RECOMBINATION IN REGIONS ADJACENT TO DELETIONS IN THE X CHROMOSOME OF *DROSOPHILA MELANOGASTER*[1,2]

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DEFICIENCIES, one of the more frequently encountered types of radiation-induced chromosome abnormalities, are widely used in genetic analysis, particularly in localizing genes to specific areas on chromosomes. Yet, remarkably little attention has been paid to their effects on synapsis and crossing over. Only in the older literature are found reports on the influence of deficiencies on recombination in regions adjacent to the deleted segment. In Drosophila, studies by BRIDGES (1917), MOHR (1923), and BRIDGES, SKOOG, and LI (1936) demonstrated a loss in recombination corresponding to the length of the missing region, but did not show, for deletions short enough to be viable and fertile in the heterozygous state, significant linkage disturbances in areas adjacent to the deletion. By contrast, STADLER (1935) and STADLER and ROMAN (1948), working with corn, concluded that heterozygous deficiencies can produce an extensive reduction in crossing over, much more than that expected from the actual length of the deficiency. This paradox remains unresolved.

The difficulty, in part, of assessing the effect of a deletion on crossing over arises from the fact that the genetic extent of the deficiency, and thus the expected loss of recombination, cannot readily be ascertained. An unambiguous demonstration of the effects of deletion on crossing over would follow from a comparison of recombination values obtained between markers surrounding a deletion when the deficiency is heterozygous and when it is homozygous.

The inviability of deficiency homozygotes would seem to make the latter test impossible; however, an insertional translocation (which occasionally arises simultaneously with a deletion when the deleted fragment is "captured" by a break in a non-homologous chromosome) provides a mechanism for "covering" the lethality of homozygous deficiencies. If an insertional translocation, acting as a duplication, has an extent only little greater than that of a deficiency it is able to cover, then the genetic imbalance of deficiency-duplication homozygotes is minimal. In this condition, recombination frequencies detected in the deficient chromosomes should permit an assessment of the reduction resulting solely from the deletion itself, i.e., its genetic extent. Then, a measure of crossing over in the

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deficiency heterozygotes should clearly reflect the magnitude of any linkage disturbance that might exist in regions adjacent to the deficiency.

Insertional translocations, moreover, can be studied independently of their associated deficiencies. As a class of chromosome rearrangements, such translocations have been as little studied as deficiencies with regard to their effects on recombination. An insertional translocation was included among several duplications whose effects on recombination were investigated by DOBZHANSKY (1934). Even earlier, RHOADES (1931) had analyzed another insertional translocation, originally discovered by DOBZHANSKY, in which a segment of chromosome 2 had been intercalated in the Y-chromosome. As in most of DOBZHANSKY's duplications, recombination was reduced in the region of the chromosome bearing homology to the duplicated segment. Competitive pairing was suggested as the mechanism by which duplications, whether insertional translocations or otherwise, affected crossing over. A more recent study by GRELL (1964) suggested that the position of the duplication in the genome, as well as its length, was important in the establishment of competitive pairing.

Because of the scarcity of information about the effects of deficiencies and insertional translocations on recombination, together with the apparently contradictory results of studies in corn and Drosophila, new experiments were undertaken to provide quantitative data on recombination in the vicinity of two deletions whose cytological extents were precisely known. An insertional translocation was used to permit a further study of recombination when one of the deficiencies was in the homozygous state. In addition, the effects of three different insertional translocations on recombination in normal chromosomes were investigated.

MATERIALS AND METHODS

Crossing over in the X chromosome was measured by using the following markers (described by BRIDGES and BREHME 1944): yellow *(y)* 0.0, white-apricot *(w^a)* 1.5, split *(spl)* 3.0, ruby *(rb)* 7.5, lozenge *(lz)* 27.7, and miniature *(m)* 36.1. These markers were contained in three laboratory stocks: (1) *$y\ w^a\ spl\ rb$;* (2) *$lz^{50l30}\ m$* (females homozygous for this *lz* allele, provided by M. M. GREEN, are fertile); (3) wild type (+) M56i. Deficiencies and balancing segments used are illustrated in Figures 1 and 2 and are described below.

Deficiency white-marbled Notch-63b Df(w^{ma}N)^{63b}: An X-ray-induced Notch deficiency extending from 3C3 through 3E2, inclusive (18 salivary chromosome bands on BRIDGES, 1938, revised salivary chromosome map), which is accompanied by a simultaneously elicited, nonuniformly pigmented white allele, called "marbled", that has properties similar to those of the allele, spotted-white. The deficiency which, in the interest of brevity, hereafter will be referred to as *Df(w^{ma}N)*, acts as a recessive lethal, expressing a Notch phenotype in the heterozygous condition, and includes the loci of roughest (*rst*, 1.7), *spl*, and diminutive (*dm*, 4.6). Heterozygous females are viable and fertile, but the deficiency is lethal in males. (LEFEVRE and WILKINS, 1966). Figures 1A and 1B illustrate salivary chromosome preparations of *Df(w^{ma}N)*/+ females. Two unsynapsed strands of *Df(w^{ma}N)* are shown in Figure 1A. Figure 1B illustrates the synapsis of the deficient chromosome with its structurally normal homologue.

