THE NATURE OF GENETIC RECOMBINATION NEAR THE THIRD CHROMOSOME CENTROMERE OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Previous studies have indicated that recombination near the third chromosome centromere is associated with negative chromosome interference, a phenomenon for which GREEN (1975) and SINCLAIR (1975) suggested gene conversion as a possible mechanism. In this report, we demonstrate that negative chromosome interference is still observed when deficiencies or translocation breakpoints are scored as the middle markers in recombination experiments and the rate of recombination is increased by interchromosomal effect. We argue that these chromosomal rearrangement breakpoints are not subject to conversion. Since neither successive premeiotic and meiotic exchanges, nor negative chromatid interference, can by themselves account for the negative chromosome interference, we conclude that a greater than expected frequency of multiple exchanges actually occurs. We further suggest that negative chromosome interference may be characteristic of all chromosomal regions normally showing very little exchange in relation to physical length.

THE proximal portion of chromosome 3 of *Drosophila melanogaster* is characterized by a low frequency of genetic recombination in proportion to physical chromosome length. The gene *radius incompletus* (*ri*) is located in the left arm at 47.0 on the standard recombination map and in region 77 (or possibly 78) on the polytene chromosome map (ARAJARVI and HANNA-ALAVA 1969). Only 1% recombination normally separates *ri* from *pink* (*p*), which maps in the right arm at 48.0 and has been localized to 85A (DUNCAN and KAUFMAN 1975; ALEXANDER 1975). Thus, the *ri* to *p* interval represents about 15% of the euchromatic portion of the third chromosome (estimated from polytene chromosome numbered units), but is associated with slightly less than 1% of the recombinational map. This region also contains the third chromosome centric heterochromatin, which probably shows little if any recombination (BAKER 1958; ROBERTS 1965).

Reciprocal exchange in Drosophila and other eukaryotes is characterized by positive chromosome interference, and multiple exchanges normally are not observed at all in intervals of 10 cM or less. MORGAN, BRIDGES and STURTEVANT

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(1925) reported that the chromosome 3 centromeric region represents an exception to this generalization; doubles were observed within an interval of only 6 cM long, and a coincidence value of 1.3 was observed.

This early observation of negative interference in the chromosome 3 centromeric region has been confirmed recently by GREEN (1975) and SINCLAIR (1975). Both investigators found apparent multiple exchanges within short intervals, and coincidence values were consistently greater than one. Both GREEN and SINCLAIR suggested that negative interference exists because of the occurrence of gene conversions, which may give products genetically equivalent to those arising from double crossovers. They noted that earlier studies showed that there is no interference across the centromere in Drosophila (GRAUBARD 1934; STEVENS 1936), so that the expectation is that the coefficient of coincidence for classical double exchanges flanking the centromere should be one. The degree to which coincidence was greater than one was considered to reflect the occurrence of conversion events. As other alternatives to a high frequency of real double exchanges, SINCLAIR (1975) suggested that premeiotic exchange and negative chromatid interference could also be responsible for the high coincidence values observed.

We have tested the hypothesis that apparent double exchanges in this region are generated by gene conversion by utilizing genetic markers that should not be subject to conversion. Thus, deficiencies and *Y*-autosome translocations have been used as middle markers in three- and four-point recombination experiments.

MATERIALS AND METHODS

The genetic variants utilized in the experiments are described in LINDSLEY and GRELL (1968) or when first mentioned below. Crosses were routinely performed in half-pint bottles, with about ten females and 15 males per bottle. Parents were subcultured after three to five days, and in some cases subcultured one additional time. Cultures were maintained at 25 ± 1° on standard corn meal-yeast-molasses-agar medium. Recombination frequencies and coefficients of coincidence, and their standard deviations, were calculated using the method of STEVENS (1936).

Recombination with Tpl *deficiencies*: A uniquely dose-sensitive region, the *Triplo-lethal* (*Tpl*) locus, is located in proximal *3R* at 83D–E on the polytene chromosome map (DENELL 1976). The locus is associated with lethality when present either in three doses or in one dose in an otherwise diploid individual. In an analysis of the mutational properties of this locus, KEPPY and DENELL (1979) recently generated a series of deficiencies and *Y*-linked duplications including the locus. Two of these deficiencies, *Df(3R)29c76* (=*Df(3R)83D;84A4,5*) and *Df(3R)30c76* (=*Df(3R)83C1,2;84B1,2*), were used in recombination experiments. (The genotype of the females tested is diagrammed in Figure 1, and the resulting data are presented in Table 1.)

