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Influence of Chromosomal Gene Position on Intragenic Recombination in Maize[1]

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Summary. Chromosome interchanges were used to relocate four alleles of the *wx* locus in order to analyze the distribution of recombination potential of the maize genome. There are two homotranslocation series: proximal, interchanges proximal to *wx*, thus moving the *wx* locus to varied positions away from a centromere; and distal, interchanges distal to *wx*, thereby lengthening the segment distal to *wx*.

1. Among the controls (homoalleles on standard chromosomes), derived from outcrosses (heterozygous sources), yield more recombination than these combinations in inbred lines.
2. All heteroallelic, homotranslocation combinations, irrespective of the extent of the locus-centromere distance, are equal or less in recombination frequency than the *wx* recombination in the standard chromosome.
3. Among all heteroalleles on homotranslocations, there is a positive linear correlation of inter-allelic recombination with increasing distance from a centromere but the value is always less than found in the standard chromosomes. This also is true for the same heteroallele at different positions; the longer distances show a higher recombination value than the shorter *wx*-centromere distance.
4. There is no effect of a lengthening distal segment on *wx* interallelic recombination.
5. The frequencies of *Wx* pollen grains arising from homoalleles on homotranslocations in most instances (except for 90/90) are not significantly different from the frequencies found with standard chromosomes.
6. The reversion frequency of the same homoallele at different positions has no relationship with the *wx*-centromere or *wx*-breakage-point distance.
7. Different homoalleles at the same position are not equally affected in *wx* reversion rate at the new position, although no trend is evident in *wx*-centromere or *wx*-breakage-point distance.
8. The most variation in percentage change of the *Wx* frequency from the controls among different heteroalleles with the same homotranslocation is found among interchanges with relatively short *wx*-breakage-point distances.
9. There is a significant environmental effect; the greatest change between years is among the heteroalleles with higher *wx* recombination.
10. Homoalleles differ from heteroalleles in response to various factors, such as *wx*-centromere and *wx*-breakage-point distances. This supports the expectation that *Wx* pollen grains arising from homoalleles have a different origin than do *Wx* pollen grains arising from heteroalleles.

Introduction

Cytogenetics attempts the correlation of genetic and cytological maps, which depends on an intimate identification of cytological sites and detailed linkage relationships (Emerson, Beadle, and Fraser, 1935; Rhoades, 1950; Anderson, Kramer, and Longley, 1955a, b; Phillips, 1969a, b; Lindsley and Grell, 1967). Examination of these maps, where specific details of gene positioning in the cytological chromosome is known, quickly establishes that the distribution of chiasmata along the length of the chromosome is not random and that the chance of chiasma forming in a given region is not proportional to the physical length of that region. This aspect of recombination was examined critically by Stephens (1961). He mustered pertinent evidence to show that the *interference* in successive chiasmata as measured by *coincidence* (number of doubles obtained over the number of doubles expected) is not restricted to contiguous regions. It follows that there are "hot spots" for recombination events and that a higher probability for *coincidence* of exchange exists in regions remote from the first point of exchange. From examination of the Weinstein data, Stephens (1961) concluded that the expected single chiasma (over 90% of the recombination occurred as single-chiasma bivalents) usually was located in the midregion of the chromosome.

The depressive effects of centromeres on recombination frequencies were established early in *Drosophila* cytogenetic studies (Bridges and Brehme, 1944; Bridges, 1935, 1937, 1938). In these instances, the contracted genetic linkage distance did not match the corresponding physical length on a cytological map. The development of correlative linkage-cytological maps confirmed the centromere effect that was demonstrated by relocating centromere-associated chromosome segments (Graubard, 1932; Beadle, 1932; and Thompson, 1964).

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[2] The authors dedicate this paper to Professor Marcus M. Rhoades on the occasion of this 70h birthday.

Since the distribution of chiasmata is not random, what causes localization of "hot spots" of recombination along the chromosome arm? An experiment designed to investigate this question should utilize a common unit of measure at several different distances from the centromere. Further, it should be possible to easily canvass large populations. Intracistronic exchange events at the waxy locus in maize fulfill these requirements. These events have been identified by numerous investigators as the same kind of events that are registered as intergenic recombination events (Meselson, 1967; Fogel and Hurst, 1967; Murray, 1969; Fogel and Mortimer, 1969; Fogel, Hurst, and Mortimer, 1971; Smith, Finnerty, and Chovnick, 1970; Chovnick *et al.*, 1970; Ballantyne and Chovnick, 1971). In addition, intracistronic and intergenic reciprocal recombination events have been shown to react identically to ultraviolet radiation and x-rays (Esposito, 1968).

A preliminary account of these experiments has been reported (Yu and Peterson, 1971).

1. Materials, Methods, Explanations

a. Gene Symbols and Definition of Terms

Allele or element	Description or phenotype
Wx	The nonwaxy starch-producing allele; expressed in endosperm and pollen grains by staining blue black with I_2-KI solution.
wx	The waxy starch-producing allele; recessive to *Wx*; expressed in endosperm and pollen grains by staining red to brownish red with I_2-KI.
wx^x or wx^y	One of the *wx* alleles specified; x (or y) as general term = C, B, 90, or H21.
N	Normal chromosomal constitution in the genome.
T	Reciprocal translocation or chromosome interchange present in the genome (as contrasted to N): the exchange of segments of chromosome 9 and another chromosome.
T_i	One of the translocations specified; i as general term for 1, 2, . . . , or 10.
T/N or N/T	Translocation heterozygote; semisterility results in pollen and ear culture.

Definition of Terms

Homoallelic combination	Homozygous for the specific *wx* allele; e.g., wx^x/wx^x or wx^y/wx^y.
Heteroallelic combination	Heterozygous for specific *wx* allele; e.g., wx^x/wx^y.
Waxy heterozygote	Heterozygous for the *wx* genotype; e.g., *wx/Wx*.
Standard chromosome	Normal chromosome 9 in maize genome.
Proximal translocation	Translocation with the breakage point proximal to the *wx* locus in the short arm of chromosome 9.
Distal translocation	Translocation with the breakage point distal to the *wx* locus in the short arm of chromosome 9.
Homotranslocation	Homozygous for the specific translocation; e.g., T_i/T_i.
wx-centromere distance	Physical distance between the *wx* locus and centromere in the chromosome.
wx-breakage-point distance	Distance between the *wx* locus and breakage point in the translocation chromosome.

Genetic stocks containing the four *wx* alleles wx^C, wx^B, wx^{90}, and wx^{H21} (hereafter referred to as C, B, 90, and H21, general terms wx^x, wx^y) were obtained from Dr. O. E. Nelson (University of Wisconsin). The 10 chromosome translocation stocks chosen for this study were obtained from the Maize Genetics Cooperative, University of Illinois. Among the 10 translocations, five involve breakage points proximal to the *wx* locus, and the other five, distal to *wx* (general terms T_1, T_2, . . . , T_i, etc.). A description and an idiogram of the 10 translocation chromosomes are shown in Table 1 and Fig. 1. The translocation chromosomes will be referred to hereafter as interchanges or chromosome interchanges.

b. Development of the wx Interchange Chromosome Combinations

Step 1. The *Wx* translocation stocks (Table 1), T_i-*Wx*/T_i-*Wx*, are crossed to the wx^x allele (N-wx^x/N-wx^x) on normal (N) chromosome 9 to recover the heterozygous F_1 hybrids T_i-*Wx*/N-wx^x.

$$\frac{T_i\text{-}Wx}{T_i\text{-}Wx} \times \frac{N\text{-}wx^x}{N\text{-}wx^x} \longrightarrow \frac{T_i\text{-}Wx}{N\text{-}wx^x}.$$

The proximal translocation stocks (T_1—T_5) are crossed to the four *wx* alleles, C, B, 90, and H21; one distal stock (T_6) is crossed to B and 90, and the other four (T_7—T_{10}), to alleles C and 90. All kernels from this cross are phenotypically nonwaxy (*Wx*).

