Pollen tube growth in vitro and in vivo — Fresh pollen grains were germinated in cavity slides in aqueous culture medium consisting of 0.45M sucrose supplemented with Ca, B and Mg. More than 50% of pollen grains germinated, and mean pollen tube lengths were obtained for the parents and F_1's of the 8x8 diallel at ½, 1, 2 and 3 hours. Pollen tube growth in live silks was studied with the help of radioactive ^{32}P (House and Nelson, J. Hered. 69:18, 1958). Actively shedding tassels were removed from plants and the cut stalk end was immediately immersed in water in a conical flask. Radioactive ^{32}P in the form of H_3PO_4 of 350 µCi activity was administered in water. Radioactive pollen from the tassels was collected after 24 hours and used for pollination on silks specially prepared by cutting back on the previous afternoon. At 2, 4 and 6 hours after pollination cobs were harvested and the tips of the silks washed to remove ungerminated hot pollen. Silks were removed, dried by pressing between blotting paper sheets, and mounted in autoradiographic frames with X-ray film in direct contact. The films were developed after 5 weeks and the lengths of pollen tubes were measured.

Significant variability for rate of pollen tube growth both in vitro and in vivo was observed in the eight lines. The ranges were 1.50 - 3.69 µ per minute in vitro and 33 - 108 micra per minute for in vivo studies. In most of the crosses pollen from the hybrids had shown either intermediate rate or slower than either parent. Heterotic effects in rate of tube growth were obtained in a few crosses only.

Correlation studies involving pollen size and rate of pollen tube growth in artificial medium revealed that larger pollen showed a higher rate of pollen tube growth than the smaller pollen. A correlation coefficient for pollen tube growth in vitro computed over two hours after inoculation was 0.84 and significant. On the other hand, negative but non-significant correlation coefficients were obtained between pollen size and rate of tube growth in live silks. There was no correspondence between rates of pollen tube growth in vivo and in vitro.

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Stabilization of freshly broken chromosome ends in the endosperm mitoses — We have previously shown that B chromosomes cause loss of knobbed segments of members of the regular chromosome complement. We hypothesized that replication of the heterochromatic knobs was delayed at, and only at, the second microspore mitosis. We suggested that a dicentric chromosome is formed at anaphase; following bridge breakage, a broken chromatid passes to one sperm cell and an intact chromatid goes to the other sperm. Data from high-loss plants carrying a knobbed chromosome 9

are particularly instructive because 9S has marker genes (C and Wx) affecting the endosperm that are situated some distance apart. When pollen from a high-loss plant with a large terminal knob on 9S and the C and Wx alleles was used in crosses to c wx female lines, a number of distinctive kernel phenotypes were produced following breaks in 9S at the second microspore anaphase. Let region (1) be the knob-C interval, region (2) the C-Wx segment and region (3) the Wx-centromere interval. Breaks in (1) followed by a bridge-breakage-fusion cycle in the endo-sperm mitoses give kernels variegated for C-c and for Wx-wx. A break in (2) produces a kernel with colorless aleurone and an endosperm mosaic for Wx and wx sectors. Rupture in region (3) yields a colorless (c) kernel with all wx starch. Data from such a cross are given below:

Distribution of breaks in the short arm of chromosome 9 based on the frequency of whole loss and variegation for the C and Wx markers.

C-c, Wx-wx kernels from breaks in (1)	c, Wx-wx kernels from breaks in (2)	c, Wx kernels from breaks in (2) No Wx-wx mosaicism	c, wx kernels from breaks in (3)	Total kernels examined
30	20	14	601	3483

The unexpected feature of the above data is the occurrence of the 14 stable c Wx kernels. We postulated in our 1973 paper (J. Heredity) that these c Wx kernels could arise in the following ways:

(1) if there is a break between C and Wx followed by healing to form stable ends, as occurs in the embryo; (2) if chromosome 9 underwent a cryptic bridge-breakage-fusion cycle throughout endosperm development but, owing to a weak fusion of the broken ends, the dicentric bridges broke at the same position at every anaphase; (3) if a mutation of C to c occurred; or (4) if there was an internal deletion of the C locus. We tentatively concluded that the c Wx class was best accounted for by assuming a weak or imperfect union of broken ends. More recent observations suggest that none of the four mechanisms was correct and that a new type of structural modification is responsible. The pertinent data follow.

Crosses were made in which pollen from a high-loss strain with a terminal knob on 9S and the Yg2, C and Wx alleles was placed on silks of yg2 C wx testers. A deficiency of the Yg locus in a sperm fertilizing the yg egg would yield a yg seedling. Scores of exceptional yg seedlings arising from the above cross were grown and testcrossed as the female parent by Yg c wx pollen. The great majority of the exceptional yg plants had, in addition to a normal chromosome 9 with the yg C wx alleles, a chromosome 9 deficient for all of the markers on 9S, which was not transmitted. Some, however, had a chromosome 9 deficient for Yg but carrying

the C and Wx loci in some instances and only the Wx locus in others. When one of these exceptional yg plants (33016-1, which had a low percentage of aborted pollen) was pollinated by a Yg c wx plant, it gave an ear with 242 C Wx and 106 C wx kernels. Since the Wx allele carried by the deficient chromosome 9 was recovered more frequently than the wx allele on the normal chromosome, this situation was deemed worthy of further study. Plant 33016-1 was not examined cytologically, and all that could be inferred from the genetic data was that it had a deficiency for the Yg allele and that some unknown mechanism was responsible for the excess of Wx kernels. C Wx kernels from the above cross were planted as family 33356, and several individuals were backcrossed reciprocally; PMC's were also taken for cytological examination.