When normal disjunction occurs, the only viable offspring that receive a *Tpl*$^-$ chromosome are males that also receive *Dp(3;Y)11a76* [=*Dp(3;Y)83D*; *84F4+99E1;100F*], marked with B^S; the sequentially normal maternal third chromosome is recovered only in females. Thus, by scoring progeny phenotypes with respect to *ri*, B^S, p^p, and *Sb*, we can recognize apparent exchanges in each of the intervals shown in Figure 1. For two reasons, the resulting crossover frequencies and coefficients of coincidence presented in Table 1 are calculated only from the female progeny. (1) There is a consistent excess of female (with respect to male) offspring among the nonrecombinant classes; we interpret this skewed sex ratio to be due to

reduced viability of males receiving a *Tpl*$^-$ third chromosome and *Dp (3;Y)11a76*. (2) A potential ambiguity associated with these experiments should be noted. The parental females arose from a cross of putative *C(1)M3, y*^{2} *bb*; *Dp(3R)21l73/ri Tpl*$^-$ *p*^{p} females and *Y*^{S}*X·Y*^{L}, *In(1)EN, y/Dp(3;Y)11a76, B*^{S}; *Sb/Df(3R)3g74* males. *Dp(3R)21l73* and *Df(3R)3g74* are respectively, a small reverse duplication and a deficiency of the *Tpl* region (KEPPY and DENELL 1979). A degree of uncertainty is associated with the presence of *ri* and *p*^{p} on the *Tpl*$^-$ chromosomes; in spite of the low frequency of crossing over in this region, which should be reduced still further by the presence of *Dp(3R)21l73*, some of the *Tpl*$^-$ chromosomes may have lost either the *ri* or *p*^{p} marker by an exchange with the duplication-bearing chromosome.

With respect to the basis of negative interference in these experiments, the class of greatest significance has apparent double crossovers in regions 1 (*ri* to *Df*) and 2 (*Df* to *p*^{p}). The frequency of recovery in this class and calculation of coincidence values are little affected by uncertainties associated with the presence of *ri* and *p*^{p} as flanking markers. The rate of crossing over in region 1 is calculated from female progeny that have received *ri*, and thus must have arisen from mothers bearing this allele. Similarly, the crossover frequency of region 2 is calculated from female offspring that receive *p*^{p}. Thus, we can recognize these recombinant progeny unambiguously and reasonably estimate coincidence values. This would not be true if we used data from male offspring as well, since females homozygous for *ri*$^+$ transmit noncrossover chromosomes to their sons, which are scored as having arisen by an exchange in region 1.

In other experiments, we have occasionally observed the loss of the *B*^{S} marker from the *Dp(3;Y)11a76* chromosomal element, presumably due to spontaneous heterochromatic exchanges. Thus, in the present experiments, it is possible that non-Bar females are recovered rarely that have the *Tpl*$^-$ third chromosome and an unmarked free duplication; conversely, males phenotypically Bar could have two *Tpl*-normal third chromosomes and a *Y*-fragment marked with *B*^{S}. In the experiment using *30c76*, all offspring that were putative double recombinants in regions 1 and 2 were progeny tested to determine if their *Tpl* genotype was consistent with respect to the expression of non-Bar or Bar; no case of a breakdown of *Dp(3;Y)11a76* was found. This result suggests that the recovery of breakdown products did not significantly affect the data in the experiment with *29c76*.

In the experiment using *29c76*, crossover progeny were counted and then all offspring were retained and the total number was estimated by the dry weight method of DORN and BURDICK (1962). In order to calculate frequencies based on female data, it is necessary to estimate the number of female noncrossover progeny. Based on the experiment with *30c76* presented here, and an additional recombination experiment using a sequentially normal *Tpl*$^-$ bearing third chromosome, we can estimate that 70% of the nonrecombinant progeny, or 3787, were females.

Recombination with an Antennapedia deficiency: Two additional recombination experiments were performed with *Df(3R)Antp*$^{Ns+R17}$ (=*Df(3R)84B1,2;84D11,12*). This deficiency fails to complement roughened eye (DUNCAN and KAUFMAN 1975), and the segregation of the deficiency in heterozygous females was followed by crossing to males homozygous for *roe*.

