FACTORS AFFECTING PREFERENTIAL FERTILIZATION IN MAIZE[1]

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THE B chromosome of maize is highly heterochromatic, relatively inert, and lacking in known genetic markers (RANDOLPH 1941). The inheritance of B chromosomes is irregular and plants within a single population may differ greatly in the number of B chromosomes they contain. LONGLEY (1927), RANDOLPH (1941), and BLACKWOOD (1956) each analyzed the progeny of reciprocal crosses between plants lacking B chromosomes and plants with one or two B's. They found that B chromosomes are transmitted in a regular fashion through the female parent, but apparently undergo nondisjunction in the male inflorescence. ROMAN (1947) was able to identify the division at which nondisjunction occurs by means of translocations between B chromosomes and members of the regular (A) complement. The chromosomes making up an A-B translocation are designated A^B (containing the centromere of the A chromosome) and B^A (containing the B centromere and A chromosome genetic markers). ROMAN found, with two different translocations, that the B^A chromosome regularly undergoes nondisjunction at the second pollen mitosis, producing sperm with zero B^A and two B^A chromosomes. As a result of double fertilization, kernels are produced in which the endosperm and embryo differ in regard to presence or absence of B^A markers. It was inferred, from these results, that nondisjunction of the normal intact B chromosome also occurs at the second pollen mitosis. Further, investigation demonstrated that sperm containing two B^A's have an advantage in fertilization of the egg over sperm lacking B^A's (ROMAN 1948). ROMAN suggested that sperm carrying normal B chromosomes also preferentially fertilize the egg, and that preferential fertilization serves, together with nondisjunction, as an accumulation mechanism for the B's, increasing their numbers in a population. Possible causes of preferential fertilization are considered in this investigation.

ROMAN (1948) proposed two explanations for preferential fertilization: B^A (or B) chromosomes may confer on sperm the ability to preferentially fertilize the egg, or they may be included in sperm that are destined to unite with the egg. The latter idea requires that the chromosomes undergoing nondisjunction at the second pollen mitosis migrate to a specific pole, whose derivative sperm usually fertilizes the egg. Such an oriented movement of chromosomes has considerable precedent, and is known to be involved in the accumulation mechanisms of B's from many species (BATTAGLIA 1964). In 1956, CATCHESIDE considered the consequences of an oriented nondisjunction of B's in maize. In plants with several B

chromosomes, the B's should often migrate to the same sperm, following non-disjunction. The distribution of B's to the progeny would, therefore, differ from that predicted on a random migration to the poles. CATCHESIDE analyzed the cytological data of RANDOLPH (1941) and BLACKWOOD (1956) on B chromosome inheritance and concluded that B's migrate to a specific pole during the second pollen mitosis. The analysis was made difficult by the fact that B's sometimes fail to undergo nondisjunction at the second pollen mitosis, and this tends to randomize distribution to the poles. In this investigation, the question of a random vs. an oriented nondisjunction of B chromosomes has been re-examined, using the A-B translocations, TB-9b and TB-4a. In addition, the behavior of an inbred line of maize, which has a striking effect on preferential fertilization, is described.

MATERIALS AND METHODS

The B chromosome of maize has been described by McCLINTOCK (1933). It is subdivided, from proximal to distal ends, into a terminal centromere, a proximal heterochromatic knob, an interstitial euchromatic section, and a large block of distal heterochromatin.

Two of the A-B translocations of ROMAN, TB-4a and TB-9b, are utilized in the present investigation. The breakpoints of TB-4a are at position 0.15 in the short arm of chromosome 4, and at the juncture of the euchromatic and distal heterochromatic regions of the B. The B^4 chromosome carries the dominant Su_1 marker from chromosome 4. In TB-9b, the breakpoint in the short arm of chromosome 9 was at 0.5, and that in the B chromosome slightly distal to the juncture of the euchromatic and distal heterochromatic regions. The B^9 chromosome contains dominant alleles for Yg, C, Sh, and Bz. The Wx allele is present on the 9^B chromosome. Crossing over between Wx and the translocation breakpoint occurs less than 0.5% of the time (ROBERTSON 1967) and the Wx allele, therefore, marks the 9^B.