Deficiency of white through echinus Df(w-ec)^{64d}: An X-ray-induced deficiency extending from 3C2 through 3F1–2 (27 salivary chromosome bands). The deleted segment was simultaneously inserted in section 37D of chromosome 2, *Dp(1;2L)(w-ec)^{64d}* (LEFEVRE and WILKINS 1966; right breakpoint corrected from the published information). In referring to this aberration,

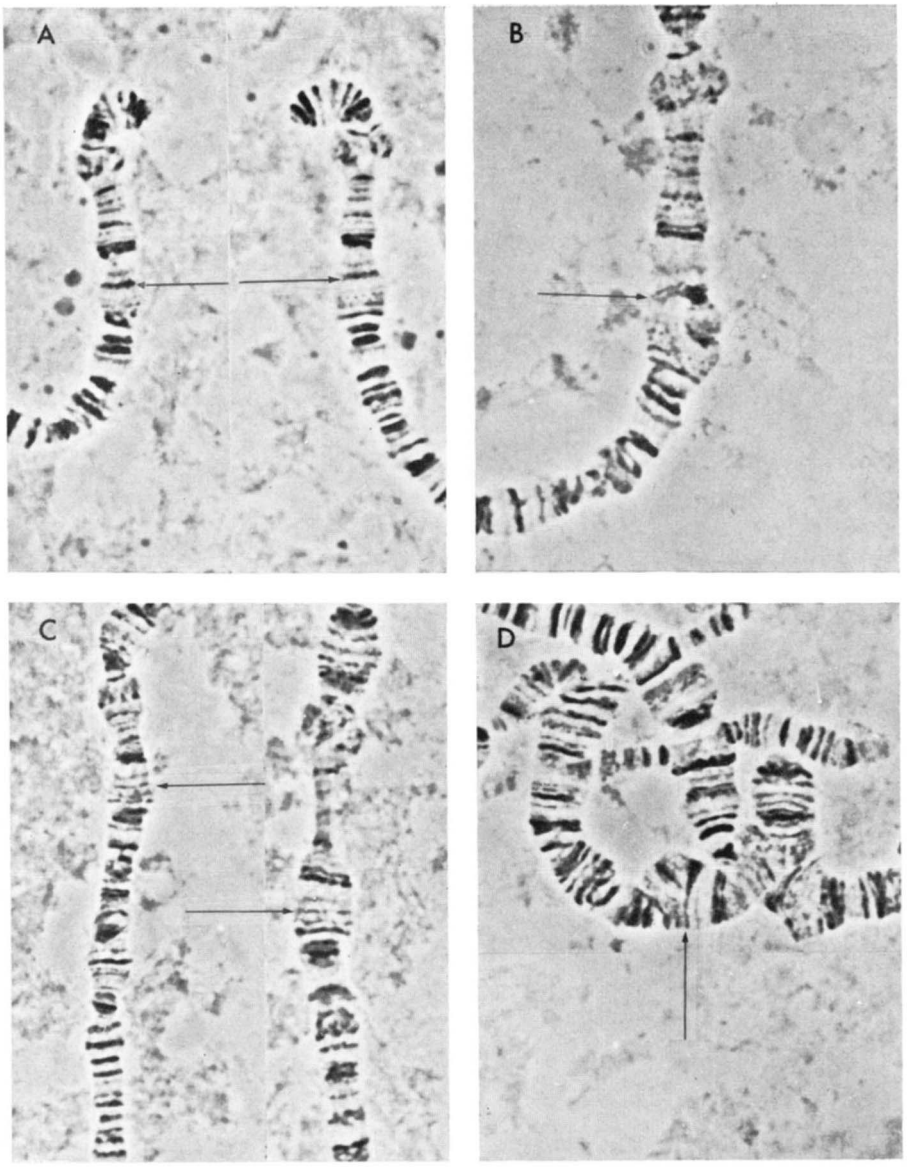

FIGURE 1.—*A*. Two unsynapsed *Df(w^{ma}N)* strands. *B*. Heterozygous female, showing synapsis between *Df(w^{ma}N)* and a structurally normal X chromosome. *C*. Two male X chromosomes, showing *Df(w-ec)*. *D*. Heterozygous female, showing synapsis between *Df(w-ec)* and a structurally normal X chromosome. (In all figures, the arrows point to the position of the deficiency.)

the abbreviation *(w-ec)* will be used hereafter, preceded by either *Df* or *Dp* to designate the deficient or duplicated condition, respectively. Males having the deficient X chromosome and the translocated segment survive and express both a white and echinus phenotype, with normal wings. Figure 1C shows two views of the *Df(w-ec)* X chromosome of a *Df-Dp* male. The left and right breakpoints of this mutant coincide closely with the loci of *w* (1.5) and *ec* (5.5),