Recombination with Y*-autosome translocations*: Additional recombination experiments were performed using two *Y*-autosome translocations: *T(Y;3)J139* and *T(Y;3)B155*, which have third chromosome breakpoints at 80 and 82C, respectively (LINDSLEY *et al.* 1972). These crosses follow the transmission of four chromosomal elements: the compound *X* chromosome, the sequentially normal third chromosome, and the two translocation elements. In each cross, approximately one quarter of the progeny arose from a meiocyte in which three of these elements passed to one pole and one element to the other at the first meiotic division (3:1 disjunctions); in the remaining cases, two chromosomes passed to each pole (2:2 disjunctions). Since an examination of the results with a contingency χ^2 test showed no differences in the frequencies of crossover progeny arising from maternal 2:2 and 3:1 disjunctions, all data were pooled to calculate the crossover frequencies and coincidence values.

Ki is known to be localized in chromosome *3R* (MERRIAM and GARCIA-BELLIDO 1969) proximal to *Antennapedia* (GREEN 1975), and our crosses show that *Ki* is clearly distal to the chromosome *3* breakpoint of *T(Y;3)B155* (see RESULTS).

In one bottle of the *T(Y;3)B155* cross (denoted mating 6), a large number of phenotypically y^2 *ri Ki* flies were recovered. This class is expected to arise from an exchange in region 3, but occurred in about six times the expected frequency. The reciprocal class (B^S p^p progeny) was recovered in the proportion expected. It seems likely that most y^2 *ri Ki* flies arose from an exceptional event, possibly a premeiotic exchange in a parental female. Thus, the results from mating 6 have been excluded from those of the remainder of the *T(Y;3)B155* cross.

In the crosses involving *Y*-autosome translocations, we intended to progeny test all male flies recovered from apparent multiple exchanges to confirm their genotypes. Because of the *Y*-chromosome translocation breakpoints, males bearing either of the translocations are sterile in the absence of additional *Y* chromosomes. The cross presented in Figure 3 was designed to insure male fertility by the presence of a compound-*XY* chromosome. Nevertheless, all males tested were sterile, presumably because of a genetic breakdown of $Y^SX{\cdot}Y^L$ in the stock yielding parental males.

RESULTS

A number of crosses were performed to characterize the recombinational behavior of the chromosome *3* pericentric region in the presence of chromosomal rearrangements. In the initial phase of this study, recombination was examined in females heterozygous for either *Df(3R)29c76* or *Df(3R)30c76*. Maternal genotypes are diagrammed in Figure 1, and the resulting data are presented in Table 1. Each of the deficiencies includes the *Triplo-lethal* region located at polytene chromosome position 83D–E, and an additional dose of this region, carried by *Dp(3;Y)11a76*, is necessary for viability (KEPPY and DENELL 1979). The parental females also carried *C(1)M3*, which increases the frequency of exchange near the chromosome *3* centromere by interchromosomal effect (SINCLAIR 1975). In spite of the presence of the deficiencies *29c76* and *30c76*, we found that *ri* and p^p were separated by 4.50 cM and 2.22 cM, respectively, whereas SINCLAIR (1975) found a value of 1.92 cM in *C(1)M3* females with sequentially normal third chromosomes. This region gives variable rates of recombination depending on genetic background (HANNAH-ALAVA 1969), so that without isogenic control crosses we cannot confidently interpret the effects of these relatively large deficiencies on recombination between *ri* and p^p. In any

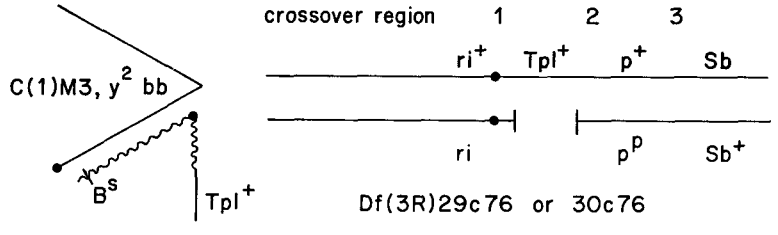

FIGURE 1.—The first and third chromosomal complements are diagrammed for females used in the crossover experiments presented in Table 1. The *Y* chromosome is depicted by a wavy line, and closed circles indicate centromeres.