The meiotic behavior of TB-9b has been analyzed by ROBERTSON (1967) and his results probably apply to other A-B translocations. One useful generalization concerning meiotic behavior of TB-9b is that plants heterozygous for the 9 and 9^B chromosomes show regular disjunction of these chromosomes, irrespective of the presence of zero, one, or two B^9's.

Cytological techniques: During development of the TB-9b/TB-4a plants, root tip preparations were used to insure the presence of the telocentric B^A chromosomes. Root tips were pre-fixed in monobromonaphthalene for four hrs and fixed overnight at room temperature in 3:1 ethanol/glacial acetic acid. They were stained by the Feulgen method and destained in tap water for one-half hour. The root tips were then macerated with a glass rod in acetoorcein and a cover slip applied. Telocentric chromosomes were identified at metaphase.

Genetic markers: The markers yg_2, c, sh, bz, and wx are located in the short arm of chromosome 9. The yg_2 phenotype is expressed in the seedling, c, sh, and wx in the endosperm and bz in both the seedling and endosperm. The order of the genes on the chromosome is as given, with yg_2 being the distal marker and wx proximal. In TB-9b, the breakpoint of chromosome 9 is between bz and wx. The recessive yg_2 gives a yellow-green plant, c a colorless aleurone, sh a collapsed endosperm, wx an endosperm starch that is deficient for amylose, and bz a plant and endosperm with bronze color.

The su marker determines an endosperm trait (shriveled appearance) and is located in the short arm of chromosome 4. In TB-4a, the breakpoint in the short arm of chromosome 4 is proximal to the su locus.

RESULTS

In order to distinguish an oriented from a random nondisjunction at the second pollen mitosis, the segregation of two different B^A chromosomes to the sperm was tested. Two of ROMAN's A-B translocations, TB-4a and TB-9b, were combined

in the same plant and cases of simultaneous nondisjunction of the B^4 and B^9 chromosomes examined. Plants of the constitution $9^{Yg\ C\ Wx}\ 9^{BWx}\ B^{9Yg\ C}$; $4^{Su}\ 4^B\ B^{4Su}\ B^{4Su}$ were crossed as male parents onto a stock containing recessive alleles for both the chromosome 9 and chromosome 4 markers. The tester stock was an F_1 between a chromosome 9 tester ($yg_2\ c\ wx$) and a chromosome 4 tester (su_1). Only cases in which nondisjunction of both the B^9 and the B^4 chromosomes was known to have occurred were examined. Nondisjunction of the B^9 was indicated by the expression of the recessive c in the endosperm (Yg embryo) or yg in the embryo (C endosperm). Since an embryo marker was not available for the B^4 chromosome, only cases of nondisjunction in which the endosperm lacked the B^4 (su_1 phenotype) could be classified. With the markers available, two classes of simultaneous nondisjunction could be identified: kernels with a $c\ su$ endosperm, and kernels with a su endosperm and yg embryo. These two classes represent the movement of both chromosomes to one pole ($c\ su$) and their migration to opposite poles ($yg\ su$). On a random distribution of B's to the poles, one expects both the $c\ su$ and $yg\ su$ classes to occur frequently, with the $c\ su$ class being the larger, due to preferential fertilization (see DISCUSSION). An oriented migration, however, predicts that the $yg\ su$ class would be relatively rare.

Results are given in Table 1 for crosses of three male parents with the same chromosomal constitution (9 9^B B^9; 4 4^B B^4 B^4). The $c\ su$ class, for the pooled data, contains 461 individuals and the $yg\ su$ class 298. The su kernels planted for classification of yg seedlings did not show perfect germination, and the corrected value for $yg\ su$ is 311. The results suggest that an oriented nondisjunction of B's does not occur, but further proof is needed.