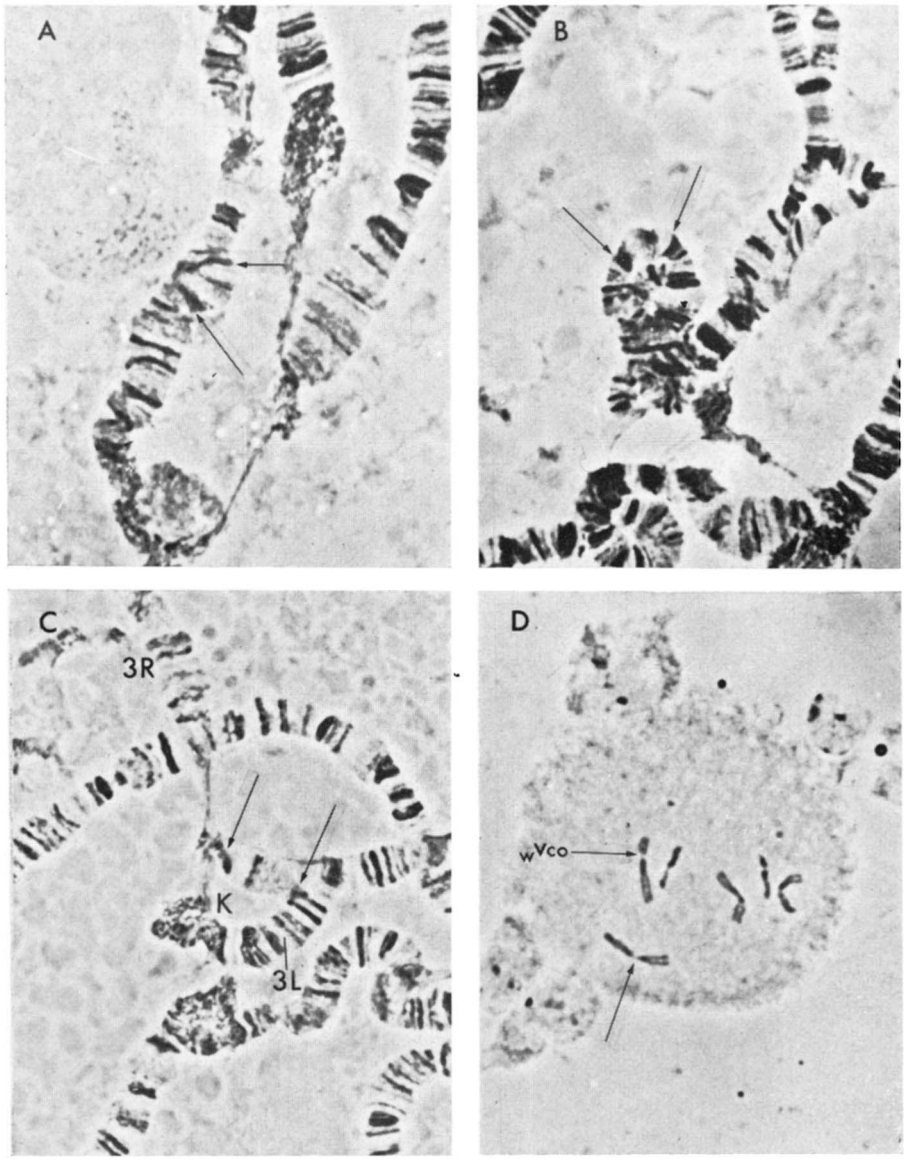

FIGURE 2.—The cytology of *Dp(w-ec)* and *Dpw*^{Vco}. A. and B. Salivary chromosome preparations of *Dp(w-ec)*. The limits of the duplication are indicated by the arrows. In A, note the intimate synapsis between duplication-bearing and normal second chromosomes. In B, the chromosomes are somewhat unsynapsed in the vicinity of the duplication. C. Salivary chromosome preparation of *Dpw*^{Vco}. Arrows mark the limits of the duplication. The third chromosome centromere region is at K. 3L and 3R are indicated. D. Larval brain preparation of *Dpw*^{Vco}. The centromere regions of the normal and duplication-bearing third chromosomes are marked. Note the eccentric location of the centromere in the chromosome with *Dpw*^{Vco}, resulting from the pericentric inversion.

respectively. Females heterozygous for the deficiency express Notch. A salivary chromosome preparation of a *Df(w-ec)*/+ female is shown in Figure 1D. Figures 2A and 2B both illustrate *Dp(w-ec)*, inserted in 2L at 37D.

Duplication w^{+51b7} Dp(1;2R)w^{+51b7}: A segment extending from 3C2 through 3D5–6, produced by irradiation of inversion white mottled-4 (w^{m4}), and including some adjacent proximal heterochromatin (but not 3C1). This deleted material was inserted in a euchromatic region of the right arm of chromosome 2 at 52F (RATTY 1954). The duplication includes the loci of *w*, *rst*, *spl*, and *dm*.

Duplication white-variegated cobbled Dpw^{Vco}: An insertional translocation involving the X chromosome and chromosome 3 found by CLAUSEN (BRIDGES and BREHME 1944). A segment of the X chromosome from 2C1 through 3C4, including the loci of kurz (*kz*, 0.7), prune (*pn*, 0.8), zeste (*z*, 1.0), *w* and *rst*, was inserted in the heterochromatic region of the right arm of chromosome 3, which itself has a short inversion across the spindle attachment with breaks at 77D4 and 81A (chromocenter); 3C4 adjoins 81A (analysis by SCHULTZ, cited in BRIDGES and BREHME 1944). Figure 2C illustrates a salivary chromosome preparation of Dpw^{Vco}. The pericentric inversion in chromosome 3 is well illustrated by a larval brain preparation shown in Figure 2D.

All test crosses were carried out following a standard procedure in order to minimize variations in recombination frequencies due to maternal age and temperature. Females heterozygous for the X chromosome to be tested were collected as virgins, aged three days, transferred to fresh food vials, and mass mated for 24 hours with males of the same age carrying recessive markers. Mating vials were discarded the following day, and females were then individually subcultured daily in fresh food vials, without males, for a period of three days (fourth, fifth, and sixth days after emergence) in order to sample eggs from the peak egg-laying period. Progeny were counted and scored for crossovers after the cultures had been incubated for 13 days and again after 17 days.

Each experiment and its control were done concurrently, and where possible control flies were drawn from among the siblings of the experimental flies. All experiments were conducted at 25 ± 1°C., using 8-dram vials containing standard Drosophila medium.

