A SIMULTANEOUS MEASURE OF INTERCHROMOSOMAL EFFECTS ON AUTOSOMAL CROSSING OVER AND SEX CHROMOSOME NONDISJUNCTION IN *DROSOPHILA*¹

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IT is a common observation in *Drosophila melanogaster* that the presence of heterozygous inversions may characteristically result in an increase in crossing over in given regions of non-homologous chromosomes. STURTEVANT (1944) made the unexpected finding that, in addition to effects on crossing over, structural heterozygosity in an autosome may also cause an increase in primary nondisjunction of sex chromosomes. COOPER *et al.* (1955) have verified and extended STURTEVANT's claim and, in addition, have shown that in the presence of marked structural heterozygosity in chromosomes X and 2, X and 3, or 2 and 3, a large increase in dominant lethals is observed.

To analyze further the ways in which structurally altered chromosomes influence such interactions between non-homologous chromosomes, experiments were undertaken to measure simultaneously crossing over in the X and in chromosome 2, as well as nondisjunction of the sex chromosomes. Since X chromosomes which fail to disjoin have rarely been involved in crossing over (BRIDGES 1916; STURTEVANT and BEADLE 1936), the influence of crossing over in the X chromosomes on crossing over in a non-homologous chromosome can be made by comparing the relative frequencies of crossing over in chromosome 2 in flies that received two X chromosomes and those that received one X chromosome through the egg. Furthermore, since heterozygosity for an autosomal inversion has an effect on crossing over and segregation of X chromosomes, the introduction of an inversion in chromosome 3 provides information on its effects on crossing over in the X chromosome and in chromosome 3, and on nondisjunction of the X chromosomes.

A first report of results from experiments of this design is presented below.

DESCRIPTION OF CROSSES AND RESULTS

In the initial experiments, crossing over was followed in the *b-en* region of chromosome 2 in XXY females heterozygous for either *In(1)sc^r*, or *In(1)B^{M1}*, with and without *Ins* (3LR)*DcxF*. Both *sc^r* and *B^{M1}* are relatively small inversions. The former is located distally in the X chromosome, the latter proxi-

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mally, and to judge from BRIDGES map (1935; see appendix BRIDGES and BREHME 1944) some 77 percent of the polytene chromosome lies uninverted to the right of the proximal breakpoint of *sc*⁷, and about 83 percent to the left of the distal breakpoint of *B*^{m1}. The *b-en* region was chosen for testing because it has been found that crossing over in the region increases markedly upon the introduction of heterologous inversions (SCHULTZ and REDFIELD 1952). In conjunction with the current experiments, tests were made with females similar in constitution to those described above but lacking a Y chromosome. Details concerning mutant genes and inversions employed will be found in BRIDGES and BREHME (1944).

The results from these experiments are summarized in Table 1. It should be noted that crossover values for chromosome 2 from regular offspring are listed separately for (a) males carrying a crossover X chromosome, and, hence, must have been derived from an exchange tetrad (column 3) and (b) for *all* regulars, a class which includes those individuals coming from exchange tetrads as well as those coming from no-exchange tetrads where crossing over in the X chromosome is lower than normal (column 4). From an examination of the results, the following conclusions may be drawn:

1. Upon the introduction of *DcxF* into regular XX females, the crossover rates in the marked regions of chromosome 2 are approximately doubled (compare lines A, columns 3,4 *vs.* B3,4; F3,4 *vs.* G3,4).

2. The addition of a Y chromosome shows no marked effect on crossing over in chromosome 2 as measured from regular offspring (A3,4 *vs.* C3,4; F3,4 *vs.* H3,4 and I3,4).

3. A combination of a Y chromosome and heterozygosity for *DcxF* give crossover rates in regulars that do not differ very much from those obtained with *DcxF* alone (B3,4 *vs.* E3,4; G3,4 *vs.* J3,4).

4. In the absence of *DcxF*, crossover rates for chromosome 2 in regulars and exceptionals are similar (C3,4 *vs.* C5; D3,4 *vs.* D5; H3,4 *vs.* H5; and I3,4 *vs.* I5). There appears to be, however, an over-all suggestion of a small yet fairly consistent difference between the rates from exceptionals and regular males carrying a crossover X chromosome the former tending to slightly higher values.