A second test was made to determine the effect of extra B chromosomes on preferential fertilization by B^A-containing sperm. If B chromosomes are randomly distributed to the poles during nondisjunction, (and confer an advantage on sperm in fertilization of the egg), plants with several B's should have pollen grains with B's in both sperm. Neither sperm should have an advantage in fertilization of the egg. Consequently, in crosses of plants containing one B^A chromosome and several intact B's, preferential fertilization by sperm containing two B^A chromosomes should not occur. On the other hand, if nondisjunction is oriented, and there is no direct effect of B's on fertilization of the egg, the presence of extra B's in a plant should not alter the frequency of fertilization of the egg by sperm carrying two B^A's. Isogenic lines of the Black Mexican variety, with and without B chromosomes, were crossed as male parents onto plants of the constitution 9 9^B B^9 B^9. The TB-9b plants all came from the same stock, which had been backcrossed for two generations to an inbred line. Among the progeny of the cross, heterozygous TB-9b plants (9 9^B B^9) were selected on the basis of pollen sterility. (As a result of meiotic segregation in TB-9b heterozygotes, 25% of the pollen receives a 9^B chromosome unaccompanied by a B^9. This pollen aborts.) Five heterozygous plants with no B chromosomes were crossed as male parents onto a $yg\ sh\ bz\ wx$ tester stock. Five other heterozygous plants, with six to eight B chromosomes, were also crossed as male parents onto a $yg\ sh\ bz\ wx$ stock. The TB-9b male parents were homozygous, in each case, for the dominant alleles Yg, Sh, Bz, and Wx. In the

TABLE 1

Endosperm and seedling classification of progeny from crosses of the following type:
$$yg\ c/++;\ su/+\ ♀\ \times\ 9^{C\ Yg}\ 9^{B}\ B^{9C\ Yg};\ 4^{Su}\ 4^{B}\ B^{4Su}\ B^{4Su}\ ♂.$$

Endosperm classification

Designation of male parent	Endosperm phenotypes			
	C Su	C su	c Su	c su
894–4	4825	1171	716	145
895–6	4691	1222	844	149
1050–3	5500	1336	828	167
	15016	3729	2388	461

Total *c su* = 461

Seedling classifications*

Designation of male parent	Type of kernels planted	Number of kernels planted	Number of seedlings recovered	Phenotype of seedling	
				yg	Yg
894–4	*C su*	1171	1121	98	1023
895–6	*C su*	1222	1179	118	1061
1050–3	*C su*	1336	1273	82	1191
		3729	3573	298	3275

Total *yg su* = 298
Corrected for germination,
    the total *yg su* = 311.

* Only kernels with the *C* phenotype in the endosperm can be *yg* in the embryo, since the *c* phenotype indicates that two B⁹'s, carrying *Yg*, are present in the embryo. Therefore, only *C su* individuals were germinated in a search for *yg su* individuals.

Results are given separately for three crosses with different male parents. The heterozygosity of the female parent for the recessive markers and the heterozygosity of the male parent for the translocations accounts for the low number of *c su* and *yg su* individuals in the progeny.

progeny, the ratio of *bz* kernels to *yg* seedlings was calculated as a measure of preferential fertilization. The results given in Table 2 demonstrate that preferential fertilization occurred at a normal rate (ROMAN 1948; BIANCHI 1961; CARLSON 1968) in the control group lacking supernumerary B's, but did not occur in the presence of extra B chromosomes. This finding supports the hypothesis that preferential fertilization depends on a selective advantage conferred on sperm by the B chromosome, and not on an oriented nondisjunction.

Another approach to the mechanism of preferential fertilization was provided by the discovery of an unusual inbred line of maize. In 1966, plants of the constitution $9^{c\ sh\ wx}\ 9^{BWx}\ B^{9C\ sh}\ B^{9C\ sh}$ were crossed as male parents onto two inbred lines, a *c sh wx* tester and a *c sh wx gl₁₅* tester. Meiotic segregation in 9 9ᴮ B⁹ B⁹ plants results in two main spore types: $9^{c\ sh\ wx}\ B^{9C\ sh}$ and $9^{BWx}\ B^{9C\ sh}$ (ROBERTSON 1967). Selection of *Wx* kernels among the progeny allowed analysis of only the 9^{BWx} $B^{9C\ sh}$ spores. (The B⁹ chromosome does not undergo nondisjunction in 9 B⁹ pollen —CARLSON 1967, 1968—and this class cannot be used in a study of preferential

TABLE 2

Preferential fertilization by B^9-containing sperm in the presence and absence of extra B chromosomes