The cytogenetic analysis of individual plants of 33356 revealed the presence of four chromosomal classes.

Class I had: 2 normal chromosomes 4 with no marker genes
              1 normal chromosome 9 with the Yg c wx alleles
              1 chromosome 9 deficient for the tip of 9S including
                the Yg locus but capped by a terminal piece of 4L
                consisting of the segment from the knob to the tip

Since the deficient segment of 9S included McClintock's wd locus, this 9-4 translocated chromosome is designated T9(wd)-4 to indicate that it is a translocated chromosome with an internal deficiency for the tip of 9S. The deficient chromosome in Class I carried the dominant C and Wx alleles.

Two classes of gametes are produced by Class I plants: N9 N4 and T9 (wd)-4 N4. Class I plants were testcrossed reciprocally. When used as the female parent, a 1:1 ratio was found for the segregating C:c and Wx:wx alleles, indicating that megaspores with the T9(wd)-4 chromosome functioned as well as those with a N9. The T9(wd)-4 N4 megaspores have a duplication for the terminal piece of 4L and are deficient for the tip of 9S, but transmission is normal despite the genic imbalance. However, when Class I plants were used as the pollen parent in testcrosses, the following data were obtained:

C Wx	C wx	c Wx	c wx	Σ
46	9	116	1081	1252

If none of the T9(wd)-4 N4 pollen functioned in competition with N9 N4 pollen, the frequency of C kernels (4.4%) measures the amount of crossing over between C and the Wd deficiency. Two male testcrosses made onto yg c wx silks gave:

(1)	(0)	(1-2)	(2)	(2)	(1-2)	(0)	(1)	
Yg	yg	Yg	yg	Yg	yg	Yg	yg	
C	C	C	C	c	c	c	c	
Wx	Wx	wx	wx	Wx	Wx	wx	wx	Σ
4	11	1	1	35	0	310	2	364

The estimated amount of wd-C crossing over, based on the assumption of no functioning of T9(wd)-4 N4 pollen, is 4.7%. That some T9(wd)-4 N4 pollen does achieve fertilization in competition with N9 N4 grains is indicated by the occurrence of yg plants in the above table. These amount to 3.8% of the total population, i.e., the T9(wd)-4 N4 pollen functions only 4.0% (14/350) as well as does N9 N4 pollen. The amount of wd-C recombination is 1.9% (7/364). The C-Wx recombination of 10.0% is comparable to the value found in the reciprocal cross.

Class II had: 1 normal chromosome 4
                 1 chromosome 4 deficient for a terminal piece of 4L
                 1 T9(wd)-4 chromosome carrying C and Wx
                 1 N9 chromosome carrying Yg c wx

Four kinds of gametes are produced: N9 N4; T9-4 N4; T9-4 Df4; and N9 Df4. The latter will abort. Class II plants used as the pollen parent in crosses with c wx females gave:

C Wx	C wx	c Wx	c wx	Σ
1014	90	135	1074	2313

C-Wx recombination = 9.7%

The ratios of C:c (1104:1209) and of Wx:wx (1149:1164) indicate a high percentage of functioning of T9-4 Df4 pollen since we know from the data of Class I plants that there is a low functioning of T9-4 N4 pollen. This is expected since there is no duplication and only a small terminal deficiency of 9S including the Yg and Wd loci.

Some crosses of Class II individuals to yg c wx females provided the following data:

(1)	(0)	(1-2)	(2)	(2)	(1-2)	(0)	(1)	
Yg	yg	Yg	yg	Yg	yg	Yg	yg	
C	C	C	C	c	c	c	c	
Wx	Wx	wx	wx	Wx	Wx	wx	wx	Σ
5	271	1	20	34	1	290	7	629

The wd-C interval has 2.2% recombination, and that for C-Wx is 8.9%.

The following data came from testcrosses using Class II plants as the female parent:

C Wx	C wx	c Wx	c wx	Σ
267	30	25	131	453

The ratios of 297 C:156 c and of 292 Wx:161 wx approximate the 242 Wx:106 wx ratio found in the original yg exceptional plant. The C-Wx recombination value is 12.1%.

Class III had: 2 normal chromosomes 4
                  1 normal chromosome 9 carrying C and Wx
                  1 normal chromosome 9 carrying c and wx

Only one plant of this constitution has been studied. Reciprocal testcrosses gave 1:1 ratios for both C:c and Wx:wx. The high C-Wx recombination value of 25%

determined from limited data is a reflection of the structural homozygosity of the two chromosomes 9.