5. However, in XXY females heterozygous for *DcxF* (a) the crossover rate in chromosome 2 in exceptionals is considerably higher than that in regulars (E3,4 *vs.* E5; J3,4 *vs.* J5), and (b) the frequency of secondary nondisjunction is markedly reduced (C7, D7 *vs.* E7; H7, I7 *vs.* J7).

It was desirable, therefore, to determine whether a discrepancy such as that indicated in item 5 is found between regular and exceptional offspring in mothers lacking a Y chromosome. A combination of X chromosome and autosome inversions was selected which would permit a measurable amount of crossing over in and nondisjunction of the X chromosomes. Accordingly, XX females were made heterozygous for *In(1)dl-49*, *γ v f car*, and *Ins(2L+2R)Cy*, *Cy*, and crossing over was followed in the uninverted portion of the X and in the *Gl-Sb* region of chromosome 3, the latter being within a region in which STEINBERG and FRASER (1944) and SCHULTZ and REDFIELD (1952) have found major crossover

TABLE 1

The results from crosses of XX and XXY females heterozygous for chromosome 2 markers *b* and *en* and for *In*(1)^{BM1} (*A-E*) or *In*(1)^{sc7} (*F-J*) in the presence or absence of *Ins*(3LR) *DcxF*, by *b en* males. Pairs of experiments run simultaneously were *A* and *B*, *D* and *E*, *F* and *G*, and *I* and *J*.

Exp.	(1) Constitution of female	Total flies (2)	(3) X-c.o. reg.	Percent c.o. <i>b-en</i> in all reg. (4)	(5) Except.	Percent c.o. in X (6)	Percent XX-O or XX-Y (7)	Equational exceptions (8)
A.	<i>v BM1/y2 w^a v; +/+</i>	2967	12.9±1.3	13.2±0.6	...	45.1±1.3	....	....
B.	<i>v BM1/y2 w^a v; DcxF/+</i>	1936	23.5±1.9	21.1±0.9	.....	50.3±1.6	0.2	.....
C.	<i>v BM1/y2 w^a v/Y; +/+</i>	6840	11.0±0.8	10.5±0.4	13.0±1.6	50.5±0.9	11.9±0.4	(2) <i>y2 v BM1; + (1)y2 v BM1; en</i> (1) <i>y2 w^a v BM1; b en</i>
D.	<i>v BM1/y2 w^a v/Y; +/+</i>	2640	10.9±1.3	11.0±0.6	11.7±2.4	45.8±1.5	13.3±0.6	(1) <i>y2 v BM1; +</i>
E.	<i>v BM1/y2 w^a v/Y; DcxF/+</i>	3484	21.7±1.5	23.1±0.7	37.0±4.2	48.8±1.3	7.5±0.4	(1) <i>y2 v BM1; +; + (1)y2 v BM1; b en; +</i> (1) <i>y2 v BM1; b en; D</i> (2) <i>y2 w^a BM1; b; +</i>
F.	<i>sc7 w^a/sc f; +/+</i>	2084	10.3±1.8	12.2±0.7	.....	28.4±1.5	.....	.....
G.	<i>sc7 w^a/sc f; DcxF/+</i>	1408	18.6±2.7	20.1±1.1	.....	29.7±1.8	.....	.....
H.	<i>sc7 w^a/y2 w^a cv m f/Y; +/+</i>	4986	10.2±1.5	13.8±0.6	13.8±1.3	29.8±1.2	26.9±0.9	.....
I.	<i>sc7 w^a/y2 w^a cv m f/Y; +/+</i>	1536	9.7±2.0	12.8±0.9	13.1±2.3	30.5±1.7	24.3±1.0	(1) <i>y2 w^a cv; +</i>
J.	<i>sc7 w^a/y2 w^a cv m f/Y; DcxF/+</i>	1644	20.0±2.0	23.1±1.1	34.4±4.6	33.6±1.8	11.8±0.8	(1) <i>sc7 w^a; b; D</i>

reg. Regular offspring: regular females from XX mothers receive one X chromosome from the female and one from the male parent; regular males receive an X chromosome from the female parent and a Y chromosome from the male parent. Regular females and males from XXY mothers derive their X chromosomes as above and may, in addition, receive a Y chromosome from the mother.