RESULTS

Recombination in the vicinity of a heterozygous deficiency. Recombination was measured in the genetic interval between *y* and *rb* in the presence of two different heterozygous deficiencies included within the marked interval (Table 1). *Df($w^{ma}N$)* reduced recombination in the overall test interval to nearly one-fourth (1.86%/7.28%) that of the control value for the same interval obtained from structurally normal chromosomes ($\chi^2 = 93.3$, $P \ll .001$, d.f. = 1). In the distal part of the interval (*y*-deficiency), recombination was reduced to 0.77%; in the region from the deficiency to *rb*, 1.09% recombination was observed.

Df(w-ec), which was also tested, reduced recombination in the total *y-rb* region to one-fifth (1.52%/7.28%) that of the control value ($\chi^2 = 55.5$, $P \ll .001$, d.f. = 1). The measured sub-intervals (*y*-deficiency; deficiency-*rb*) showed values of 0.56% (7/1251) and 0.96% (12/1251) respectively.

Although *Df(w-ec)* is 9 bands longer than *Df($w^{ma}N$)*, the amount of recombination between *y* and *rb* (1.52% and 1.86%) in the two experiments was not significantly different ($\chi^2 = .56$, $.25 < P < .50$, d.f. = 1). Nonetheless, the lower value of recombination between *y* and *rb* was observed with the longer deficiency, as would be expected.

Recombination in the vicinity of a homozygous deficiency. Females possessing *Df($w^{ma}N$)* in both X chromosomes were also tested for recombination in the interval from *y* to *rb*. Females carrying two deficient chromosomes, one marked

TABLE 1

Crossing Over in the Vicinity of Deficiencies

	y-w	*w-spl*	*spl-rb*	*y-rb*
Normal chromosomes $\frac{y\ w^a\ spl\ rb}{+\ +\ +\ +}$ (n=9116)	189 (1.86%) $\chi^2 = 18.4$* P<<.001**	112 (1.23%)	363 (3.98%) $\chi^2 = 47.5$ P<<.001**	664 (7.28%) $\chi^2 = 93.3$ P<<.001**
Deficiency heterozygotes $\frac{+\ Df(w^{ma}N)\ +}{y\ w^a\ spl\ \ rb}$ (n=2472)	19 (0.77%) $\chi^2 = .52$ .25<P<.50	.....	27 (1.09%) $\chi^2 = .14$ .50<P<.75	46 (1.86%) $\chi^2 = .56$ .25<P<.50
$\frac{+\ Df(w\text{-}ec)\ +}{y\ w^a\ spl\ \ rb}$ (n=1251)	7 (0.56%)	.....	12 (0.96%)	19 (1.52%)
Deficiency homozygotes $\frac{y\ Df(w^{ma}N)\ +}{+\ Df(w^{ma}N)\ rb}; \frac{Dp(w\text{-}ec)}{+}$ (n=1784)	.....	.....	.....	70 (3.92%) $\chi^2 = .044$ .75<P<.90
$\frac{y\ Df(w^{ma}N)\ +}{+\ Df(w^{ma}N)\ rb}; \frac{Dp(w\text{-}ec)}{Dp(w\text{-}ec)}$ (n=854)	.....	.....	.....	35 (4.10%)

* χ^2 and P values lie between entries, the significance of whose differences they test.
** These values are highly significant.

with *y* and the other with *rb*, were provided with *Dp(w-ec)* in order to cover the deleted region; such *Df-Dp* females were viable and fertile. As an examination of Table 1 indicates, a value of 3.92% for the surrounding *y-rb* interval was evidenced in homozygous deficient females when the balancing segment was present in only one member of the pair of second chromosomes: *y Df($w^{ma}N$) +/+ Df($w^{ma}N$) rb; Dp(w-ec)/+*. In this cross, it was impossible to determine whether any given *y-rb* recombinant resulted from crossing over in the distal or proximal sub-interval. The recombination value represents a reduction to almost one-half (3.92%/7.28%) that obtained for the same interval in structurally normal chromosomes.

A similar recombinational value (4.10%) was evidenced in the encompassing *y-rb* interval of *Df($w^{ma}N$)* homozygotes when the balancing segment was present in both second chromosomes: *y Df($w^{ma}N$) +/+ Df($w^{ma}N$) rb; Dp(w-ec)/Dp(w-ec)*. The values obtained for recombination in the *y-rb* interval in the deficiency homozygotes, whether heterozygous or homozygous for *Dp(w-ec)*, (3.92% *vs.* 4.10%), did not differ significantly from one another ($\chi^2 = .044$, $.75 < P < .90$, d.f. = 1). When the data from both experiments are combined, a value of 4.03% (105/2638) for the *y-rb* interval results. This value is significantly

TABLE 2

Crossing Over in a Region Homologous to Insertional Duplications

	y-w	*w-spl*	*spl-rb*
Control $\frac{y\ w^a\ spl\ rb}{+\ +\ +\ +}$ (n=2178)	50 (2.30%)	21 (0.96%)	90 (4.13%)
	$\chi^2 = .96$* .25<P<.50	$\chi^2 = .31$ .50<P<.75	$\chi^2 = .48$ .25<P<.50
$\frac{Dpw^{+51b7}\ y\ w^a\ spl\ rb}{+\ +\ +\ +\ +}$ (n=1282)	23 (1.79%)	10 (0.78%)	56 (4.29%)
Control $\frac{y\ w^a\ spl\ rb}{+\ +\ +\ +}$ (n=3732)	83 (2.22%)	63 (1.66%)	164 (4.39%)
	$\chi^2 = .81$ .25<P<.50	$\chi^2 = 1.5$ .10<P<.25	$\chi^2 = .58$ .25<P<.50
$\frac{Dp(w\text{-}ec)\ y\ w^a\ spl\ rb}{+\ +\ +\ +\ +}$ (n=1433)	38 (2.65%)	31 (2.16%)	56 (3.91%)
Control $\frac{y\ w^a\ spl\ rb}{+\ +\ +\ +}$ (n=3206)	56 (1.75%)	29 (0.90%)	109 (3.40%)
	$\chi^2 = 9.8$ P = .002**	$\chi^2 = .73$ .25<P<.50	$\chi^2 = 1.3$ .10<P<.25
$\frac{Dpw^{Vco}\ y\ w^a\ spl\ rb}{+\ +\ +\ +\ +}$ (n=900)	3 (0.33%)	11 (1.22%)	38 (4.22%)