Step 2. The semisterile (SS) F_1 plants from the step 1 cross, heterozygous for waxy and the interchange chromosome (T_i-*Wx*/N-wx^x), are then backcrossed to the same parental *wx* alleles to recover the desirable crossover chromosome strands — T_i-wx^x (Figure 1). These events occur with a variable frequency and are dependent on the *wx*-breakage-point distance

$$\frac{T_i\text{-}Wx}{N\text{-}wx^x} \times \frac{N\text{-}wx^x}{N\text{-}wx^x} \longrightarrow \frac{T_i\text{-}wx^x}{N\text{-}wx^x} \ (<1\%).$$

The proximal *wx*-linked interchange chromosome is derived from a crossover occurring between the *Wx* locus and the breakage point (Fig. 2a), and the distal one is obtained if the crossover is in the interstitial segment, distal to the *Wx* locus in chromosome 9^x (Fig. 2b). For any confirmed T *wx* strand, only one isolate was used in further crosses.

The resultant ear culture will include half waxy and half nonwaxy kernels. The desirable T_i-wx^x/N-wx^x combination is among the waxy (*wx*) seeds. A large number of maize plants (e.g., 10^2—10^3 plants from *wx* progeny of step 2) usually are needed to procure a single desired T_i-wx^x chromosome strand.

Step 3. The purpose of this step is to isolate the T_i-wx^x chromosome strand from the T_i-wx^x/N-wx^x genotype. The field-identified semisterile plant is crossed to an inbred line (N-*Wx*/N-*Wx*) for this isolation.

$$\frac{T_i\text{-}wx^x}{N\text{-}wx^x} \times \frac{N\text{-}Wx}{N\text{-}Wx} \longrightarrow \frac{T_i\text{-}wx^x}{N\text{-}Wx} \ (50\%).$$

It is important that no *Wx* kernel is mixed among the *wx* seeds in planting. Most misclassified *Wx* seeds will appear as semisterile plants; therefore, *wx* homozygotes among the selected plants are confirmed by examining the semisterile pollen with I_2-KI solution. All kernels resulting from the step-3 cross are expected to be of the nonwaxy type.

Step 4. The desirable heteroallelic combination, T_i-wx^x/T_i-wx^y, homozygous for the specific chromosome interchange (T_i), is produced by intercrossing the appropriate semisterile plants from step 3.

$$\frac{T_i\text{-}wx^x}{N\text{-}Wx} \times \frac{T_i\text{-}wx^y}{N\text{-}Wx} \longrightarrow \frac{T_i\text{-}wx^x}{T_i\text{-}wx^y}\ (25\%)\ .$$

The homoallelic genotypes, homozygous for both the *wx* allele and the interchange chromosome (T_i-wx^x/T_i-wx^x) are produced by self- or sibcrossing the semisterile plants within individual lines.

$$\frac{T_i\text{-}wx^x}{N\text{-}Wx} \times \frac{T_i\text{-}wx^x}{N\text{-}Wx} \longrightarrow \frac{T_i\text{-}wx^x}{T_i\text{-}wx^x},$$

$$\frac{T_i\text{-}wx^x}{N\text{-}Wx}, \frac{N\text{-}Wx}{N\text{-}Wx}\ (1:2:1)\ .$$

Again, the *wx* kernels (about 25%) are usually of the desired types. The *Wx* kernels (about 75%) from this homoallelic cross can be used to further identify the translocation. Two-thirds of the *Wx* progeny plants should be semisterile because the proportion of genotypes, T_i-wx^x/N-*Wx* and N-*Wx*/N-*Wx*, is 2:1.

Step 5. The desirable combination, heteroallelic or homoallelic for the *wx* allele and homozygous for the specific chromosome interchange is obtained from T_i-$wx^x/_iT$-wx^y or T_i-wx^x/T_iwx^x crosses, respectively, and represent the sources of pollen for the recombination assay. From each heteroallelic line, at least two plants used in the tassel collection are outcrossed to normal (N) plants to confirm the presence of the translocation.

$$\frac{T_i\text{-}wx^x}{T\text{-}wx^y} \times \frac{N\text{-}Wx}{N\text{-}Wx} \longrightarrow \frac{T_i\text{-}wx}{N\text{-}Wx}\ (100\%)\ .$$

All the progeny plants from these testcrosses should be semisterile.

To assure a uniform environment for the interchange event, seed samples of all the desirable combinations from step-4 crosses were alloted to 16-foot rows, with a completely randomized design, and planted on the same date, May 13, 1971.

c. The Location of the wx Locus and the Length of the Rearranged Chromosome

The precise position of the *wx* locus in the short arm of chromosome 9 (9S) has never been determined. McClintock (1941) reported that *wx* is located at approximately the middle of the short arm and is distal to the dark-staining third of the arm adjacent to the centromere. It was also placed distal to *Ds* as illustrated by her photographs and diagram (McClintock, 1951). According to Roman and Ullstrup (1951), the approximate positions of the breakage point of TB-9b is 9S.40. Because the *wx* locus is not uncovered by Tb-9b, it can be inferred that the cytological distance between *wx* and the breakage point of such a translocation is negligible (Bianchi and Borghi, 1966). Genetic data suggest that the crossover distance also is small; the *wx* locus is about 0.1 map unit from the breakage point of Tb-9b (Bianchi, 1968). Rhoades (personal correspondence) suggested that the *wx* locus could not be any nearer than 9S.40. Because of these considerations, 9S.40 is arbitrarily adopted as the chromosome position of the *wx* locus in this study.

The length of the rearranged chromosomes (Fig. 1) is determined from the information on breakage points of the two chromosomes involved in the interchange. For

Table 1. *The constitution of interchange chromosomes*

Chromosome interchange	Mean position of break-point[a]	Approximate distances[b] (u)		Length of chromosome (u)
1	2	3	4	5
Proximal		*wx*-ct[c]	*wx*-bk[d]	
T1 8–9 5391	8L.07;9S.33	3.61	1.08	24.18
T2 5–9 4871	5L.71;9S.38	22.56	0.31	60.31
T3 5–9a	5L.69;9S.17	25.17	3.55	62.92
T4 6–9b	6L.10;9S.37	4.15	0.46	25.30
T5 4–9 8636	4L.94;9S.09	38.79	4.79	70.66
Distal		*wx*-dt[e]	*wx*-bk[d]	
T6 8–9 5300	8L.85;9S.43	5.89	0.46	39.86
T7 3–9f	3L.63;9S.69	19.77	4.48	53.75
T8 6–9	6L.06;9S.73	39.73	5.10	73.70
T9 7–9 6482	7L.01;9S.97	42.93	8.80	79.90
T10 7–9 7074	7L.03;9S.80	39.61	6.18	73.59
Standard		*wx*-ct[c]	*wx*-dt[e]	
Chromosome 9		6.18	9.27	43.24

[a] from Longley (1961).
[b] Modified from Rhoades (1950).
[c] Waxy-centromere distance.
[d] Waxy-break-point distance.
[e] Waxy-distal end distance.

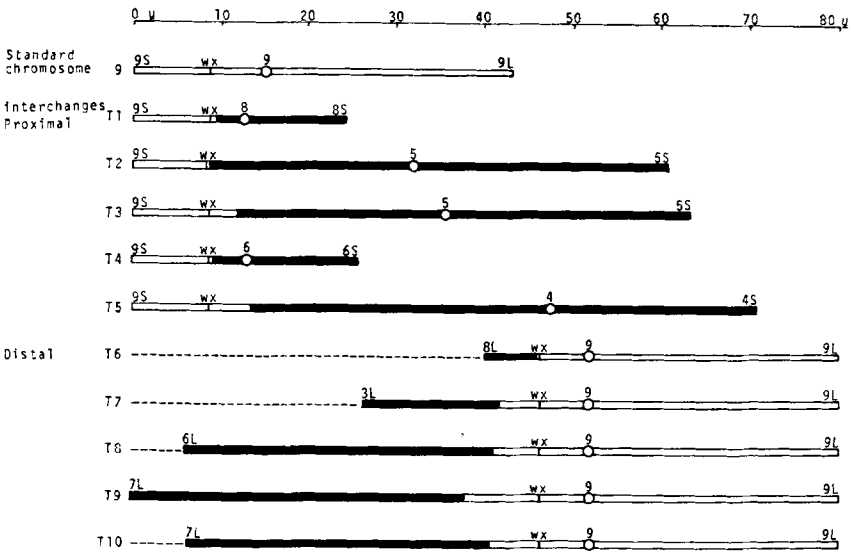

Fig. 1. The standard and rearranged chromosomes; interchanges involve chromosome 9 of maize with breakage points proximal (T_1–T_5) and distal (T_6–T_{10}) to the *wx* locus (interchanges listed in Table 1)