TABLE 1

Crossing over in females bearing C(1)M3, y^2 bb/Dp(3;Y)11a76, B^S; Sb/ri Tpl$^-$ p^p *mated to* $XY^S \cdot Y^L$, y^2 su-w^a w^a $Y^S \cdot Y^L$, y+/Y; ri p^p/ri *males*

Crossover interval	Progeny phenotype		*N* for crosses with the indicated *Tpl*$^-$ chromosome *29c76*	*30c76*
0	y^2 + + + *Sb* ♀		3787*	1955
	+ *ri* B^S p^p + ♂		1623*	861
1	y^2 *ri* + + *Sb* ♀		119	31
	+ + B^S p^p + ♂		166	80
2	y^2 + + p^p + ♀		42	5
	+ *ri* B^S + *Sb* ♂		16	3
3	y^2 + + + + ♀		569	196
	+ *ri* B^S p^p *Sb* ♂		246	114
1,2	y^2 *ri* + p^p + ♀		3	2
	+ + B^S + *Sb* ♂		5	2
1,3	y^2 *ri* + + + ♀		36	8
	+ + B^S p^p *Sb* ♂		36	8
2,3	y^2 + + p^p *Sb* ♀		2	1
	+ *ri* B^S + + ♂		9	1
		Σ	6659	3267
	Crossover frequencies ± SD (cM)†			
1			3.47±0.27	1.86±0.29
2			1.03±0.15	0.36±0.13
3			13.32±0.50	9.33±0.62
		Σ	17.82	11.55
	Coincidence ± SD†			
1,2			1.84±1.10	13.40±8.13
1,3			1.71±0.24	2.09±0.65
2,3			0.32±0.22	1.34±1.26

* In this experiment, the total number of progeny was estimated by dry weight; thus the number of male and female noncrossover classes was estimated as described in the MATERIALS AND METHODS.

† For reasons discussed in the text, these values were calculated from the female data only.

event, the genetic background of *29c76* and *30c76* should be very similar, and as expected there is a lower frequency of exchange with the larger deficiency (*30c76*) than with the smaller one.

In both experiments presented in Table 1, negative interference obtains for all classes in which double exchanges flank the deficiencies. That is, the occurrence of a higher than expected frequency of multiple exchanges previously described in this region is still observed despite the presence of relatively large deficiencies as middle markers.

The crosses used in these initial experiments were genetically complex, and many progeny classes were not recovered because of inviability due to aneuploidy involving the Triplo-lethal region or the sex chromosomes. In order to

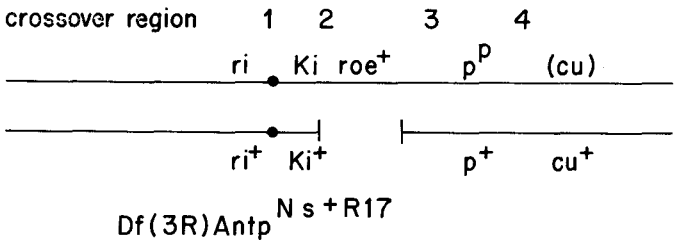

FIGURE 2.—The third chromosome complement is diagrammed for females used in the crossover experiments presented in Table 2.

examine a more straightforward genetic system, similar recombination experiments were performed using females with a normal sex chromosome complement and heterozygous for another proximal deficiency, *Df(3R)Antp*$^{Ns+R17}$. The maternal genotype is shown diagrammatically in Figure 2, and the resulting data are presented in Table 2. In the absence of an interchromosomal effect, the recombinational values between *ri* and p^p were 0.71 cM and 0.51 cM in the two experiments performed. No progeny resulting from multiple exchange were recovered.