* χ^2 and P values lie between entries, the significance of whose differences they test.
** This value is highly significant.

greater than the value of 1.86% obtained for the same interval with *Df(w^{ma}N)* heterozygotes ($\chi^2 = 20.0$, P $\ll$ .001, d.f. = 1).

Recombination in the presence of insertional duplications. Tables 2 and 3 record the results of the effects of the presence of three different insertional duplications on recombination in normal X chromosomes, both in homologous regions falling within the *y-rb* interval and in a nonhomologous region *(lz-m)*.

A. Effects on homologous regions: The results of tests of the three duplications on chromosomal region homologous to themselves, together with their control values, are listed in Table 2. Since statistical analysis indicated that the control data were not homogeneous and therefore could not be pooled, crossover values obtained in the presence of each duplication were compared only with their respective control values. Neither *Dpw^{+51b7}* nor *Dp(w-ec)* produced any significant effect on recombination values in any of the test intervals (*y-w; w-spl; spl-rb*) as compared with those found in their respective controls. In contrast, *Dpw^{Vco}* produced a significant decrease (0.33%/1.75%; $\chi^2 = 9.8$, P = .002, d.f. = 1) in the distalmost interval *(y-w)* as compared with the values found in its control. The other intervals tested, *w-spl* and *spl-rb*, showed no statistically significant linkage disturbances in the presence of *Dpw^{Vco}*.

B. Effects on a nonhomologous region: Table 3 contains recombination values obtained from crosses made to investigate the effects of the same three insertional

TABLE 3

Crossing Over in a Region of Nonhomology to Insertional Duplications

			lz +	+ *m*	Total	Tests of significance
Control	$\frac{lz\ m}{++}$	(n=7527)*	289	309	598 (7.94%)	.....
Dpw^{+51b7} / +	$\frac{lz\ m}{++}$	(n=2351)	74	86	160 (6.81%)	$\chi^2 = 3.0$ $.05<P<.10$
Dp(w-ec) / +	$\frac{lz\ m}{++}$	(n=866)	41	35	76 (8.77%)	$\chi^2 = .69$ $.25<P<.50$
Dpw^{Vco} / +	$\frac{lz\ m}{++}$	(n=3185)	103	124	227 (7.13%)	$\chi^2 = 1.9$ $.10<P<.25$

* Total of the separate controls done simultaneously with the three experiments. Statistical tests showed them to be homogeneous ($\chi^2 = 3.95$, $.10<P<.25$ d.f. $= 2$).

duplications on a nonhomologous *(lz-m)* interval of the X chromosome. Since the control values in this experiment were shown to be homogeneous ($\chi^2 = 3.95$, $.10 < P < .25$, d.f. $= 2$), they were pooled, and the control value given is the average of all three control experiments done simultaneously with their respective test crosses. None of the duplications tested produced any significant effect on recombination values in the nonhomologous chromosomal region.

DISCUSSION

Recombination in normal chromosomes. The interpretation of the experimental results recorded above requires that normal recombination values within the *y-rb* interval of the X chromosome be compared with those obtained from females heterozygous and homozygous for deficiencies and duplications. Standard map distances might have been used as the norms for the pertinent intervals: *y-w*, 1.5; *w-spl*, 1.5; *spl-rb*, 4.5. Because of the well-known variability from stock to stock in recombination frequencies (REDFIELD 1955), control values were obtained from *y* w^a *spl rb* / + females. All experimental values were obtained from crosses with the same *y* w^a *spl rb* stock.

As shown in Table 2, the control values for the three measured intervals varied considerably, tending to be higher than the standard map distance for the *y-w* interval, but lower for the *w-spl* and *spl-rb* intervals. Overall, however, recombination for the entire *y-rb* interval in all control crosses combined yielded a close approximation to the standard map distance (7.28 *vs.* 7.5). Under these circumstances, it seems improbable that unsuspected chromosome abnormalities were present in the control stock, causing the abnormal values in the *y-rb* subintervals. More likely, as a stock characteristic, the distribution of crossovers in the *y* w^a *spl rb* stock is somewhat distorted, as compared with other stocks. Therefore, the recombination frequencies used as standards for comparison were those obtained from the combined control crosses. These values, as listed in Table 1, are: *y-w*, 1.86; *w-spl*, 1.23; and *spl-rb*, 3.98.

Recombination in deficiency heterozygotes. Recombination in marked regions of a chromosome encompassing a deficiency in the heterozygous condition should be reduced by at least the genetic length of the missing segment. If, in addition, the deficient chromosome encounters difficulties in pairing with its structurally normal homologue in the regions adjacent to the missing segment, an even greater reduction would be anticipated. This latter expectation was realized in the present study.

The amount of recombination observed for the entire *y-rb* interval in females heterozygous for *Df(w^{ma}N)* was 1.86%; a control value of 7.28% was obtained for the same interval in structurally normal chromosomes. If there were no synaptic difficulties present in regions adjacent to the deficiency, then these results could be used to estimate the genetic length of the missing segment. Under this assumption, the genetic length of *Df(w^{ma}N)* would be 5.42 crossover units (7.28–1.86).

