and the hyperploid gamete with the polar nuclei.

Similar results have been obtained with TB-9b, an interchange involving chromosome 9 of the basic set. The break in chromosome 9 occurred about halfway along the short arm, between C and Wx; the B-type chromosome was broken within the distal heterochromatic segment. In the cross to be described, the B^9 chromosome carried C (for anthocyanin-colored endosperm) and 9^B chromosome carried Wx (for starchy endosperm). The TB-9b pollen parents used in the cross were homozygous for the 9^B chromosome and hemizygous for the B^9 chromosome (with respect to chromosome 9, their constitution was $9^B 9^B B^9$). Such a plant is equivalent, as regards male transmission, to the homozygote (with two B^9 chromosomes), since only the microspores possessing both a B^9 and a 9^B chromosome develop into functional pollen grains. The microspores that are deficient for the B^9 chromosome produce aborted pollen grains with a distinctly reduced starch content.

The seed parents used in the cross were cytologically normal and homozygous for c and wx. In the TB-9b pollen parent, the B^9 chromosome undergoes non-disjunction in the division of the generative nucleus. A pollen grain is thus produced in which one gamete has two B^9 chromosomes

and the other has none (and therefore lacks C). The union of the hyper-
ploid gamete with the polar nuclei and the deficient gamete with the egg
would give a kernel with a hyperploid, colored endosperm and a deficient
embryo (analogous to the Class I seed obtained from the crosses involving
TB-4a). The reciprocal order of fertilization would produce a seed with a
deficient, colorless endosperm and a hyperploid embryo (Class II). The
results of six crosses, involving two TB-9b parents are given in table 3.
Here, as with TB-4a, the seeds with a deficient endosperm are clearly in
the majority. With rare exception, the seeds of both classes were starchy,
an indication of the regularity of disjunction of the 9^B chromosome in
the division of the generative nucleus.

TABLE 3

SEE TEXT FOR DESCRIPTION OF CROSS

CROSS	SEEDS WITH COLORLESS ENDOSPERM	TOTAL SEEDS	% COLORLESS	±S.E.
119-4 × 96-8	96	148	64.9	3.9
119-11 × 96-8	235	356	66.0	2.5
2264-7 × 96-8	198	334	59.3	2.7
119-5 × 96-15	214	305	70.2	2.6
119-10 × 96-15	150	249	60.2	3.1
2264-11 × 96-15	220	353	62.3	2.6

Scutellum color was used as an indicator of the presence of the B^9
chromosome in the embryos of the colored and colorless seeds. C, in
addition to certain other known genes, is required for anthocyanin color
in the scutellum.[4] Two of the crosses gave an appropriate complementary
genotype and the seeds obtained from these were classified for scutellum
color in relation to endosperm color. It was anticipated that the Class I
seeds should have a colorless embryo and the Class II seeds should have a
colored embryo. The results given in table 4 show that over 97% of the

TABLE 4

RELATION OF SCUTELLUM COLOR TO ENDOSPERM COLOR IN TWO CROSSES OF TABLE 3

CROSS	COLORED ENDOSPERM*		COLORLESS ENDOSPERM†	
	COLORED SCUTELLUM	COLORLESS SCUTELLUM	COLORED SCUTELLUM	COLORLESS SCUTELLUM
119-4 × 96-8	0	52	95	1
119-11 × 96-8	9‡	108	232	2
Totals	9	160	327	3

  * Three others lacked an embryo and one other was also unclassifiable for scutellum
color.
  † One other lacked an embryo.
  ‡ One had a colorless endosperm sector.

seeds fall into these two categories and that the Class II seed outnumbers
its reciprocal counterpart by approximately 2:1.

Twelve other seeds, not of Class I nor II, were also obtained. Three had a colorless endosperm as well as a colorless embryo; they were therefore deficient in both endosperm and embryo for the B^9 chromosome. They may be accounted for as cases of heterofertilization in which deficient gametes from two different pollen grains were involved. The nine seeds with a colored endosperm and a colored embryo may also be the result of heterofertilization, this time involving two hyperploid gametes. Or they may represent fertilization involving a pollen grain in which both gametes contain one B^9 chromosome, as a consequence of normal disjunction of this chromosome (thus equivalent to the Class III seeds of table 2). The frequency of normal disjunction appears to be much lower for the B^9 chromosome than for the B^4 chromosome. Whether this is a characteristic difference between the two chromosomes or is an expression of a difference in genotype in the two parental types is a question for further investigation.