Recombination was further examined in females bearing *C(1)M3* and *T(Y;3)J139* or *T(Y;3)B155*, which have chromosome *3* breakpoints in the centric heterochromatin and at 82C, respectively. The genotype of the *B155* females is presented diagrammatically in Figure 3, and the data from both crosses is presented in Table 3. A considerable amount of exchange occurs in the pericen-

TABLE 2

Crossing over in ri Ki p^p/Df(3R)Antp^{Ns+R17} *(I) and* ri Ki p^p cu/Df(RR)Antp^{Ns+R17} *(II) females crossed to* ri roe p^p *and* ri roe p^p cu *males, respectively*

Crossover interval	Progeny phenotype		N(I)	N(II)
0	*ri Ki* + p^p (*cu*)		3131	7047
	+ + *roe* + (+)		2717	7639
1	*ri* + *roe* + (+)		21	26
	+ *Ki* + p^p (*cu*)		15	33
2	*ri Ki roe* + (+)		1	0
	+ + + p^p (*cu*)		0	1
3	*ri Ki* + + (+)		0	5
	+ + *roe* p^p (*cu*)		5	11
4	*ri Ki* + p^p +		–	96
	+ + *roe* + *cu*		–	83
		Σ	5890	14,941
	Crossover frequencies ± SD (cM)			
1			0.61±0.10	0.39±0.05
2			0.02±0.02	0.01±0.01
3			0.08±0.04	0.11±0.03
4			–	1.20±0.09

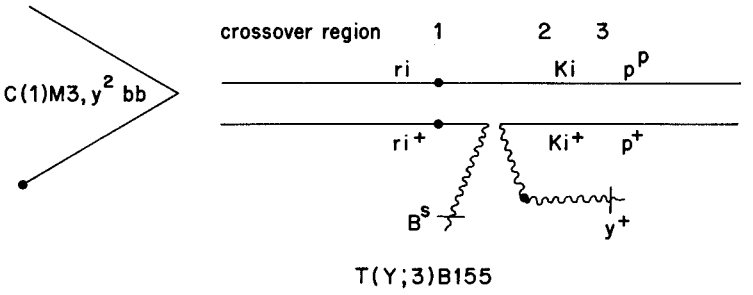

FIGURE 3.—The first and third chromosomal complements are diagrammed for females bearing *T(Y;3)B155*, used in the crossover experiments presented in Table 3.

tric region in the presence of these two translocations. For double exchanges in which the translocation breakpoints were scored as middle markers, all classes but one show negative chromosome interference.

DISCUSSION

Negative interference near the chromosome *3* centromeric region was first reported by MORGAN, BRIDGES and STURTEVANT (1925), who found a coefficient of coincidence of 1.3 for crossing over in this region. Both GREEN (1975) and SINCLAIR (1975) have recently confirmed this earlier observation, and have suggested that negative interference may arise because apparent multiple crossover classes are generated by gene conversion. Alternatively, SINCLAIR (1975) suggested that successive premeiotic and meiotic exchanges (the "two-step" mechanism) or negative chromatid interference could also be responsible for the high coincidence values observed. Although in some respects SINCLAIR's data were consistent with a two-step mechanism, an analysis of the progeny of individual females did not fulfill the prediction that there should be clusters of singles in regions where doubles were observed. In the present investigation, the progeny of individual females were not followed. In one case (mating 6, Table 3), however, an apparent cluster arising from a premeiotic exchange was recognized and the data from this bottle were excluded. Under the mating regime utilized here, smaller clusters would not be recognized. Nevertheless, based on SINCLAIR's (1975) data, we conclude that the two-step mechanism does not provide a sufficient explanation for the negative chromosome interference characteristic for the region.

No evidence exists concerning the presence or absence of negative chromatid interference in proximal chromosome *3*. If this phenomenon does occur, it would generate an excess of two-strand doubles and thus raise coincidence values. However, the high negative chromosome interference in proximal chromosome *3*

TABLE 3

Crossing over in females bearing C(1)M3, y^2 bb; ri Ki p^p/T(Y;3)J139, y^+ B^S *or* T(Y;3)B155, y^+ B^S *mated to* $Y^SX \cdot Y^L$, In(1)EN, y; ri roe p^p/ri roe p^p *males*