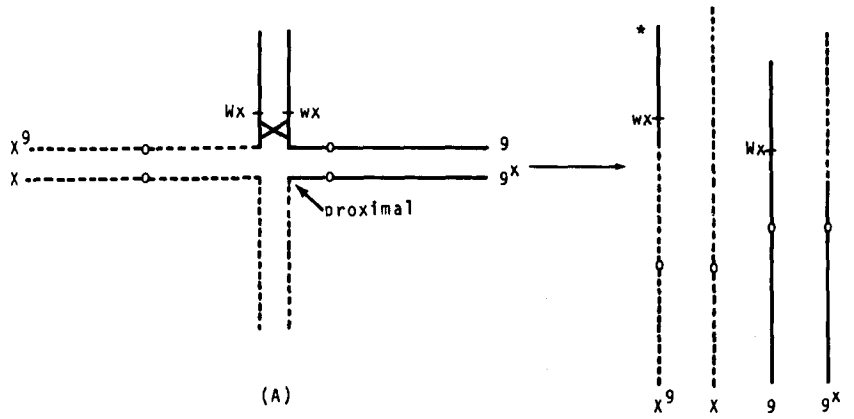

Fig. 2 A. The desirable (proximal) crossover events and recovered chromosomes including the desirable interchange chromosome containing *wx* from the translocation heterozygote involving chromosone 9 of maize; 9 = standard chromosome 9; x = standard other chromosome; x^9 = interchange of x chromosome with segment from 9-x centromere (the arm length of chromosome 9 and the site of *wx* are not drawn to scale)

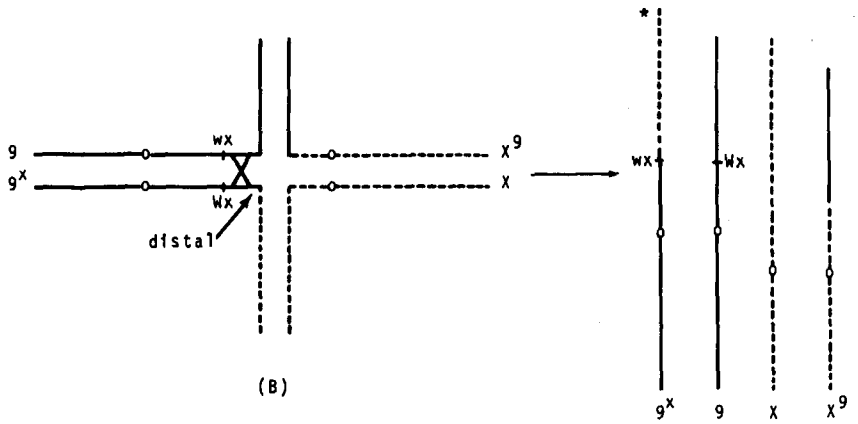

Fig. 2 B. The desirable (distal) crossover events and recovered chromosomes including the desirable interchange chromosome containing *wx* from the translocation heterozygote involving chromosome 9 of maize; 9 = standard chromosome 9; x = standard other chromosome; 9^x = interchange of 9 with other chromosome-9 centromere (the arm length of chromosome 9 and the site of *wx* are not drawn to scale)

example, the proximal interchanges contain *wx* in the noncentromere 9S segment, distal to the breakage point on 9S, and another chromosome with a centromere, but without a segment of one arm. Thus, the whole length of the rearranged chromosome will be the sum of the distal 9S segment, plus the main portion of the chromosome involved in the interchange, including its centromere. The interchanges involved with the breakage point distal to *wx* contain the long arm and proximal portion of the short arm (including *wx*) of chromosome 9, as well as a distal segment from the other chromosome involved in the interchange. The physical length (in microns) of maize chromosomes and their arm ratios are based on Rhoades' calculations (1950), and the position of breaks are from Longley's data (1961). The arm ratio of chromosome 6 is believed to be 3.1:1.0 (Maguire, 1962; Rhoades and Dempsey, personal correspondences), although Rhoades (1950), Neuffer *et al.* (1968), and W. L. Brown (unpublished notes) gave ratios of 7.1, 7.0, and 6.2:1.0 for this chromosome, respectively, that do not include the satellite and "gap" portion of the nuclear organizer region (Phillips, personal communication).

d. Pollen Collection and the Recombination Assay

Tassel segments used in pollen assays are collected the day before anthesis and consist of parts of the main spike of the tassel adjacent to freshly shed anthers. These are cured in 70-percent ethanol for several weeks before staining. The solution used to stain pollen is a modification of that suggested by Nelson (1968): 25 ml H_2O. 250 mg KI, and 45 mg I_2. One drop of Tween 80 is stirred into the I_2-KI solution, which is then mixed with 0.3 g of soluble Baker's gelatin. Fresh stain is prepared weekly.

For slide preparation, about 60 anthers (3 from each of 20 florets) are picked, placed on the surface of a 3.25" × 4" lantern slide (Esco groundedge cover glass) containing several drops of stain. The anthers are cut apart and gently pressed so that the pollen grains are released into the solution. After debris is removed, the pollen grains are distributed evenly in an area then covered by a 50 × 75 mm cover glass (Corning). The preparation, which is ready for counting shows maximum differential staining at this time. A slide may average more than 100,000 pollen grains and frequently can be stored a few days by coating the edges of the cover slip with clear nail polish.

An AO Cycloptic binocular (Stereoscopic Microscope, Series 58) with adequate illumination under the slide is used for scanning pollen grains. Estimation of the total pollen population on the slide is obtained by multiplying the average of 10 counts from randomized sites on the slide by a "constant", 940. The grid in the eyepiece covers 4 mm^2 (= 2 mm × 2 mm) on the glass stage of the binocular (when magnification is set at 25X), which is 1/937.5 of the total area of the cover slip, 3750 mm^2 (= 50 mm × 75 mm). Since pollen grains distributed on the edge of the control area are likely to be counted, 940 has been used to compensate for this minor bias.

2. A Description of the Combinations of *wx* with Standard and Interchanged Chromosomes

Four *wx* alleles amd 10 translocations, five proximal (T_1—T_5) and five distal (T_6—T_{10}), are used in this study. With four *wx* alleles, C, B, 90, and H21, there are six possible heteroallelic combinations, C/B, C/90,

Table 2. *The possible heteroallelic homotranslocation combinations: the recovered lines (X); unavailable (—)*

Proximal	T1	T2	T3	T4	T5	Control
C/B	X	X	—	X	X	X
C/90	X	—	—	X	X	X
C/H21	—	X	—	—	—	X
B/90	X	—	—	X	X	X
B/H21	—	X	X	—	—	X
90/H21	—	—	—	—	—	X
Distal	T6	T7	T8	T9	T10	Control
B/90	X	—	—	—	—	X
C/90	—	X	X	X	X	X

C/H21, B/90, B/H21, and 90/H21, and, with five proximal chromosome interchanges (breaks on the chromosome proximal to *wx*), 30 T-*wx* combinations are possible. In the distal series (breaks distal to *wx*), one B/90 and four C/90 heteroallelic combinations are tested on five chromosome interchanges. Of these 35 (30 proximal and 5 distal), 18 are complete (Table 2). Included are 13 of the proximal series and five of the distal series.

Data on the frequency of *Wx* in wx^x wx^y stocks are obtained from several allelic combinations: homoallelic, standard chromosomes; heteroallelic, standard chromosomes; homoallelic, homotranslocation chromosomes; and heteroallelic, homotranslocation chromosomes. The homoallelic standard serves as the control. The *Wx* frequency values of the 4 *wx* homoalleles on the standard chromosome, both from the inbred source and from the crosses of waxy heterozygous parents, are shown in Table 3. The recombination frequencies of 6 heteroallelic combinations at the *wx* locus of the standard chromosome (controls) and 18 heteroallelic combinations on interchange chromosomes are shown in Table 4. The *Wx* frequencies of the homoallelic homotranslocations are shown in Table 5. In each instance, more than one-half million pollen grains for each combination (Tables 3 and 5, column 5; Table 4, column 5) from five plants were sampled to obtain the *wx* reversion results.