Female parent	Male parent (TB-9b with no extra B's)	bz	yg*	Percent bz	χ^2 value for deviation from 50%
yg sh bz wx	1028–5	351	170	67.5	64**
yg sh bz wx	1029–1	516	310	62.5	51**
yg sh bz wx	1030–3	283	126	69.1	61**
yg sh bz wx	1156–2	337	190	64.2	41**
yg sh bz wx	1157–2	505	253	66.6	84**
		1992	1049	65.5%	294**

Female parent	Male parent (TB-9b with 6-8 extra B's)	bz	yg*	Percent bz	χ^2 value for deviation from 50%
yg sh bz wx	886–2	207	212	49.4	0.09
yg sh bz wx	886–4	223	220	50.2	0.04
yg sh bz wx	887–2	257	251	50.8	0.07
yg sh bz wx	887–5	344	296	53.7	3.60
yg sh bz wx	1031–3	231	240	49.0	0.13
		1262	1219	51.0%	0.71

* The yg value was corrected to account for kernels that did not germinate. Since more than 97% of the kernels produced seedlings, only slight adjustments were necessary, and distortions due to viability effects of the recessive alleles are negligible.
** Significant difference at the 1% level.

fertilization.) Wx kernels were classified for $c\ sh$ vs. $C\ Sh$ in the endosperm. The data for two different male parents are given in Table 3. In both cases, the $c\ sh\ wx\ gl_{15}$ stock shows a lower frequency of $c\ sh\ Wx$ kernels in the progeny than do crosses with the $c\ sh\ wx$ stock. The $c\ sh\ Wx$ kernels resulted from nondisjunction of the B^9 in the pollen, followed by fertilization of the egg by the sperm carrying two B^9's. An influence of the female parent on the rate of preferential fertilization could account for the difference in $c\ sh\ Wx$ frequencies. An embryo classification of the $C\ Sh\ Wx$ kernels was made to identify cases of nondisjunction in which the sperm with two B^9 chromosomes united with the polar nuclei. Plants derived from $C\ Sh\ Wx$ kernels were crossed to a $c\ sh\ wx$ tester, and the presence or absence of the $C\ Sh$ alleles determined in progeny kernels. In the $c\ sh\ wx ♀ \times$ 678–5 ♂ cross, 88 out of 93 $C\ Sh\ Wx$ kernels tested lacked the $C\ Sh$ alleles in the embryo and had resulted from nondisjunction. When this information is combined with the data for the $c\ sh\ Wx$ kernels (Table 3), the calculated rate of nondisjunction in the cross was 98%. In the $c\ sh\ wx\ gl_{15} ♀ \times$ 678–5 ♂ cross, 86 out of 91 plants grown from $C\ Sh\ Wx$ kernels lacked the B^9 chromosome in the embryo. The overall rate of nondisjunction in this cross was 96%. The rates of nondisjunction in the two crosses of plant 678–5 were, therefore, the same. From the data on nondisjunction, the rates of preferential fertilization for each of the crosses can be calculated. In the $c\ sh\ wx ♀ \times$ 678–5 ♂ cross, fertilization

TABLE 3

Endosperm classification of progeny from c sh wx ♀ × hyperploid TB-9b ♂ crosses

	χ^2 value for deviation of $c\ sh\ Wx$ value from population average in progeny of 678-5
1. $c\ sh\ wx \times 678\text{-}5\ (9^{c\ sh\ wx}\ 9B^{Wx}\ B9^{C\ Sh}\ B9^{C\ Sh})$	
$c\ sh\ \ Wx = 422\ (62.5\%)$	
$C\ Sh\ Wx = 252$	33^{**}
$C\ sh\ Wx = \ \ \ 1$	
$\overline{675}$	
$c\ sh\ wx\ gl_{15} \times 678\text{-}5$	
$c\ sh\ \ Wx = 224\ (38.5\%)$	
$C\ Sh\ Wx = 356$	39^{**}
$c\ Sh\ Wx = \ \ \ 1$	
$\overline{581}$	