Class IV had: 1 normal chromosome 4
                 1 deficient chromosome 4
                 1 normal chromosome 9 carrying yg C wx
                 1 T9(wd)-4 chromosome carrying C and Wx
                 1 normal chromosome 9 carrying Yg c wx

This class includes infrequent trisomics arising from nondisjunction of the N9 and T9-4 chromosomes in plant 33016-1. A single plant was studied (33356-14). When used as the female parent in testcrosses, it gave 88.4% C and 70.4% Wx kernels. The reciprocal cross gave 61.1% C and 23.6% Wx kernels. Without knowledge of the chromosomal constitution of 33356-14, the above genetic data defy comprehension, but they are readily understood on the basis of cytological observations. In the absence of crossing over in 9S the following kinds of meiotic products are formed on the assumption of random segregation:

n spores				n + 1 spores		
1.	N9 (C wx)	N4		7.	$\dfrac{\text{T9-4 (wd C Wx)}}{\text{N9 (c wx)}}$	N4
2.	N9 (C wx)	Df4	aborts	8.	$\dfrac{\text{T9-4 (wd C Wx)}}{\text{N9 (c wx)}}$	Df4
3.	N9 (c wx)	N4		9.	$\dfrac{\text{N9 (C wx)}}{\text{T9-4 (wd C Wx)}}$	N4
4.	N9 (c wx)	Df4	aborts	10.	$\dfrac{\text{N9 (C Wx)}}{\text{T9-4 (wd C Wx)}}$	Df4
5.	T9-4 (wd C Wx)	N4	low in ♂	11.	$\dfrac{\text{N9 (C wx)}}{\text{N9 (c wx)}}$	N4
6.	T9-4 (wd C Wx)	Df4		12.	$\dfrac{\text{N9 (C wx)}}{\text{N9 (c wx)}}$	Df4 aborts

Of the n spores, one-third will abort (2 and 4) while T9-4 (wd C Wx) N4 pollen will have a low transmission rate even though filled with starch. If none of the n+1 spores function in male crosses, the C:c ratio should approximate 2:1 (61% C was observed), while the percentage of wx kernels should be greater than that of Wx (76% wx was observed). When class IV plants were used as the female parent in testcrosses, where we may assume equal viability of all megaspores except those of the aborted types (2, 4 and 12), the percentage of C kernels should be greater than in the male data since all of the functioning n + 1 spores have the C allele. The observed percentage of C in the female data is 88.4% as compared with 61% in the male gametes. With the Wx gene, 70.4% Wx was observed in female tests and 23.6% in the male backcrosses. The greatly different frequencies of Wx:wx in the reciprocal crosses could be accounted for by a number of speculative hypotheses,

but they are readily explained by the predicted array of gamete types given above. Ascertainment of the true mechanism came only from cytology.

The establishment of the four classes in the offspring of 33016-1 makes it possible to reconstruct its genotype and the events occurring at the second microspore division. 33016-1 had a N9 and a N4 contributed by its female parent. The fertilizing sperm possessed a Df4 and the T9(wd)-4 chromosome. These two structurally changed chromosomes arose at one second microspore division when both chromosomes 9 and 4 formed dicentric bridges. Chromosome 9 was ruptured in anaphase close to the terminal knob, producing a freshly broken end which united with a freshly broken end of an acentric fragment derived from breakage of the dicentric chromosome 4.

Plant 33016-1 therefore had a N9, a N4, a T9(wd)-4 and a Df4. It was pollinated by a N9 N4 strain. Class I came from an egg with a N4 and the T9(wd)-4 chromosomes to give a N9 T9(wd)-4 N4 N4 zygote. Class II arose from an egg with the Df4 and T9(wd)-4 chromosomes. Class III was derived from a N9 N4 egg, and Class IV originated following nondisjunction of the N9 and T9(wd)-4 chromosomes, which passed to the same pole at anaphase I of meiosis as did the Df4 chromosome. Nondisjunction of the N9 and T9(wd)-4 chromosomes presumably would be increased by the piece of 4L on the T9(wd)-4 chromosome.

While the above account is a good example of how a combined cytological and genetical attack led to the elucidation of a complex genetic situation, the demonstration that chromosomes comparable to the T9(wd)-4 chromosome can arise suggests a new explanation of the stable C Wx class discussed in the introduction of this note. Let us assume that the dicentric bridge involving chromosome 9 broke between the C and Wx loci. The C allele would be eliminated in the acentric fragment. Further assume that another chromosome of the complement was involved in a dicentric bridge, because it too was knobbed, and that breakage produced an acentric fragment with a broken end. The union of the two freshly broken ends produced a translocated chromosome deficient for C, possessing the Wx allele and capped by a piece from the heterologous chromosome. If such a chromosome were present in the endosperm, it would not undergo a bridge-breakage-fusion cycle, and the mature endosperm would be colorless and would not show Wx-wx mosaicism. Such a mechanism is, we believe, responsible for the stable C Wx class.

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