3. Comparison of the *Wx* Frequencies on Standard Chromosomes

a. Between Inbred and Outcross Sources of Parental Homoalleles

Parental stocks gave low, but measurable, frequencies of *Wx* pollen grains. Lines from the inbred sources (self-crossed or sib-crossed) and from the outcrosses (N-wx^x/N-*Wx'* × N-wx^x/N-*Wx''*) vary. The value of the reversion rate of any mutant may be affected by back mutation, suppressor mutation, and even

Table 3. *The Wx frequencies of four parental homoalleles on standard chromosomes, both from inbred parents (A) and outcrossed parents (B)*[a]

Line		Allele	*Wx* frequency ($\times 10^{-5}$)	$s_{\bar{x}}$[b]	No. of gametes sampled ($\times 10^3$)
1		2	3	4	5
A	1	C/C	0.95	0.07	633
	2	B/B	0.77	0.10	647
	3	90/90	0.29*	0.18	680
	4	H21/H21	1.15	0.58	609
B	5	C/C	0.97	0.37	615
	6	B/B	1.54	0.83	651
	7	90/90	3.86*	0.96	596
	8	H21/H21	3.07	0.51	561

[a] Inbred parents = N-wx^x/N-wx^x × N-wx^x/N-wx^x, outcrossed parents = N-wx^x/N-*Wx* × N-wx^x/N-*Wx'*.

[b] $s_{\bar{x}} = (s^2/n)^{1/2}$, standard error; where "s^2" is the sample variance and "n" is the number of observations on the mean.

* Difference is significant at 0.05 level (t-test, for the difference of the same homoallele from two parental sources).

Table 4. *The Wx frequencies of heteroallelic combinations on standard (A) and interchange (B and C) chromosomes of four wx alleles*[a]

	Line	Allelic combination	*Wx* frequency ($\times 10^{-5}$) Observed	Adjusted[b]	No. of gametes sampled ($\times 10^3$)	$s_{\bar{x}}$[c]
	1	2	3	4	5	6
A	1	C/B	41.00	39.75	737	5.43
	2	C/90	88.25	85.84	740	9.76
	3	C/H21	51.85	49.83	627	4.57
	4	B/90	0.88	(−1.82)	793	0.43
	5	B/H21	37.03	35.73	651	5.01
	6	90/H21	17.88	14.42	783	2.40
B	7	T1-C/B	29.43	28.87	751	1.46
	8	T1-C/90	33.84	33.33	632	3.39
	9	T1-B/90	0.52	0.01	769	0.32
	10	T2-C/B	26.92	24.18	773	3.37
	11	T2-C/H21	50.68	49.16	734	2.91
	12	T2-B/H21	28.77	26.12	813	3.20
	13	T3-B/H21	30.38	27.66	770	4.08
	14	T4-C/B	41.84	39.31	722	3.94
	15	T4-C/90	3.04	(−0.98)	1084	0.94
	16	T4-B/90	35.57	32.06	827	4.66
	17	T5-C/B	33.96	33.00	689	2.31
	18	T5-C/90	68.62	67.06	721	10.48
	19	T5-B/90	0.71	(−0.34)	707	0.44
C	20	T6-B/90	1.83	0.39	711	0.81
	21	T7-C/90	44.55	43.55	631	6.80
	22	T8-C/90	49.63	47.40	514	2.87
	23	T9-C/90	47.73	46.32	631	4.40
	24	T10-C/90	59.15	57.07	561	3.64

[a] A = controls (standard chromosomes), B and C = proximal and distal interchanges.

[b] Adjusted = (observed value) − $(P_1 + P)/2$; adjusted for reversion (figure with "—" symbol should be counted as "0").

[c] $s_{\bar{x}}$ = standard error.

Table 5. *The Wx frequencies of the four parental homoalleles on the proximal (A) and distal (B) interchange chromosomes*

	Line	Allelic combination	*Wx* frequency ($\times 10^{-5}$)	$s_{\bar{x}}$ [a]	No. of gametes sampled ($\times 10^3$)
	1	2	3	4	5
A	1	T_1-C/C	0.57	0.45	701
	2	T_1-B/B	0.56	0.11	716
	3	T_1-90/90	0.46	0.08	864
	4	T_2-C/C	1.61	0.42	746
	5	T_2-B/B	3.87	1.08	646
	6	T_2-H21/H21	1.43	0.69	838
	7	T_3-B/B	1.73	0.08	636
	8	T_3-H21/H21	3.72	1.55	752
	9	T_4-C/C	3.04	0.81	724
	10	T_4-B/B	2.03	0.72	541
	11	T_4-90/90	5.00	1.12	580
	12	T_5-C/C	1.48	0.12	677
	13	T_5-B/B	0.45	0.06	660
	14	T_5-90/90	1.65	0.49	668
B	15	T_6-B/B	1.26	0.43	796
	16	T_6-90/90	1.62	0.46	924
	17	T_7-C/C	0.90	0.34	557
	18	T_7-90/90	1.10	0.10	544
	19	T_8-C/C	2.86	1.17	524
	20	T_8-90/90	1.60	0.05	561
	21	T_9-C/C	0.79	0.09	506
	22	T_9-90/90	2.03	0.74	599
	23	T_{10}-C/C	1.80	0.65	611
	24	T_{10}-90/90	2.35	0.72	510

[a] $s_{\bar{x}}$ = standard error.

contamination from wind-blown pollen grains lodged in one of the glumes to be sampled.

The frequencies of occurrence of *Wx* pollen grains among the four inbred source homoallelic combinations are from 0.29 to 1.15 $\times 10^{-5}$, and range from 0.97 to 3.86 $\times 10^{-5}$ in lines developed from the crosses of heterozygous for waxy parents (Table 3).

The frequency of *Wx* of each individual homoallele from the inbred source, in every case, is slightly lower than that obtained from outcross sources. The value for line N-wx^{90}/N-wx^{90} shows a significant difference (t-test) between the two seed origins (Table 3, column 3, lines 3 vs. 7).

It seems that the variability in genetic background does influence the rate of reversion. All the inbred source stocks were inbred for no less than 2 generations, which made them more homogeneous. The parental stocks developed from outcrosses produced higher *Wx* values, which may represent a kind of heterosis, the result of the stimulation of the heterogeneous genetic background.

b. Between Homoallelic and Heteroallelic Combinations

To compensate for back-mutation (reversion), suppressor mutation, and other factors, the *Wx* frequency from heteroallelic combinations has been adjusted. This was done by using the factor $(P_1 + P_2)/2$ as the average of *Wx* arising from homoalleles. The P_1 and P_2 represent the frequencies of *Wx* originating from the two homozygous parental stocks (either on standard or on interchange chromosomes), which are shown in Tables 3-B and 5. The mean of the *Wx* frequencies arising from heteroalleles is reduced because the *Wx* frequency of the F_1 cross includes both the real recombination between two mutant sites, as well as back mutation, and (or) other factors as expressed by the parental stocks (P_1 and P_2). The adjusted values are shown in Table 4, column 4.

The adjusted recombination frequencies of the heteroallelic combinations on standard chromosome (Table 4-A, column 4, lines 1—6) range from lower (e.g., B/90 combination) than the average *Wx* frequency of its two parental homoallelic stocks to many-fold higher. Five (of six) heteroallelic combinations, which show higher recombination values than those of the parental mean values, are significantly different (Table 6). The other heteroallelic combination, B/90, shows a highly significant lower *Wx* value than the mean value of the parental *Wx* only because its adjusted figure has been estimated as "0" (the adjusted value is negative, -1.82×10^{-5}, which should be treated as "0"). Therefore, the alleles used in the heteroallelic crosses occupy different sites within the *wx* region in the chromosome.