	χ^2 value for deviation of $c\ sh\ Wx$ value from population average in progeny of 808-1
2. $c\ sh\ wx \times 808\text{-}1\ (9^{c\ sh\ wx}\ 9B^{Wx}\ B9^{C\ Sh}\ B9^{C\ Sh})$	
$c\ sh\ \ Wx = 238\ (58.4\%)$	
$C\ Sh\ Wx = 170$	5.7^{*}
$\overline{408}$	
$c\ sh\ wx\ gl_{15} \times 808\text{-}1$	
$c\ sh\ \ Wx = 131\ (43.5\%)$	
$C\ Sh\ Wx = 170$	8.3^{*}
$\overline{301}$	

* Significant at the 5% level.
** Significant at the 1% level.

of the egg by the B^9-containing sperm occurred 64% of the time (422/660), a common value for preferential fertilization (ROMAN 1948; BIANCHI *et al.* 1961; CARLSON 1968). However, in the $c\ sh\ wx\ gl_{15}$ ♀ × 678-5 ♂ cross, fertilization of the egg by the hyperploid sperm took place at a rate of only 40% (224/561), and indeed the hypoploid sperm more often united with the egg than did sperm hyperploid for the B^9. There was no preferential fertilization of the egg nucleus by the B^9-containing sperm.

A similar result was found in the crosses of plant 808-1. Classification of $C\ Sh\ Wx$ kernels from the cross $c\ sh\ wx$ ♀ × 808-1 ♂ showed 57 cases of nondisjunction out of 92 individuals tested. The overall rate of nondisjunction was 84%. In the cross $c\ sh\ wx\ gl_{15}$ ♀ × 808-1 ♂, 51 out of 75 $C\ Sh\ Wx$ kernels came from nondisjunction of the B^9 chromosome. The total rate of nondisjunction in this cross was 82%. No difference in the rate of nondisjunction between the two crosses of 808-1 was observed. When preferential fertilization was calculated, however, a difference between the crosses was found. In the $c\ sh\ wx$ ♀ × 808-1 ♂ cross, fertilization of the egg by B^9-containing sperm occurred at a rate of 69%

(238/343). But, in the $c\ sh\ wx\ gl_{15}$ ♀ × 808–1 ♂ cross, fertilization of the egg by this class of sperm was found only 53% (131/247) of the time.

Further tests were made in 1967 to insure that the influence of the $c\ sh\ wx\ gl_{15}$ stock on preferential fertilization was repeatable. Plants of the constitution 9 9^B B^9 B^9 were again crossed as male parents onto the $c\ sh\ wx\ gl_{15}$ stock and onto one of three other chromosome 9 tester lines. In each case, a single pollen shedding was used for crosses of the male parent with both female parents. Wx kernels were again selected from the progeny and classified in the endosperm for $c\ sh$ vs. $C\ Sh$ or $bz\ sh$ vs. $Bz\ Sh$, with the results shown in Table 4. Preferential fertilization was again reduced in crosses with the $c\ sh\ wx\ gl_{15}$ stock. In cross number 1, the difference in progeny data between the two female parents is so great that a reversal of preferential fertilization on the $c\ sh\ wx\ gl_{15}$ ears is suspected. A *direct* measure of preferential fertilization was obtained in the crosses listed as numbers 3, 4, 5, and 6. Here the embryo constitution of the $Bz\ Sh\ Wx$ kernels obtained in crosses with the $yg\ sh\ bz\ wx$ and $sh\ bz\ wx\ B\ Pl$ stocks could be identified in the seedling stage. In the $yg\ sh\ bz\ wx$ background, the presence of the B^9 chromosome in the embryo was indicated by the Yg phenotype (embryo constitution 9$^{yg\ wx}$ 9$^{B^{Wx}}$ B^{9Yg}) and its absence by the yg phenotype (embryo constitution 9$^{yg\ wx}$ 9$^{B^{Wx}}$). The corresponding classification in the $sh\ bz\ wx\ B\ Pl$ background depended on the bz vs. Bz seedling phenotypes. The embryo classification of $Sh\ Bz\ Wx$ kernels from crosses 3–6 is given in Table 5, together with the calculated rates of nondisjunction and preferential fertilization. In addition, values for preferential fertilization are given for the corresponding $c\ sh\ wx\ gl_{15}$ crosses. These values are calculated on the assumption that nondisjunction of the B^9 chromosome is constant per male parent. This was previously shown to be the case in crosses of