The evidence from the crosses with TB-4a and TB-9b may be summarized as follows. Plants carrying either of these interchanges produce, in the main, one type of pollen grain. This contains one gamete deficient for the aberrant chromosome (B^4 or B^9, as the case may be) and another gamete carrying the chromosome in duplicate. Fertilization involving the two gametes may proceed in either of two ways: (1) the deficient gamete may unite with the polar nuclei to form the primary endosperm nucleus and the hyperploid gamete fertilizes the egg or (2) the hyperploid gamete may unite with the polar nuclei and the deficient partner with the egg. The first fertilization pattern is much more prevalent than the second. In the case of TB-4a, it was estimated that the first occurred three times as frequently as the second; with TB-9b, it was twice as frequent. The preference of one pattern over the other will be referred to by the term "directed fertilization."

Two possible explanations that will account for directed fertilization are being tested. The first of these assumes a specific orientation of the deficient and the hyperploid gametes within the pollen grain or at some time prior to fertilization. According to this hypothesis one of the gametes would have a positional advantage over the other that would result in the preferential union of the hyperploid gamete with the egg and the deficient gamete with the polar nuclei. The other explanation assumes a physiological difference between the hyperploid and deficient gametes that results in directed fertilization.

The assumption that the gametes are oriented within the pollen grain presupposes that one of the oriented gametes receives both of the nondisjoining chromosomes more often than the other. Evidence for a directed distribution such as this has been demonstrated cytologically in other organisms in which mitotic non-disjunction occurs. In *Sciara co-*

prophila, the sex chromosome moves to a specific pole in the division of the secondary spermatocyte.[5] In rye, the supernumerary chromosome, which undergoes non-disjunction in the first division of the microspore, is found more often in the generative nucleus than in the vegetative nucleus.[6] The products of non-disjunction of the supernumerary chromosome in sorghum are directed to the generative pole in the exceptional division of the vegetative nucleus.[7]

It is doubtful that the deficiency or duplication of A-chromatin (the segment of chromosome 4 in the B[4] chromosome or chromosome 9 in the B[9] chromosome) in the gametes is an essential factor in determining directed fertilization. An indication of directed fertilization is also found in the results of a cross in which the pollen parent had two intact B-type chromosomes and a normal basic complement. When the seed parent was devoid of B-types, most of the progeny possessed either two B-types or none in their embryos. The progeny with two B-types were in a marked majority.[2,8] This may be interpreted as follows: (1) the 2B parent produces a pollen grain in which one gamete has two B-types and the other has none; and (2) the 2B gamete has an advantage over its OB partner in the fertilization of the egg.

Directed fertilization of the egg by the 2B gamete is also of interest in connection with the problem of the survival of the B-type chromosome. This chromosome is subject to fragmentation and also to loss in meiosis.[2,9] Yet it persists in a number of maize varieties and is particularly prevalent in certain sugary strains, notably the Black Mexican. The preferential fertilization of the egg by the gamete containing the B-types, after non-disjunction, would provide an excess of B-types to counteract the fragmentation and loss of this chromosome. The common occurrence of the B-type in certain varieties could then be accounted for if the mechanism of directed fertilization is more highly developed in these strains, as compared with others in which the B-type is absent or rarely found.

[1] Roman, H., *Genetics,* **32,** 391–409 (1947).

[2] Randolph, L. F., *Ibid.,* **26,** 608–631 (1941).

[3] Sprague, G. F., *Ibid.,* **17,** 358–368 (1932).

[4] Sprague, G. F., *U. S. Dept. Agr. Tech. Bull.,* **292,** 1–43 (1932).

[5] Metz, C. W., *Am. Naturalist,* **72,** 485–520 (1938).

[6] Müntzing, A., *Hereditas,* **32,** 97–119 (1946).

[7] Darlington, C. D., and Thomas, P. T., *Proc. Roy. Soc. London,* **130,** 127–150 (1941).

[8] Supplementary data (unpublished) were kindly furnished by Dr. A. E. Longley. Among the examined progeny from seven OB × 2B crosses, 31 had 2B's and 13 had none.

[9] Darlington, C. D., and Upcott, M. B., *J. Genet.,* **41,** 275–296 (1941).