Table 6. *Comparison of the Wx frequencies ($\times 10^{-4}$) between parental homoallele (average value) and heteroallelic combination (adjusted value) on standard chromosome*[a]

Heteroallelic combination	Recombination value	Mean value of the two parents	X^2-test
C/B	39.75	1.26[b]	**[c]
C/90	85.84	2.42	**
C/H21	49.83	2.02	**
B/90	0[d]	2.70	**
B/H21	35.73	2.31	**
90/H21	14.42	3.47	**

[a] Chi-square test (data from Tables 3-B and 4-A).
[b] 1.26 = (0.97 + 1.54)/2 (from Table 3-B, lines 5—6).
[c] ** = significantly different at .01 level.
[d] Any figure compared with 0 is considerated**.

The adjusted *Wx* frequencies of the heteroallelic combinations (Table 4-A, column 4) are the expression of the recombination events within the *wx* cistron. It seems that the hetereoallelic combination, C/90, has the highest recombination frequency (85.84 $\times 10^{-5}$). Therefore, *wx* alleles C and 90 should be farthest apart among the four alleles in this study. Since the *Wx* frequencies of both combinations C/H21 and 90/H21 (49.83 and 14.42 $\times 10^{-5}$) are lower than that of C/90, the location of allele H21 according to this data is considered to be between C and 90. These results are in agreement with Nelson's (1959).

The very low recombination value between alleles B and 90 (negative, after adjustment) indicates that the B and 90 alleles lie very close to each other or are overlapping in the cistron. Contrary to expectation, however, the recombination relationships of these

two alleles with the others are not similar. The inclusion of the B allele does not permit an additive evaluation and makes mapping of the *wx* region difficult. The linear order of the *wx* alleles, deduced from a composite of the data (Table 13-A, lines 1—6), is C-H21-90(B).

The adjusted recombination values of combination B/90, whether on standard chromosomes (Table 13-A, line 4) or on chromosome interchanges (Table 13-B and C, lines 7, 14, 15), are too low to detect a real difference. For this reason the heteroallelic combination, B/90, generally will not be included for comparative purposes.

Table 7. *Comparison of all Wx frequencies ($\times 10^{-5}$) of the heteroalleles on chromosome interchanges with that of controls*[a]

Allele	Control	Chromosome interchanges				d′ .05	d′ .01
C/B		(T4)[b]	T5	T1	T2**		
	39.75	—	33.00	28.87	24.18	10.96	14.99
		T5	T10*	T8**	T9**		
C/90	85.84	67.06	57.07	47.40	46.32	22.47	29.19
		T7**	T1**	(T4)			
		43.55	33.33	—			
		T2					
C/H21	49.83	49.16				11.49	17.71
		(T4)	T6**	T1**	T5		
B/90	0[c]	—	0.39	0.01	0	1.68	2.30
		T3	T2				
B/H21	35.73	27.66	26.12			12.42	17.72

[a] Dunnett's procedure, d′ = t (Dunnett) s/ (data from Table 4, column 4).
[b] (T4) figure is not used for comparison.
[c] Any figure compared with 0 is considered to be **.
* Significant difference from control at 0.05 level.
** Significant difference from control at 0.01 level.

4. Comparison of *wx* Recombination Values

a. Heteroallelic Homotranslocations versus Heteroallelic Standard Chromosomes

Recombination at the *wx* locus has been studied both in standard and in interchange (homotranslocation) chromosomes. The *Wx* frequencies of the interchange chromosome combinations, both the proximal and the distal series (Table 4-B and C, column 4), generally are lower than those of the controls (Table 4-A, column 4). This is evident among the data listed in Table 4, column 4, where 13 of 15 combinations under consideration (lines 14—16 are not included) have values lower than or equal to those of the standard chromosomes. The recombination values of the three combinations, C/B, C/90, and B/90, involving T_4 (Table 4-B, lines 14—16) are not consistent with the values of other interchange combinations and will not be discussed further.

When the recombination values of all interchange groups are compared with their controls, significant differences are evident in the C/B, C/90 (lower), and B/90 (higher) groups (Table 7). By utilizing Dunnett's procedure (Steel and Torrie, 1960), it is evident that the adjusted recombination values of heteroallelic combinations, C/B on T_2, and C/90 on T_1, T_7, T_8, and T_9, are significantly lower than those of controls at the 0.01 level, and T_{10}-C/90 combination is significantly lower at the 0.05 level. The heteroallelic combinations C/H21 and B/H21 on interchanges T_2 and T_3 (Table 7, lines 3 and 5) are not significantly different from the controls.

The heteroallelic combination B/90 on the standard chromosome (control) has an adjusted *Wx* frequency of "zero" (Table 4-A, line 4). Since any figure divided by zero is "infinity", the values in the B/90 interchange group are interpreted as significantly different (higher) from that of the control (Table 7), although all three figures in the interchanges are extremely small.

b. The same Heteroallelic Combination at Different Positions

i. Proximal Series. The distribution of the recombination frequencies of a heteroallelic combination among different interchange chromosomes is an independent event. Duncan's "new multiple-range test" (Steel and Torrie, 1960) provides a method of comparison with all size differences. This depends on the closeness of the means after ranking, with the smallest value for adjacent means and the largest for the extremes. With this test, the combinations that show significant differences among interchanges are C/B and C/90 heteroallelic groups (Table 8).

The recombination values of the C/90 heteroallelic combination on the relocated positions show highly significant differences between T_1 (33.33×10^{-5}) and T_5 (67.06×10^{-5}); significant differences are found between T_5 and T_7, T_8, and T_7 (43.55, 47.40, and 46.32×10^{-5}, respectively) as well as between T_1 and T_{10} (57.07×10^{-5}) combinations (Table 8). In the C/B heteroallelic group, a significant difference is evident between T_2 (24.18×10^{-5}) and T_5 (33.00×10^{-5}), and no significant difference is found between other interchange pairs.

From the distribution of the *Wx* frequencies of C/B and C/90 heteroallelic groups in the chromosome interchanges, it seems that the distance between the *wx* locus and the centromere does influence the rate of recombination. The linear correlation between the *wx*-centromere distances and the *wx*-recombination frequencies on the rearranged chromosomes, combined values of the C/B and C/90 combinations, is shown by the solid line in Fig. 3. It seems that the longest *wx*-centromere distance of T_5 (38.79 *u*) results in the greatest recombination values, and the hetero-

Table 8. *Comparison of the Wx frequencies ($\times 10^{-5}$) of the same heteroallelic combination among different homotranslocations*[a]

Allele	Homotranslocations					
C/B	T5	T1	T2			
	33.00	28.87	24.18			
	a	a				
		b	b			
	T5	T10	T8	T9	T7	T1
C/90[b]	67.06	57.07	47.40	46.32	43.55	33.33
	a	a				
		b	b	b	b	
			c	c	c	c
	T6	T1	T5			
B/90	0.39	0.01	0			
	a	a	a			
	T3	T2				
B/H21	27.66	26.12				
	a	a				

Least significant ranges (LSR)

Value of "p"	1	2	3	4	5	6
C/B	.05	7.72	8.09			
	.01	10.82	11.40			
C/90	.05	17.21	18.10	18.57	18.98	19.34
	.01	23.35	24.41	25.00	25.53	25.88
B/90	0.5	1.74	1.82			
	.01	2.44	2.57			
B/H21	.05	11.96				
	.01	17.39				

[a] Duncan's new multiple-range test on the adjusted data (Table 4, column 4), figures with different letters are significantly different at 0.05 level; data of T4 group are not included.

[b] Among the interchanges T1 and T5 are significantly different at 0.01 level.