The left breakpoint of *Df(w^{ma}N)*, as shown by cytological analysis (LEFEVRE and WILKINS 1966), lies just to the left of 3C3, leaving 3C2 (and the *w* locus it contains) undeleted. As a consequence, the *y-w* interval remains completely intact in the deficient chromosome. This circumstance provides an opportunity to test recombination in a structurally normal region lying immediately adjacent to the breakpoint of the deleted segment. Recombination in the *y-w* interval in controls was 1.86%; the same interval adjoining *Df(w^{ma}N)* exhibited a value of 0.77%. Thus, the *y-w* region, when immediately adjacent to the breakpoint of a deficiency, suffered a 59% reduction in recombination.

The second deficiency tested, *Df(w-ec)*, provided additional evidence for reduced recombinational values in the vicinity of deleted segments. Unlike *Df(w^{ma}N)*, *Df(w-ec)* includes one band of the *y-w* interval, 3C2. This loss, only one band out of a total of 98 in the *y-w* interval, is small enough to ignore in making comparisons with the results obtained with *Df(w^{ma}N)*. The recombinational value observed with *Df(w-ec)* heterozygotes for the *y-w* region (0.56%) was, in fact, not significantly different from that obtained with *Df(w^{ma}N)*.

Both deficiencies tested remove band 3C7 and, with it, the *spl* locus found there. Consequently, the *spl-rb* region tested actually comprised the interval remaining between the right breakpoints of the respective deletions and the ruby locus. *Df(w^{ma}N)* removes 14 bands from the *spl-rb* interval; *Df(w-ec)* lacks 8 more, making a total of 22 bands deleted from the *spl-rb* interval. A recombinational value of 1.09% for the *spl-rb* interval was evidenced with *Df(w^{ma}N)* in heterozygous condition; a value of 0.96% for the same interval was found with *Df(w-ec)*. Although both of these values contrast sharply with the control value of 3.98% found with normal chromosomes, the two values do not differ significantly from one another ($\chi^2 = .14$, $.50 < P < .75$), even though one would expect the value with *Df(w-ec)* to be lower than that observed with the shorter deficiency, *Df(w^{ma}N)*.

BRIDGES (1917), in the initial observation of the deficiency phenomenon, reported a loss of recombination in deficiency heterozygotes no greater than the genetic length of the deficiency, although its actual cytological extent was not

then known. Later, salivary chromosome analysis by SUTTON (1943) revealed that the deletion BRIDGES studied (f^{257-6}, a segment removed from the interval between rudimentary, 55.1, and fused, 59.5) had occurred in a chromosome carrying the tandem duplication, Bar. The actual amount of missing material, when compared with a normal (nonduplicated) chromosome, was found to be only 10 salivary chromosome bands (15Fl-9). BRIDGES credited the deficiency with removing 0.7 of the 4.4 crossover units in the surrounding rudimentary to fused interval. The short extent of the deficiency may have contributed to his failure to conclude that the reduction in recombination in the measured interval might have been greater than the actual length of the deficiency.

The Notch-8 *(N^8)* deficiency, lying within the *y-rb* interval of the X chromosome and of the same cytological length (18 bands) as *Df($w^{ma}N$)*, was studied by MOHR (1923). He reported that N^8 in heterozygous condition reduced recombination by 3.8% in the surrounding region. This value seemed to be no greater than the presumed genetic length of the deficiency.

Later, BRIDGES, SKOOG, and LI (1936) reported that a relatively long deficiency in 2R (Notopleural) yielded a close correlation between the amount deleted and the reduction in recombination observed. Although the results of genetic tests were somewhat erratic, the 50-band deficiency was estimated to reduce recombination by 1.5% in the area of the second chromosome from which it was deleted. An expected recombinational value of 1.2% for the missing segment was obtained by multiplying the genetic length of the whole chromosome arm (in crossover units) by the fraction of the total length of the salivary chromosome map of 2R that was comprised by the deficient region (measured in microns). The authors noted the inexactness of the method used to calculate the expected value and admitted the possibility that recombination may have been reduced in the immediate vicinity of the deficiency. Nonetheless, reduction in recombination by more than the genetically measured extent of deficiencies in Drosophila would not be anticipated from the conclusions of either BRIDGES or MOHR. Unpublished data from later genetic studies indicate that in some cases, at least, a greater reduction does occur (B. H. JUDD, personal communication). Further, two of three *a* deficiencies in corn studied by STADLER and ROMAN (1948) showed a great reduction in crossing over in adjacent regions; the other yielded approximately normal values for the same region.

Recombination in deficiency homozygotes. Complete homology exists between two X chromosomes when *both* are lacking the same deleted segment. As a consequence, the deficient chromosomes should exhibit no loss of recombination as a result of asynapsis in the chromosomal regions that surround the breakpoints of the deleted segments; recombination values should reflect more accurately the genetic length of the missing segment than would values obtained from deficiency heterozygotes.

Homozygous *Df($w^{ma}N$)* females, however, carried a balancing segment, *Dp(w-ec)*. As a result, although the X chromosomes were homologous, the presence of *Dp(w-ec)* in only one of the members of the second chromosome pair established nonhomology between the two second chromosomes. In this condition,

a value of 3.92% for recombination in the *y-rb* interval was found, as compared with the control value of 7.28%.