X-c.o. reg. Regular males receive a crossover X chromosome, or a crossover X chromosome and a Y chromosome, from the female parent, and a Y chromosome from the male parent.

Except. Exceptional offspring: exceptional females receive two X chromosomes from the female parent and a Y chromosome from the male parent, exceptional males derive their X chromosome from the male parent, and no sex chromosome from the female parent if the mother is XX, and a Y chromosome if the mother is XXY.

changes. Heterozygous dl-49 females are known to give about seven percent crossing over in the uninverted section extending to *car* (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954) in the absence of the *Cy* inversions, and in combination with *Cy* would be expected to give a higher value, and about 3–5 percent XX–O segregations (COOPER, ZIMMERING and KRIVSHENKO 1955).

The results from these experiments are given in Table 2. It may be seen that

TABLE 2

The effect of In(1) dl-49 and/or Ins(2L+2R)Cy, Cy on crossing over in the Gl-Sb region of chromosome 3, and on crossing over and nondisjunction of X chromosomes. All experiments were run simultaneously

Exp.	(1) Constitution of female	(2) Total flies	(3) all reg.	Percent c.o. Gl-Sb in (4) X-c.o.reg.	(5) Except.	Percent c.o. in X (6)	Percent XX-O (females) (7)
K.	$\gamma^2 v f/+; +/+$	2078	13.8 $\pm$ 1.6	12.6 $\pm$ 0.7	.....	50.9 $\pm$ 1.8	.....
L.	$\gamma^2 v f/+; Cy/+$	1961	18.3 $\pm$ 0.9	16.5 $\pm$ 1.5	.....	66.8 $\pm$ 1.6	.....
M.	γ dl-49 $v f car/+; +/+$	2208	17.6 $\pm$ 0.8	16.7 $\pm$ 3.0	1 of 4	7.3 $\pm$ 0.8	0.8 $\pm$ 0.3
N.	γ dl-49 $v f car/+; Cy/+$	11028	25.0 $\pm$ 0.5	23.5 $\pm$ 1.4	26.5 $\pm$ 2.3	13.1 $\pm$ 0.5	3.5 $\pm$ 0.3

the presence of the *Cy* or dl-49 inversions results in an appreciable increase in crossing over in chromosome 3 as measured in the regular offspring (K3,4 *vs.* L3,4 and M3,4), and that an approximate doubling of the control rate is found when both inversions are present (K3,4 *vs.* N3,4). The crossover rates for the γ -*v* and *v*-*f* regions from females with normally arranged X chromosomes which did not carry *Cy* are 30.3 ± 1.5 and 20.6 ± 1.3 , respectively; in the presence of *Cy*, the respective regions show 39.5 ± 1.5 and 27.3 ± 1.3 percent crossing over. For the dl-49 heterozygotes not carrying *Cy*, crossover frequencies in the region from γ to the distal breakpoint of dl-49, and from the proximal breakpoint of the inversion to *car*, are 0.5 ± 0.2 , and 6.8 ± 0.8 , and in the presence of *Cy*, 1.1 ± 0.2 and 12.0 ± 0.5 , respectively. The rate of primary nondisjunction from dl-49 heterozygotes without *Cy* found in the present experiments is in good agreement with those reported by others (STURTEVANT and BEADLE 1936; COOPER 1948), as is the crossover rate for the uninverted section of the X chromosome (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954). In connection with the results from the current experiments, the following points should be noted. (1) Exceptional offspring come from situations in which (a) X chromosomes failed to separate whereas separation in chromosomes 2 and 3 was normal, or (b) the failure of X chromosomes to separate was associated with non-homologous pairing with chromosome 2, resulting in a random separation of the X and of 2 chromosomes (COOPER *et al.* 1955); from the difference between values for nondisjunction of X chromosomes with and without *Cy*, such pairing is inferred to have occurred in 66–87 percent of all tetrads from which exceptionals were derived; while (2) males carrying a crossover X and the vast majority of noncross-

over regulars come from situations in which chromosomes X, 2, and 3 separated normally.