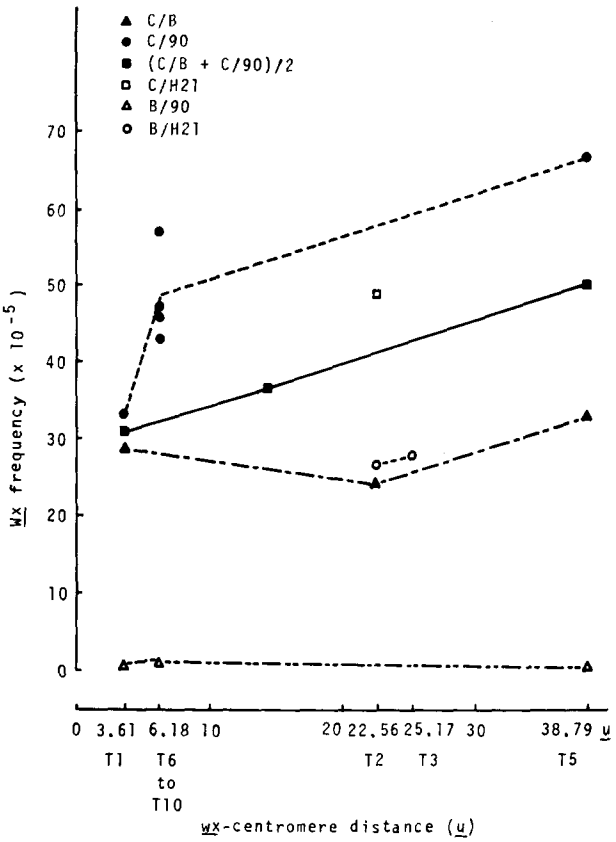

Fig. 3. Test of hypothesis of centromere effect on recombination frequency. Correlation between the *wx*-centromere distance and *Wx* recombination on the rearranged chromosomes; *wx*-centromere distance from Table 1, *Wx* frequency from Table 4, column 4, lines 7—13 and 17—24 (solid line shows the value for the combined averages of the C/B and C/90 heteroallelic combinations)

allelic combinations in shorter *wx*-centromere distances; i.e., T_2, T_6—T_{10}, and T_1 (22.56 *u*, 6.18 *u*, and 3.61 *u*), result in fewer recombinations (Table 8 and Figure 3).

This linear correlation is also true for the B/H21 combination. Translocations T_2 and T_3, which have similar *wx*-centromere distances (22.56 *u* and 25.17 *u*), show similar rates of recombination frequencies (26.12 and 27.66 $\times$ 10^{-5}, Table 8 and Figure 3). Both interchanges T_2 and T_3 involve chromosomes 5 and 9. The B/90 combination does not show a significant difference in *Wx* frequency among the relocated chromosome positions.

ii. Distal Series. The study of the distal translocation series offers an opportunity to examine the effect of an extended distal segment on *wx* recombination frequency. There are four distally lengthened segments containing the heteroallelic combination C/90 that can be compared in several ways (Table 4-C, lines 21—24). With Duncan's method, there is no statistical difference among the four adjusted recombination values, although there was a highly significant difference (X^2-test) between the adjusted *Wx* values of T_7 and T_{10} combinations (55.26 and 94.52 $\times$ 10^{-5}) in 1970 (Table 13-C, lines 16—17). This indicates that the nature of the *wx* locus is not influenced by the changing situation of the distal segment of the chromosome arm, as long as the *wx*-centromere relationship remains unchanged on the original chromosome 9.

c. Homoallelic Homotranslocations versus Homoallelic Standard Chromosomes

For the homoallelic series, there are 24 combinations among the translocations (Table 5) and four combinations among the controls (Table 3). Among the mutant homozygotes, sizable differences in *Wx* frequencies are found with the relocated positions. Within each homoallelic group, at least one or more interchange combinations show numerically higher *Wx* values than that of the standard chromosome (Table 9). This indicates that the new chromosomal position stimulates reversions in the new location within the genome.

In comparing the *Wx* frequencies among homoalleles of all interchange combinations with those of

Table 9. *Comparison of the Wx frequencies ($\times 10^{-5}$) of all homoalleles on interchange chromosomes with that of controls*[a]

Allele	Control	Interchange chromosomes				d'	
						.05	.01
C/C	0.97	T4 3.04	T8 2.86	T10 1.80	T2 1.61	2.42	2.94
		T5 1.48	T7 0.90	T9 0.79	T1 0.57		
B/B	1.54	T2 3.87	T4 2.03	T3 1.73	T6 1.26	2.50	3.09
		T1 0.56	T5 0.45				
90/90	3.86	T4 5.00	T10 2.35	T9 2.03	T5 1.65	2.78	3.36
		T6 1.62	T8 1.60	T7 1.10	T1** 0.46		
H21/H21	3.07	T3 3.72	T2 1.43			3.61	4.89

[a] Dunnett's procedure (data from Tables 3-B and 5).
** Significantly different from control at 0.01 level.

Table 10. *Comparison of the Wx frequencies ($\times 10^{-5}$) of the same homoallele among different translocations*[a,b]

Allele	Homotranslocations							
C/C	T4 3.04 a	T8 2.86 a	T10 1.80 a	T2 1.61 a	T5 1.48 a	T7 0.90	T9 0.79	T1 0.57
		b	b	b	b	b		
			c	c	c	c	c	c
B/B	T2 3.87 a	T4 2.03 b	T3 1.73 b	T6 1.26 b	T1 0.56 b	T5 0.45 b		
90/90	T4 5.00 a	T10 2.35 b	T9 2.03 b	T5 1.65 b	T6 1.62 b	T8 1.60 b	T7 1.10 b	T1 0.46 b
H21/H21	T3 3.72 a	T2 1.43 a						

Least significant ranges (LSR)

Value of "p"		2	3	4	5	6	7	8
C/C	.05	1.77	1.87	1.92	1.97	2.00	2.03	2.05
	.01	2.39	2.49	2.56	2.59	2.65	2.68	2.71
B/B	.05	1.72	1.81	1.86	1.90	1.93		
	.01	2.33	2.44	2.50	2.55	2.59		
90/90	.05	1.84	1.94	1.99	2.04	2.07	2.10	2.12
	.01	2.48	2.61	2.65	2.69	2.75	2.78	2.81
H21/H21	.05	3.90						
	.01	5.67						

[a] Duncan's new multiple-range test (data from Table 5, column 3); figures with different letters are significantly different at 0.05 level.
[b] Among interchanges T1—T2, T2—T5 and T2—T6 in the B/B group as well as T4 with all other interchanges in 90/90 group are significantly different at 0.01 level.

controls (Table 9), only the 90/90 group shows a highly significant difference. According to Dunnett's procedure, the difference between T_1-90/90 ($0.46 \times \times 10^{-5}$) and the control ($3.86 \times 10^{-5}$) is significant. None of the C/C, B/B, and H21/H21 groups show significant differences between values among all interchange combinations and the controls.

d. The same Homoallelic Combination at Different Positions

When the *Wx* reversion frequency of the same *wx* allele in the homozygous condition is analyzed at different chromosomal positions, there are differences within the C/C, B/B, and 90/90 groups (Table 10). In the 90/90 group, the value of T_4-90/90 ($5.00 \times \times 10^{-5}$) is significantly different from all the other combinations. The B/B group shows highly significant differences between T_2 (3.87×10^{-5}) and T_1, T_5, and T_6 combinations (0.56, 0.45, and 1.26×10^{-5}), and significant differences between T_2 and T_3 ($1.73 \times \times 10^{-5}$) as well as between T_2 and T_4 (2.03×10^{-5}). Significant differences also are found in the C/C group between T_4 (3.04×10^{-5}) and T_1, T_7, and T_9 (0.57, 0.90, and 0.79×10^{-5}), and between T_8 (2.86×10^{-5}) and T_1 as well as between T_8 and T_9 combinations in the same group. The H21/H21 group contains T_2 and T_3 combinations that do not show a statistical difference between each other. These results indicate that there is little relationship between the *Wx* frequency arising from the homoallelic combinations and the *wx*-centromere or *wx*-breakage-point distance.

5. Variation in Percentage Change of the *Wx* Frequency from the Control among Different Allelic Combinations with the Same Translocation

a. Heteroallelic Combinations

There are four translocations (T_1, T_2, T_4, and T_5), and with each, there is more than one heteroallelic *wx* combination available. Among the different heteroallelic combinations for each interchange group, there is a wide range in the reduction of the *Wx* frequencies from that of the controls (Table 11). Because of this, arcsine transformation ($\mathrm{Sin}^{-1} x^{1/2}$) is applied (Steel and Torrie, 1960). Also, statistical analysis requires that experimental errors be independently and normally distributed with a common variance.