TABLE 4

Endosperm classification of the progeny from sh wx ♀ × *hyperploid TB-9b* ♂ *crosses*

Female parent	Male parent	Percent of $c\ sh\ Wx$ or $bz\ sh\ Wx$ per total Wx	χ^2 value for deviation of ratio in selected population from ratio obtained for total progeny of male parent
1. a) $c\ sh\ wx$	1122–2	65.4 (237/362)	136**
b) $c\ sh\ wx\ gl_{15}$	1122–2	27.5 (332/1204)	38**
2. a) $c\ sh\ wx$	1119–1	66.7 (239/358)	100**
b) $c\ sh\ wx\ gl_{15}$	1119–1	49.9 (124/249)	11**
3. a) $yg\ sh\ bz\ wx$	1018–2	57.5 (260/452)	14**
b) $c\ sh\ wx\ gl_{15}$	1018–2	40.0 (182/456)	13**
4. a) $yg\ sh\ bz\ wx$	1020–2	51.0 (211/417)	7*
b) $c\ sh\ wx\ gl_{15}$	1020–2	38.0 (159/418)	7*
5. a) $yg\ sh\ bz\ wx$	1019–1	60.5 (374/619)	9*
b) $c\ sh\ wx\ gl_{15}$	1019–1	42.6 (135/300)	19**
6. a) $sh\ bz\ wx\ B\ Pl$	1017–3	66.7 (614/925)	48**
b) $c\ sh\ wx\ gl_{15}$	1017–3	43.0 (260/660)	66**

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 5

Observed rates of nondisjunction and preferential fertilization for the crosses 3a, 4a, 5a, 6a of
Table 4, and the inferred rates of preferential fertiliaztion for 3b, 4b, 5b, and 6b

Female parent	Male parent	Embryo classification of progeny kernels with a *Sh Bz Wx* endosperm phenotype*	Overall rate of nondisjunction (calculated from data in this table and data in Table 4) (percent)	Rate of preferential fertilization (calculated from data in this table and data in Table 4) (percent)	Rate of preferential fertilization in the cross of the same male parent with the *c sh wx gl₁₅* female parent (Table 4). (Calculated on the assumption that the rate of nondisjunction is constant per male parent) (percent)
yg sh bz wx	1018–2	147 *yg*	90	64	44
		45 *Yg*			
yg sh bz wx	1020–2	166 *yg*	93	55	42
		29 *Yg*			
yg sh bz wx	1019–2	196 *yg*	92	65	45
		35 *Yg*			
yg sh bz wx	1017–3	183 *bz*	97	69	44
		18 *Bz*			

* The values given are not corrected for germination, but calculations were made with corrected values. The percent of germination of the *Sh Bz Wx* kernels in the crosses listed above were, respectively, 100%, 94%, 99%, and 91%. In the first three crosses, all the *Sh Bz Wx* kernels available were classified for embryo constitution. In the last cross, 222 of the 311 kernels were classified.

678–5 and 808–1. The lack of preferential fertilization in crosses with the *c sh wx gl₁₅* stock is apparent. The consistency of these results with those found earlier strongly suggests that preferential fertilization never occurs in crosses with *c sh wx gl₁₅* female parents. However, the data could also be explained if there were abortion of specific kernel types (i.e. *c sh Wx* class) in the *c sh wx gl₁₅* crosses. In cross number 1 of Table 3 abortion on the *c sh wx gl₁₅* ears must occur at a rate of 50%, in order to account for the lowered *c sh Wx* percentage. When these ears were examined, only 13% abortion was found (649 kernels per 747 ovules). For an inbred line, this rate of abortion is not unusually high, and it certainly does not account for the reduced frequency of *c sh Wx* kernels.

DISCUSSION

Preferential fertilization of the egg by sperm containing B chromosomes was discovered by ROMAN in 1948. He proposed two possible explanations for the phenomenon. On the one hand, B's may directly confer on sperm the ability to preferentially fertilize the egg. Alternatively, B's may be preferentially included in sperm that are predestined to fertilize the egg. The second idea requires an oriented nondisjunction at the second pollen mitosis with the B chromosomes migrating to a specific pole. Two experiments were performed as tests of ROMAN's proposals.