An unexpected finding was the recovery from XXY B^{M1} mothers of ten of the 742 exceptional females which, upon testing, proved to be equational exceptions (Table 1, column 8). The seven $\gamma^2 v B^{M1}$ carried a noncrossover chromatid, $\gamma^2 w^a v$, and a crossover chromatid, $\gamma^2 v B^{M1}$, indicating an exchange in the γ^2 - w^a interval, while the constitution of the X chromosomes of the three $\gamma^2 w^a v B^{M1}$ females was $\gamma^2 w^a v / \gamma^2 w^a v B^{M1}$, indicating an exchange between w^a and B^{M1} . As BRIDGES (1916) first concluded, such crossovers must occur at an effectively four stranded stage. Now, since half the crossover products from such situations give homozygosis for the wild type allele of γ^2 following an exchange between γ^2 and w^a , and for both of these markers following an exchange between w^a and B^{M1} , the inferred frequency of equationals among all exceptional females is 2.7 percent (20/742). Two equational exceptions were found among 1009 exceptional females from the XXY sc^7 series; both proved to be crossovers to the left of m , i.e., between m and the proximal breakpoint of the sc^7 inversion. No correction for undetected crossover products is necessary in these cases since an exchange between γ^2 and the distal breakpoint of sc^7 appears to be a very rare event; hence, the frequency of equationals is inferred to be 0.2 percent (2/1009). The results from experiments such as those with $DcxF$ to determine the effect of inversions in chromosomes 2 and/or 3 on the frequency of equational exceptions from $B^{M1}/+/Y$ females are as yet inconclusive, although it has been found that of a total of 31 equationals recovered, 22 carried crossovers for the very short γ^2 - w^a region.

In addition to his finding that heterozygosity for a single autosomal inversion may increase in the frequency of primary nondisjunction of X chromosomes, STURTEVANT (1944) reported that the rate of secondary nondisjunction is decreased under similar conditions. The effects of autosomal structural heterozygosity on secondary nondisjunction has been under intensive investigation by COOPER, KRIVSHENKO, and ZIMMERING, and the results will be dealt with at length in another publication.

DISCUSSION

The work of SCHULTZ and REDFIELD (1952) and others has shown that marked increases in crossing over may occur in the heterochromatic centromere regions of chromosomes 2 and 3 in the presence of complex structural heterozygosity in non-homologues. The results from the current experiments are in agreement with these findings in that the $DcxF$ inversions result in an approximate doubling of the crossover rate in the centromere region of chromosome 2, and the Cy inversions have a similar effect in the mid-region of 3. That crossing over in the proximal region of the X chromosome is increased in the presence of Cy has also been reported previously (SCHULTZ and REDFIELD 1952). In this paper, the authors present data concerning the effects on crossing over of the Y chromosome alone and in combination with autosomal inversions. They find that the Y

chromosome has little effect when the X chromosomes and autosomes are in normal sequence, or when X chromosomes are in normal sequence while chromosome 2 is heterozygous for the *Pm'* inversion. It should be noted that they measured crossover rates in the *Gl-Sb* region of 3 in exceptionals and regulars and found no significant difference between them although the presence of the *Pm'* inversion resulted in an approximate doubling of the crossover value for this region as compared with that obtained from females which did not carry the inversion. From the results of the current experiments, it seems clear that crossover rates in exceptionals are appreciably higher than those in regulars in the presence of the *DcxF* inversions, and that the frequency of secondary nondisjunction is markedly reduced. In this connection, it would be of interest to determine the effect of the *Pm'* inversion on secondary nondisjunction.