The two heteroallelic combinations, C/B and C/90 (B/90 is not included in this comparison) that are relocated to the same T_1 position (Table 4-B, lines 7 and 8), show reduced values of 27.37% and 61.17% from the controls. Therefore, a highly significant difference exists between the two lines (Table 11). Three heteroallelic combinations, C/B, C/H21, and B/H21, are relocated at T_2 (Table 4-B, lines 10—12) and show reduced values of 39.17%, 1.34%, and 26.90%, respectively, from the controls. This represents a highly significant difference between C/B and C/H21 and a significant difference between C/H21 and B/H21. With translocation T_4 (Table 4-B, lines 14—16), the percentage changes of C/90 and B/90 combinations from the controls are drastic (greater

Table 11. *Comparison of percentage reduction of the Wx frequency from control among different heteroallelic combinations at the same translocation*[a, b]

Interchange	Heteroalleles[c,d]		
T1	C/90	C/B	B/90
	61.17%	27.37%	(−)
	a	b	
T2	C/B	B/H21	C/H21
	39.17%	26.90%	1.34%
	a	a	b
T5	C/90	C/B	B/90
	21.88%	16.98%	(−)
	a	a	

Least significant ranges (LSR)

Value of "p"		2	3
T1	.05	7.24	—
	.01	10.53	—
T2	.05	18.66	19.57
	.01	26.17	27.56
T5	.05	29.86	—
	.01	43.41	—

[a] Duncan's new multiple-range test on the arcsine transformation data; figures with different letters are significantly different at 0.05 level.

[b] Among the percentages C/90−C/B on T1 and C/B−C/H21 on T2 are significantly different at 0.01 level.

[c] Data of T4 as well as B/90 combinations are not included.

[d] 61.17% = (1 − 33.33/85.84) × 100% (from Table 4, column 4, lines 2 and 8).

than 100%), and the differences obviously are highly significant.

The three interchanges (T_1, T_2, and T_4), which caused significant differences in percentage change of the *Wx* frequency from the controls among different heteroallelic combinations, have relatively short *wx*-breakage-point distances (1.08 *u*, 0.31 *u*, and 0.46 *u*). In comparison with T_5 (Figure 1), which has a longer *wx*-breakage-point distance (4.79 *u*), no statistical difference was found in degree of reduction of the *Wx* value from the control between C/B (16.98%) and C/90 (21.88%) (Table 11). Therefore, a greater difference in the degree of change of recombination frequencies from controls among heteroallelic combinations at the same relocated position is mainly influenced by the closeness of the *wx*-breakage-point distance; thus, with a shorter *wx*-breakage-point distance, a greater change occurs in intraallelic recombination among the relocated segments.

b. Homoallelic Combinations

The different homoalleles at the same chromosome position show some variation in the relative percentage changes of *Wx* frequencies from the controls. In a comparison of 10 interchanges, nine show a high degree of difference (Table 12). The one that does not show a difference is translocation T_3, which involves two homoalleles, B and H21. Three combinations have percentage changes greater than 100% from the controls; i.e., T_2-B/B, T_4-C/C, and T_8-C/C (+151.3%, +213.4%, and +194.8%). There, arcsine transformation cannot be applied; instead Chi-square test is utilized. It shows that different homoalleles have great differences in the frequency of reversion. No particular trend was noted from the variables associated with the rearranged chromosomes; i.e., the differential *wx*-centromere and *wx*-breakage-point distances or the length of the segment distal to the *wx* locus.

Table 12. *Percentage changes of the Wx frequency from control among different homoallelic combinations at the same interchange chromosome*[a]

Interchange	X^2-test	C/C	B/B	90/90	H21/H21
T1	**[b]	−41.2	−63.6	−88.1	—
T2	**	66.0	151.3	—	−53.4
T3	ns[c]	—	12.3	—	21.2
T4	**	213.4	31.8	29.5	—
T5	**	52.6	−70.8	−57.3	—
T6	**	—	−18.2	58.0	—
T7	**	− 7.2	—	−71.5	—
T8	**	194.8	—	−58.6	—
T9	**	−18.6	—	−47.4	—
T10	**	85.6	—	−31.9	—

[a] Chi-square test (data from Tables 3-B and 5).

[b] **Significantly different at 0.01 level.

[c] ns = Not significant in difference.

6. Effect of Environment: The Effect of Different Years on the *Wx* Reversion Frequencies among Various Allelic Combinations

The *Wx* frequency characteristic of a cross between two *wx* alleles is influenced by environmental factors as well as by genetic background. All available data show some level of change in values among different years (Table 13). Among the heteroallelic combinations on standard chromosomes showing a change in 1970, three combinations involving the C allele increased, and the other combinations decreased. In 1971 all recombination values decreased unidirectionally. In a between-year comparison, the greatest changes from 1969 to 1970 are in C/90 (which increased from 69.60 to 109.99 $\times 10^{-5}$) and in B/H21 (which decreased from 94.20 to 52.11 $\times 10^{-5}$). These changes are highly significant. In 1971, only the C/B combination showed a highly significant decrease (from 73.79 to 39.75 $\times 10^{-5}$), and the C/H21 and 90/H21 decreased significantly (from 72.59 to 49.83 $\times$ $\times 10^{-5}$ and from 27.89 to 14.42 $\times 10^{-5}$, respectively) from 1970.

Among the chromosome interchanges, all the 1970 recombination values, for which the corresponding data of the previous season are available, show a decrease from 1969. In 1971, however, there are three combinations (T_1-B/890, T_2-C/H21, and T_6-B/90) that increased while the rest decreased from 1970 in recombination frequencies (Table 13). The recombi-

Table 13. *Comparison of the Wx frequencies ($\times 10^{-5}$) of heteroallelic combinations on standard (A) and interchange (B and C) chromosomes in different years*[a]

Line		Combination	1969[b]	1970	1971[c]
A	1	C/B	63.19	73.79	39.75**
	2	C/90	69.60	109.99**	84.85
	3	C/H21	52.21	72.59	49.83*
	4	B/90	1.33	0	0
	5	B/H21	94.20	52.11**	35.73
	6	90/H21	37.22	27.89	14.42*
B	7	T1-B/90	1.02	0	0.01
	8	T2-C/B	36.52	27.48	24.18
	9	T2-C/H21	48.17	40.87	49.16
	10	T2-B/H21	47.79	28.31*	26.12
	11	T3-B/H21	41.41	28.63	27.66
	12	T5-C/B	—	45.06	33.00
	13	T5-C/90	—	68.86	67.06
	14	T5-B/90	—	0	0
C	15	T6-B/90	—	0	0.39
	16	T7-C/90	—	55.26	43.55
	17	T10-C/90	—	94.52	57.07**

[a] A = controls; B and C = proximal and distal interchanges.
[b] Unadjusted data of 1969.
[c] From Table 4, column 4.
* *Wx* frequency significantly changed from the previous year at 0.05 level.
** *Wx* frequency significantly changed from the previous year at 0.01 level.

nation frequencies of T_2-B/H21 decreased significantly (from 47.79 to 28.31 $\times 10^{-5}$) in 1970, and T_{10}-C/90 declined sharply from 94.52 to 57.07 $\times 10^{-5}$ in 1971.

Table 14 summarizes all results obtained from 1969 to 1971 of both homoallelic and heteroallelic combinations. Among heteroallelic combinations, a comparison of the *Wx* values of all interchanges with the controls shows a significant difference in most instances. Both the C/B and C/90 groups showed highly significant differences (lower) in 1970 and 1971, but the C/H21 group was highly significantly different in 1970 only. Values of B/H21 on interchanges that did not show differences from the control in 1971 did show highly significant differences in the previous years. The B/90 group shows highly significant differences (higher) in recombination in 1971 because the control value is zero. The actual recombination values in T_1, T_5, and T_6 are 0.01, 0.00, and 0.39×10^{-5}, respectively (Table 13, lines 7, 14, and 15), which represent the lowest figures in the interchange series.

These results indicate that the frequencies of *wx* intragenic recombination of heteroalleles on the rearranged chromosomes are generally lower than those of the controls. But this is not consistent with the reversion rate with the homoallelic combinations. Ten of 24 combinations on interchanges have higher reversion values than that on standard chromosomes (Table 9). Only the 90/90 group contains a significant difference in reversion values between all interchanges and the control (Table 14-A).