Plants containing both TB-9b and TB-4a were crossed as male parents to a tester stock and progeny selected in which simultaneous nondisjunction of the B⁴

and B^9 chromosomes was indicated. Two such classes were identifiable: $c\ su$ and $yg\ su$. The $c\ su$ individuals resulted from pollen in which the B^4 and B^9 chromosomes had moved to the same pole at the second pollen mitosis, while the $yg\ su$ class was produced by pollen in which the two chromosomes migrated to opposite poles. On the theory of an oriented nondisjunction of B's, $yg\ su$ individuals should seldom be found. In this experiment, however, 311 $yg\ su$ individuals were found, and 460 $c\ su$ (Table 1). The results are consistent with a random distribution of B's to the poles. The $c\ su$ class is expected to be larger than the $yg\ su$ class, following random distribution, because of preferential fertilization. In the pollen grains that produced $c\ su$ progeny, one sperm lacked B^A chromosomes, while the other carried two B^9's and two B^4's. The sperm with four B^A chromosomes should preferentially fertilize the egg and the $c\ su$ class should be larger than the reciprocal class (embryo deficient for B^4 and B^9), which was not analyzed in these crosses. In the pollen that gave rise to the $yg\ su$ class, one sperm contained two B^9 chromosomes and one contained two B^4's. Neither sperm is expected to have an advantage over the other in fertilization of the egg, and the reciprocal class of nondisjunction, which was not analyzed, should be equivalent in size to the $yg\ su$ class. Therefore, with a random migration of B's to the poles, the size of the $c\ su$ class should be larger than that of the $yg\ su$ class. The exact values expected for the two classes depend on the frequency of preferential fertilization. However, the percentages predicted for the $c\ su$ and $yg\ su$ groups closely approximate the data obtained if the following assumptions are made: (1) Fertilization of the egg by B^A-containing sperm occurs twice as often as fertilization by sperm lacking B^A chromosomes. This ratio has been found by ROMAN (1948), BIANCHI *et al.* (1961), and CARLSON (1968), although variations have been reported (ROMAN 1948; BIANCHI 1961). (2) Sperm with four B^A chromosomes show the same ability to fertilize the egg as sperm with two B^A chromosomes. On the above assumptions, and with a random distribution of B's at the second pollen mitosis, the $c\ su$ class should comprise 57% of the total, and the $yg\ su$ class 43% ($c\ su$ = $2/3 \times 50\% = 33\%$; $yg\ su = 1/2 \times 50\% = 25\%$: $\dfrac{33}{33+25} = 57\%$). The actual results obtained in the experiment were $c\ su = 461/772 = 60\%$ and $yg\ su = 311/772 = 40\%$. The agreement of expected and observed values is good suggesting that B chromosomes are randomly distributed to the poles at the second pollen mitosis.

Still, the data do not rule out an oriented nondisjunction, if certain assumptions are made. It is known that fertilization of the egg by sperm carrying B chromosomes usually occurs at a rate of 60–70%, and theories of nondisjunction must explain this value. One idea for oriented nondisjunction is that B chromosomes migrate 100% of the time to a specific spindle pole, and the derivative sperm fertilizes the egg at a rate of 60–70%. This explanation predicts the complete absence of $yg\ su$ individuals in the data, and is, therefore, not correct. An alternative idea is that B's move to a specific pole 60–70% of the time, and the sperm produced at the pole are 100% effective in preferential fertilization. This hypothesis allows for a number of $yg\ su$ individuals in the data and its predictions

need to be considered. Since segregation of the B^4 and B^9 chromosomes at the second pollen mitosis should be independent, the proportion of $c\,su$ and $yg\,su$ individuals expected is the same as the overall proportion of c to yg. In other words, the selection of su kernels for classification of c and yg should not affect the value obtained for preferential fertilization by B^9-containing sperm. Therefore the progeny of the TB-9b/TB-4a plants were classified for yg and c, without regard to su, and the overall rate of preferential fertilization was found to be 58% $\left(\dfrac{2849\,c}{2849\,c + 2021\,yg}\right)$. One expects from this theory that the $c\,su$ class also equals 58% of the total and $yg\,su$ 42%. And this is approximately true: $\dfrac{461\,c\,su}{461\,c\,su + 311\,yg\,su} = 60\%$. The data are, therefore, consistent with one theory of oriented nondisjunction as well as with a random distribution of B's.