The finding that the exceptional offspring from dl-49; *Cy* heterozygotes show a crossover rate for the *Gl-Sb* region of chromosome 3 at least as high as that from regulars, would seem to indicate that the chromosomal associations between chromosomes X and 2 which generate nondisjunctional X's (and 2's) need not lessen the effect of these structurally heterozygous chromosomes on crossing over in chromosome 3. Furthermore, the absence of a statistical difference for crossing over in chromosome 3 between exceptionals and regular males carrying a crossover X may suggest the absence of any large difference in the kinds of chromosomal pairing configurations preceding regular or irregular chromosome separation in such females. It is supposed that X-autosome pairing leading to nondisjunction takes place when, owing to structural heterozygosity, tendencies for homologous pairing between the X or between the second chromosomes are very weak or absent entirely. That the majority of recovered crossovers for the uninverted proximal region of the X chromosome may come from situations in which a major portion of the X, i.e., from the proximal breakpoint of dl-49 to the γ end, is for the most part unpaired would appear to be a reasonable assumption based on the following considerations. (1) The frequency of exchange within the limits of the dl-49 inversion has been found to be about 10–12 percent (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954). From the report of STEINBERG (1936), the crossover value for the same region, i.e., from *cv* to *v*, from X chromosomes in normal sequence was about 22 percent higher in the presence of *Cy*; this would give an expected 12–15 percent exchange frequency for this region in the current experiments. (2) The exchange frequency between γ and the distal breakpoint of dl-49 was found to be approximately 2.2 percent in the presence of *Cy*; thus, the total exchange value for the region γ to the proximal breakpoint of dl-49 would be expected to be between 14 and 17 percent, or about 20–25 percent of the value when chromosomes are in normal sequence. (3) Crossovers in the uninverted proximal region are, in fact, not recoverable as such if a simultaneous exchange occurs within the limits of the inversion, since the chromatids involved in the latter type of exchange form a dicentric bridge at anaphase I and are not included within the egg nucleus (STURTEVANT and BEADLE 1936; NOVITSKI 1951). (4) It would appear unlikely that the rate of crossing over in chromosome

2 in the presence of *Cy* would be appreciably increased from its low level (less than one percent [BRIDGES and BREHME 1944]) upon the introduction of *dl-49*; it may be assumed, therefore, that chromosome 2 under these conditions is, for the major part of its length, ordinarily unpaired.

On the basis of this line of thought, larger crossover differences between regulars and exceptionals would be expected from cases in which an X chromosome inversion reduced the exchange frequency in the X less drastically, such that the majority of regulars come from situations in which the X chromosomes are more completely paired, and most, if not all of the exceptionals from situations in which homologous pairing between X chromosomes and a pair of autosomes is absent. This would appear to be consistent with the observed discrepancy between these classes found in the *XXY B^{M1}* and *sc^r* series upon the introduction of *DcxF*, assuming that the recovered exceptionals come for the most part from irregular separations following non-homologous pairing. Such a scheme would account also for the approximate 50 percent reduction in secondary nondisjunction if normally an X-Y-X trivalent precedes XX-Y segregation, each arm of the Y being paired with an X (COOPER 1948), and that a minimum of XY-X segregations occur from such a configuration. Thus, if only one arm of the Y chromosome were paired with an X, and either the other arm of the Y, or the other X were paired with chromosome 3, then only half the recovered offspring would be exceptional, the remainder regulars.

In connection with the appearance of equational exceptions in the present experiments, it may be noted that L. V. MORGAN (1937) reported briefly on the recovery of a small percentage of such females which were crossovers for a "distal region" from mothers carrying (presumably) normal X chromosomes, and a deleted X (*Dp 100*; see BRIDGES and BREHME 1944) as a free fragment. What occurs to bring about such an event, and what, if any, the relationship between such an event and the interchromosomal effect on crossing over and nondisjunction may be, is at present unclear.

SUMMARY

From experiments designed to measure simultaneously interchromosomal effects on crossing over and sex chromosome nondisjunction, the following results have thus far been obtained:

1. In *XXY* females heterozygous for a relatively small X chromosome inversion located either distally (*sc^r*) or proximally (*B^{M1}*), crossover rates in the centromere region of chromosome 2 are substantially the same when measured in exceptional and regular offspring.
2. On the other hand, a sizable discrepancy in such rates appears upon the introduction of a third chromosome inversion (*DcxF*), the rate from exceptionals being considerably higher than that from regulars.
3. Perhaps related to this discrepancy is the finding that the rate of secondary nondisjunction is markedly reduced in the presence of the *DcxF* inversions.

4. In the presence of the dl-49 inversion in the X, and the Cy inversions in chromosome 2, crossing over in the centromere region of chromosome 3 did not give a statistically higher rate in exceptionals than in regulars. Speculation is made as to a possible explanation accounting for the appearance of this discrepancy in one case and its absence in the other.

5. A number of equational exceptions were recovered from XXY mothers employed in these experiments; for females heterozygous for B^{M1} , a tally of all available data shows that a majority of such females (22/31) are crossovers for the very short γ^2-w^a region.

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