Two types of comparison can be made for the allelic combinations in interchanges: the same heteroalleles at different translocations and different heteroalleles at the same relocated position. In comparing the same heteroallelic combination at different translocations, each heteroallelic group shows a different level of variation. For example, the C/90 group shows highly significant differences in *Wx* frequencies while the C/B group shows a difference (only at significant level) in both 1970 and 1971 (Table 14-B). Recombination values of B/90 and B/H21 show no differences in consecutive years. It has been pointed out previously that the only B/H21 combinations recovered are T_2 and T_3, which are involved in the same 5—9 chromosome interchanges. These two interchanges also are represented by similar *wx*-centromere distances (22.56 *u* and 25.17 *u*) as well as by similar chromosome lengths (60.31 *u* and 62.92 *u*; Table 1). For B/90 combinations, however, the differences among the adjusted values are always too low

Table 14. *Comparison of the Wx frequencies of homoallelic and heteroallelic combinations on the same as well as the different homotranslocations in different years*

Pattern of comparison		Cross	Heteroallele			Homoallele
			1969	1970	1971	1971
A.	Control vs. interchanges	C/B	—	**[a]	**	—
		C/90	—	**	**	—
		C/H21	ns[b]	**	ns	—
		B/90	ns	ns	**	—
		B/H21	**	**	ns	—
		C/C	—	—	—	ns
		B/B	—	—	—	ns
		90/90	—	—	—	**
		H21/H21	—	—	—	ns
B.	Same alleles at different interchanges[d]	C/B	—	*[c]	*	—
		C/90	—	**	**	—
		B/90	—	ns	ns	—
		B/H21	ns	ns	ns	—
		C/C	—	—	—	*
		B/B	—	—	—	**
		90/90	—	—	—	**
		H21/H21	—	—	—	ns
C.	Different alleles on the same interchange	T1	—	—	**	**
		T2	**	ns	**	**
		T3	—	—	—	ns
		T4	—	—	**	**
		T5	—	ns	ns	**
		T6	—	—	—	**
		T7	—	—	—	**
		T8	—	—	—	**
		T9	—	—	—	**
		T10	—	—	—	**

[a] **Significantly different at 0.01 level.
[b] ns = Not significant in difference.
[c] *Significantly different at 0.05 level.
[d] Data of T4 as well as C/H21 combinations are not included.

to detect. Either of these possibilities might be the cause of the nonsignificant differences among the B/H21 and B/90 interchange combinations. Considering the chromosome constitution and the distribution of *Wx* frequencies, the distance between the *wx* locus and the centromere seems the main reason for variation in values among the same heteroallele. This is indicated by the linear correlation evident in Figure 3.

A comparison among the same homoalleles at different translocations also shows different levels of variation. There are highly significant differences in the B/B and 90/90 groups, and a significant difference in the C/C group (Table 14-B). The H21 homoallele, relocated at T_2 and T_3, shows no significant difference. Interchange homoallelic combinations, however, indicate no relationship between the *wx*-centromere distances and the variation of recombination values.

Different *wx* alleles occupy different mutant sites in the *wx* cistron; thus, different single crosses produce different *wx* recombination frequencies. For this reason, a comparison among the relative recombination values of different heteroalleles at the same relocated position has to be made by comparing the relative percentage changes from their respective controls. In heteroallelic combinations, this comparison indicates highly significant differences at interchanges T_1, T_2, and T_4, but shows no significant difference at the T_5 location for consecutive years (Table 14-C). The reduction in the percentages of the T_2 group, which do not show a difference in 1970, shows highly significant differences in both 1969 and 1971.

Because of the reconstructed chromosome constitutions, the *wx*-breakage-point distance must influence the variation of percentage changes in recombination values from the controls among different heteroalleles at the same position. The physical *wx*-breakage-point distances of all T_1, T_2, and T_4 interchange chromosomes are shorter than 1.1 *u*, whereas T_5 is 4.8 *u* (Table 1). This phenomenon, however, is not true for homoalleles at different positions. The *wx*-breakage-point distance does not influence the change in *Wx* reversion rate from the control among the different homoalleles at the same position.

7. Discussion

In tests of the effect of chromosome location on recombination, use is made of intracistronic recombination at the waxy locus. The measurable variable is the distance of the site of recombination from the centromere. Several questions were posed. 1. Are there changes in the frequencies of intragenic recombination at these different locations, and are correlative patterns associated with these changes? 2. Are the recombination frequencies of each of the heteroallelic combinations affected equally? 3. Are the events registered with homoallelic combinations constant in frequency within each case at varied locations?

In answer to the first question, all the heteroallelic combinations at the relocated positions (except the B/90 group) showed lower recombination values than did the controls (standard chromosome). These lowered values among the interchange chromosomes occurred despite the measurably longer *wx*-to-centromere distances in three of the five interchange chromosomes used in the proximal series (Fig. 1). No obvious explanation is evident for this decrease among relocated segments. Perhaps, in the origination of these translocations, additional chromosomal changes too subtle to be detected may occur. In *Drosophila*, such changes may be recorded as lethals or mutations, but gametophytic lethals are effectively eliminated by the gametophyte screen in plants. Other changes include position effects (White, 1948; Catcheside, 1947). If the change included a small displacement of chromosomal material, the tightness of synapsis during meiotic prophase might be lessened. For the same translocation, different allelic-interchange chromosome combinations are recovered from chromosome strands of different crosses. This would make the heteroallelic homotranslocation chromosomes virtually a pair of heteromorphic homologues. Influence of the tightness of synapsis also is evident in an analysis of the effect of distance between the *wx* locus and the breakage point on recombination. In a comparison of percentage reduction of the *Wx* frequency from the control among different heteroallelic combinations at the same translocation, relatively short *wx*-breakage-point distances cause greater reductions in *wx*-interallelic recombination. Furthermore, when the breakage point is close to *wx* in the interallelic exchange event, all recovered combinations that involved T_5 (an interchange with the longest *wx*-breakage-point distance in addition to the longest *wx*-centromere distance in the proximal series) resulted in rates of *Wx* occurrence more like the control than of any other heteroallelic combinations in the same group (Table 7).

These experiments provide evidence for a linear correlation between the length of *wx*-centromere distances and *wx* recombination frequencies for the combined values of different heteroallelic combinations. This supports the concept of a continuing distribution of exchange events proportional to distance and subject to limitations imposed by the centromere effect on linked exchange and, in this instance, on intragenic recombination. As in previously cited studies (Graubard, 1932; Beadle, 1932), the longer the distance between the segment (here, the *wx* locus) and the centromere, the higher is the recombination value.

The following evidence provides an affirmation to the question concerning the recombination frequencies of each of the heteroallelic combinations being affected equally. Significant differences in percentage change in recombination value from the control were

found for different heteroallelic combinations at the same proximal relocated position. The same arguments used to answer question 1 can be applied here: the heteroallelic-exchange event is principally influenced by the closeness of the *wx*-breakage-point distance so that a greater diversity in degree of change from the control is associated with the shorter distance between the *wx* locus and the breakage point. This short *wx*-breakage-point distance effect has the strongest influence on the longer intra-allelic distances, C/B or C/90 vs. C/H21 or B/H21 as shown in Table 4B, lines 7 and 8 (T_1), lines 10, 11, and 12 (T_2), and lines 14, 15, and 16 (T_4), which compares different heteroalleles at the same position.

The third question is concerned with frequencies of *Wx* pollen grains arising among the homoallelic series. Generally, the occurrence of *Wx* among homotranslocations is not significantly different from the controls except for one interchange with the 90/90 group. When the same homoallele is analyzed at different positions or when different homoalleles are analyzed at the same position, significant differences are evident, although these differences cannot be assigned to the influence of the *wx*-centromere or *wx*-breakage-point distances. It seems that the origin of *Wx* pollen grains among homoalleles is not influenced by the same factors that affect recombination events. Perhaps factors localized at the site of the *wx* locus are important for the origin of *Wx* pollen grains rather than generalized cell factors such as suppressor mutations that would effect all homoalleles irrespective of position.

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