The existence of a structurally nonhomologous pair in a nucleus is known to be able to increase recombination between the remaining homologous chromosome pairs in the same nucleus. Such an "interchromosomal effect" (SCHULTZ and REDFIELD, 1951) could contribute to the recombinational value observed in the homozygous deficient females when only one of the second chromosomes contained *Dp(w-ec)*. If this were case, the observed value would include, to some degree, an artificial increment in recombination.

This kind of interaction between nonhomologous chromosome pairs, however, was not present when females were homozygous both for deficient X chromosomes and for second chromosomes containing the inserted segment. Such females possessed complete structural homology between the members of the two pairs of chromosomes involved. When tested, they gave a value for the *y-rb* interval no different from that obtained when only one second chromosome possessed the duplication. When the pooled value (4.03%) is compared with that found for the same interval in $Df(w^{ma}N)$ heterozygotes (1.86%), a highly significant difference is evidenced ($\chi^2 = 20.0$, $P \ll .001$, d.f. $= 1$). Clearly, the value for the *y-rb* interval obtained from the test of homozygous deficient females provides a more accurate estimate of the actual genetic length of $Df(w^{ma}N)$ than do the results of testing heterozygous deficient females. The genetic length of $Df(w^{ma}N)$, using data from deficiency homozygotes, is 3.25%. The much greater loss of recombination evidenced by $Df(w^{ma}N)$ heterozygotes clearly shows that crossing over is reduced in regions adjacent to the deficiency in heterozygous condition.

Cytological and genetic correlations. The lack of correspondence, except for linear order, between genetic and cytological maps necessitates finding a method by which the two units of measurement can be combined to give an accurate picture of the recombinational nature of the chromosome. BRIDGES (1937) noted that the distances between genes at the distal end of 2R as measured on the salivary chromosome map were poorly correlated with the distances that separated them on the gene map. He described coefficients of crossing over that reflect regional differences in crossing over per unit chromosome length. REDFIELD (1955), studying the *y-spl* region of the X chromosome, was struck by the remarkable fact that the map distance between *y* and *w* is equal to that between *w* and *spl;* whereas, the number of salivary chromosome bands between *y* and *w* is 20 times greater than the number between *w* and *spl*.

Figure 3 illustrates this disparity between gene maps and cytological maps for the region of the X chromosome that includes the test interval used in the present study *(y-rb)*. The values were calculated for intervals delimited only by genes whose cytological positions and standard map locations have been firmly established. For this reason, the region shown extends beyond the most proximal marker used in the present study, ruby (whose exact location is not known), to the nearest more accurately localized gene, rugose. The cytological extents of the deficiencies and duplications used in these investigations (including N^8 for comparison) are superimposed on the graph.

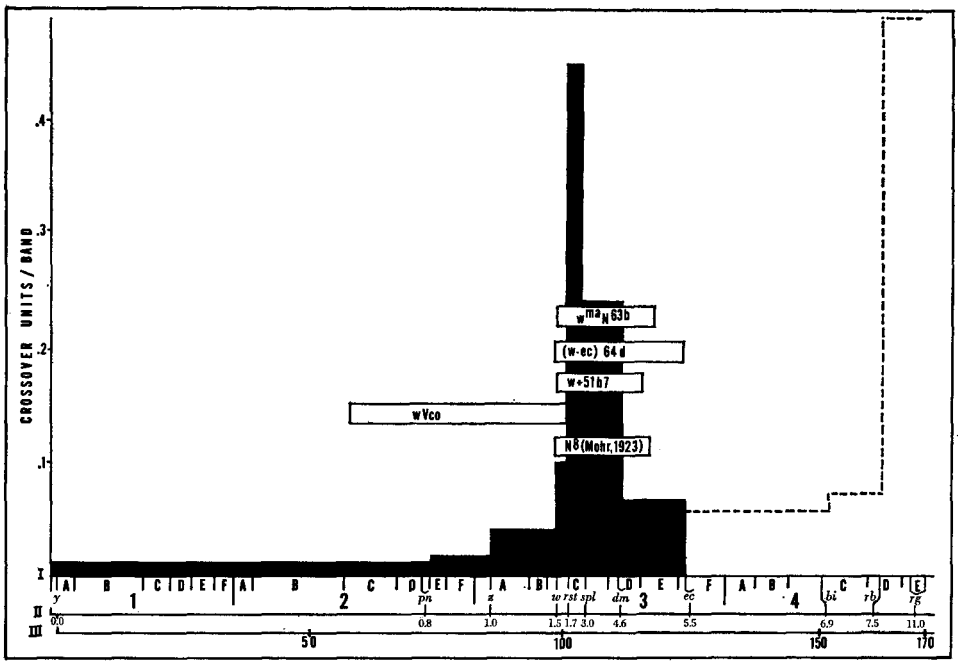

FIGURE 3.—Diagrammatic representation of the left (distal) end of the X chromosome, correlating the cytological, genetic, and recombinational properties of the region between yellow and rugose. Abscissa scales: I, salivary chromosome sections; II, linkage map; III, cumulative band totals (*y* arbitrarily placed at 0.)

The genetic length of a particular deficiency or duplication can be calculated by multiplying the number of bands within its extent by the crossover frequency per band for that particular interval (i.e., its coefficient of crossing over). By this method a calculated value of 3.5% is obtained for the 18 bands removed by *Df(w^{ma}N)*. This figure is in close agreement with the genetic length of *Df(w^{ma}N)* estimated from the reduction in recombination in the *y-rb* interval obtained with the deficiency homozygotes (3.25%).