To decide between the alternatives, plants containing TB-9b and several extra B chromosomes were synthesized, and preferential fertilization by sperm containing two B^9 chromosomes tested. If B-type chromosomes undergo an oriented nondisjunction at the second pollen mitosis, the presence of extra B's should not affect preferential fertilization by B^9-containing sperm. However, if B's are randomly distributed to the poles, and can increase the ability of sperm to unite with the egg, the added B chromosomes should eliminate preferential fertilization by sperm containing the B^9's. The data of Table 2 show that preferential fertilization does not occur in the presence of extra B chromosomes, supporting the second hypothesis.

The results of Table 2 are relevant to some other problems. Nondisjunction and preferential fertilization probably act together as an accumulation mechanism for the B chromosome. This accumulation mechanism is expected to raise the number of B chromosomes in a population to a level at which detrimental effects begin. RANDOLPH (1941) produced plants with high numbers of B's by inbreeding and found no harmful effect on the plants until 10 or more B's were present. The number of B's in a natural or unselected population should, therefore, be close to 10 per plant. However, this number is seldom found by investigators, and plants with B's sometimes occur in the same population with plants lacking B's (HUMPHREY 1934). The discrepancy can be accounted for if preferential fertilization fails to occur in the presence of several B's.

The suppressive effect of extra B chromosomes may also account for certain results of RHOADES, DEMPSEY, and GHIDONI (1967). When sperm cells differing in the presence of a normal or a deficient chromosome 3 were produced in the same pollen grain, no advantage of one sperm over the other in fertilization of the egg was found. The results suggested that variation in the A chromosome content of a sperm cannot cause preferential fertilization. However, in their experiments, the plants carried large numbers of B chromosomes, and these may have eliminated any effect of the A chromosome content on fertilization.

Finally, an inbred line of maize with an unusual effect on preferential fertilization was found. This stock, when crossed as a female parent to plants containing TB-9b, suppresses preferential fertilization of the egg. No other lines tested

or previously reported in the literature have this effect. Further study of this stock may provide information about the mechanism of preferential fertilization. The female inflorescences of the plants must be chemically or structurally different from those found in other stocks. Sectioning of the ears may reveal whether or not a structural abnormality is involved. Preliminary data (CARLSON 1968 and unpublished) suggest that the factor responsible for blocking preferential fertilization is inherited as a simple recessive, which is not expressed, through meiotic segregation, in the embryo sacs of an F_1 heterozygote.

SUMMARY

Preferential fertilization of the egg by sperm containing B^A chromosomes was discovered by ROMAN in 1948, and it was inferred that sperm with B chromosomes behave similarly. Two explanations were given for the phenomenon. One was that B chromosomes confer on sperm the ability to preferentially unite with the egg. The other idea was that B's become included in specific sperm that are predestined to fertilize the egg. Evidence presented indicates that the first explanation is the correct one. If the second explanation were correct, B chromosomes should migrate to a specific spindle pole during nondisjunction at the second pollen mitosis. However, when the segregation of two translocated B chromosomes was followed genetically at this division, no evidence for migration to a specific pole was found. The first explanation predicts that preferential fertilization will not occur when B's are present in both sperm of a pollen grain. And, indeed, when the pollen parent of a cross contained several intact B chromosomes in addition to the B^A, preferential fertilization was no longer observed. It seems likely, therefore, that B chromosomes confer on sperm the ability to preferentially fertilize the egg. The elimination of preferential fertilization in the presence of several B chromosomes also explains why large numbers of B's do not accumulate in natural populations. The accumulation mechanism which selects sperm carrying B chromosomes for transmission to future generations, breaks down before large numbers can be reached.—Further information on the process of preferential fertilization has been obtained through the discovery of an unusual inbred line of maize. In contrast to the results from tests of other stocks, there is no preferential fertilization when this line is crossed as a female parent to plants containing the A-B translocation B-9b. The female inflorescences of this line must differ structurally or chemically from the norm. A cytological study may reveal the nature of the difference and further explain the mechanism of preferential fertilization.

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