Calculations based on the crossover units per band of *Df(w-ec)* show it to encompass 4.0 crossover units. (Its breakpoints coincide closely with the loci of *w* and *ec* at 1.5 and 5.5, respectively, on the standard map.) Unfortunately, difficulty in obtaining sufficient numbers of properly marked fertile *Df(w-ec)*; *Dp(w-ec)* homozygotes made it impossible to determine the genetic length of *Df(w-ec)* experimentally.

The highly localized variability in crossover frequencies within the interval used for the present study, as illustrated in Figure 3, suggests that all chromosome regions should be similarly evaluated in combined genetic-cytological units before realistic estimations of the genetic extents of other deficiencies can be made. The extreme distal location of the chromosomal region used for these investigations minimizes any possible centromere effect; however, the influence of the telomere, if any, should be strongly felt. In contrast, more proximal regions, such as those

previously investigated, may be under the influence of the centromere. This effect might result in as great, or greater, variability in coefficients of crossing over as those observed in the *y-rb* interval.

Recombination in the presence of insertional duplications. The duplicating segment employed in this study, *Dp(w-ec)*, introduced a variable with which no previous study on the recombinational effects of deficiencies has dealt. As a consequence, further experiments were devised to test the effects of *Dp(w-ec)* and two other insertional duplications on recombination in homologous and nonhomologous regions of structurally normal chromosomes. The results of these experiments insured that the outcome of the present study was not influenced by any interchromosomal effects resulting from the presence of the duplication.

Figure 3 includes the three duplications tested. Multiplying the number of bands found in each duplication by the crossover frequency for their respective regions permits an estimation of their genetic lengths. Using this method, one can conclude that Dpw^{+51b7} ought to contain 3.4 crossover units in its 17 bands; *Dp(w-ec)* 4.0 crossover units in its 27 bands. The physically longest duplication, Dpw^{Vco}, proves to be shortest genetically, containing only 1.0 crossover unit in 43 bands. The presence of either Dpw^{+51b7} or *Dp(w-ec)*, both inserted in euchromatic areas of chromosome 2 (2R and 2L, respectively), produced no significant effect on recombination in homologous regions of structurally normal chromosomes. However, Dpw^{Vco}, inserted in the centric heterochromatin of chromosome 3R, elicited a decrease in the *y-w* interval. None of the three duplications provoked any effect on a nonhomologous region, *lz-m*.

The lack of a general increase in recombination in the presence of these insertional duplications excludes the possibility of their generating measurable interchromosomal effects. The nonhomology created between the normal homologue and its partner carrying one of the duplications tested must be insufficient to cause such an effect. In the particular case of Dpw^{Vco}, however, the third chromosome in which it is inserted also possesses a short pericentric inversion. Many inversions are known to produce interchromosomal effects (SCHULTZ and REDFIELD 1951). The failure to detect an effect in the presence of this inversion can most likely be traced to the nature of the inversion. The breakpoints occur at 77D4 and 81A (chromocenter), the centromere being inverted. Although the left breakpoint occurs just outside the heterochromatin-euchromatin junction of 3L at the locus of inturned (*in*, 47.0), the rearrangement primarily involves only heterochromatin. The relationships between the euchromatic elements of the third chromosome remain essentially unaltered by the presence of the inversion. The lack of an interchromosomal effect may stem from this fact; however, studies on recombination in neighboring regions of the third chromosome would be desirable to verify it.

Duplications were shown by DOBZHANSKY (1934) to decrease recombination in homologous regions in proportion to their length. The majority of the duplications that he studied existed as free or translocated fragments; only one insertional duplication was included in his tests. It proved to have a slight effect, which he attributed to its small size. An insertional duplication studied by

RHOADES (1931), however, produced a significant reduction. All results were viewed as consistent with the theory of competitive pairing. Later, GRELL (1964) found that both length and location of a duplication in relation to its region of homology were important in competitive pairing. This provides an additional explanation for the different effects observed in the presence of the insertional duplications studied by DOBZHANSKY and RHOADES.

The decreased recombination in the *y-w* region elicited by Dpw^{Vco} agrees with these findings. Its physical length is apparently great enough to allow at least some competitive pairing to occur with the homologous region of the normal X chromosome. Because of the smaller physical dimensions of Dpw^{+51b7} and *Dp(w-ec)*, their insertional locations, or the combination of both, little if any pairing occurs with normal X chromosomes in the regions of homology. The inability of all three duplications to affect recombination in the nonhomologous *lz-m* interval is to be expected from the conclusions of previous investigators concerning competitive pairing.

SUMMARY

Recombination frequencies were determined in regions adjacent to X-chromosomal deletions, both in the heterozygous and the homozygous condition. In heterozygous condition, two separate deletions lying within a region marked by yellow (0.0) and ruby (7.5) produced a striking reduction as compared with values obtained in control experiments with structurally normal chromosomes. One deficiency in the homozygous condition, provided with a duplication to prevent lethality, exhibited significantly more recombination in the marked interval than did its heterozygous counterpart. Results from the experiment with the homozygous deficiency were used to provide an accurate estimate of the genetic extent of the deleted region. The observed value closely corresponded with the expectation derived from a combined genetic-cytological unit of measurement, crossover frequency per salivary chromosome band, calculated from existing cytogenetic data.—Tests of recombination in homozygous deficient females required the introduction of an insertional translocation to permit the survival of the females. Accordingly, the possible interchromosomal effects of three different insertional translocations on recombination in both deficient and structurally normal chromosomes were tested. No interchromosomal effects were shown to stem from their presence. However, a decrease in recombination was obtained in one homologous region of structurally normal chromosomes in the presence of the longest duplication tested, a finding consistent with the theory of competitive pairing. None of the inserted fragments affected recombination in a nonhomologous region of structurally normal chromosomes.

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