Crossover interval	Progeny phenotype	*T(Y;3)J139* *N*	*T(Y;3)B155* *N*†	*T(Y;3)B155* *N* (mating 6)
0	y^2 + *ri Ki* p^p ♀	1935	4098	348
	+ B^S + + + ♂	1594	4124	362
	+ B^S + + + ♀*	420	1360	97
	y + *ri Ki* p^p ♂*	586	2047	137
1	y^2 + + *Ki* p^p ♀	47	98	4
	+ B^S *ri* + + ♂	20	74	3
	+ B^S *ri* + + ♀*	7	42	3
	y + + *Ki* p^p ♂*	17	39	3
2	y^2 + *ri* + + ♀	26	26	1
	+ B^S + *Ki* p^p ♂	4	24	2
	+ B^S + *Ki* p^p ♀*	2	13	0
	y + *ri* + + ♂*	2	16	4
3	y^2 + *ri Ki* + ♀	33	77	37
	+ B^S + + p^p ♂	12	60	6
	+ B^S + + p^p ♀*	3	18	1
	y + *ri Ki* + ♂*	5	32	14
1,2	y^2 + + + + ♀	0	1	0
	+ B^S *ri Ki* p^p ♂	4	2	0
	+ B^S *ri Ki* p^p ♀*	0	1	0
	y + + + + ♂*	0	2	0
1,3	y^2 + + *Ki* + ♀	0	1	2
	+ B^S *ri* + p^p ♂	1	7	0
	+ B^S *ri* + p^p ♀*	0	0	0
	y + + *Ki* + ♂*	0	1	0
2,3	y^2 + *ri* + p^p ♀	2	0	0
	+ B^S + *Ki* + ♂	3	0	0
	+ B^S + *Ki* + ♀*	0	0	0
	y + *ri* + p^p ♂*	0	0	1
		Σ 4723	12163	1025
	Crossover frequencies ±SD(cM)			
1		2.03±0.21	2.20±0.13	
2		0.91±0.14	0.70±0.08	
3		1.25±0.16	1.61±0.11	
		Σ 4.19	4.51	
	Coincidence ± SD			
1,2		4.58±2.14	3.20±1.25	
1,3		0.83±0.82	2.08±0.67	
2,3		9.31±3.76	0	

* Progeny arising from maternal 3:1 disjunctions.

† Data from mating 6 not included.

cannot be attributed solely to negative chromatid interference. Compared to a case in which two-, three- and four-strand double-exchange tetrads are in the ratio of 1:2:1 and coincidence is one, the coincidence value is only two if all double-exchange tetrads involve two-strand events. Coincidence values observed by SINCLAIR (1975) and in the present report were often higher than two, and sometimes dramatically so.

Since it appears that neither the two-step mechanism nor negative chromatid interference provides a good explanation for the negative chromosome interference observed, it is the main purpose of this report to examine the hypothesis that the excess of apparent doubles occurs because of gene conversion of middle markers. As shown in Table 1, negative interference is present in recombination experiments involving either *Df(3R)29c76* or *Df(3R)30c76*. If the coincidence values are higher than one because of gene conversion, it is necessary to assume that these deficiencies can be converted to wild type. Conversions of intragenic deletions of several hundred base pairs have been reported in yeast (FINK and STYLES 1974). The two deficiencies used here delete about 20 and 35 bands, based on BRIDGES' (1941) polytene chromosome map. Using BEERMANN's (1972) estimate of 30,000 base pairs per average band, these deficiencies are roughly 6×10^5 and 1×10^6 base pairs in length. CHOVNICK *et al.* (1973) estimate from studies of co-conversion in Drosophila that the maximum length of chromosome involved in a conversion event is several hundred base pairs. Thus, conversions of deficiencies of the size of *29c76* and *30c76* would be inconsistent with previous studies of such nonreciprocal events, and gene conversion can be rejected as the sole basis of negative interference in the proximal third chromosome.

An attempt was also made to examine exchange in the presence of *Df(3R)* $Antp^{Ns+R17}$. No interchromosomal effect was present, and in two experiments this heterozygous deficiency is associated with only 0.71 % and 0.51% recombination between *ri* and p^p (Table 2). Based on the assumption of no chromosome interference, progeny arising from a double exchange within the *ri*—p^p interval and flanking the deficiency are expected in a frequency of 5×10^{-6} for experiment I, and no such progeny were observed among 5,890 offspring. In experiment II, double exchanges flanking the deficiency and within the *ri*—*cu* interval are expected with a frequency of 5×10^{-5}, and again no such progeny were recovered among 14,941 offspring. Thus, the frequencies of exchange were so low in these experiments that the recovery of progeny from double exchanges is unlikely, even if negative chromosome interference were present.

NOVITSKI (1975) has performed experiments examining recombination in the presence of two different *Y;3* translocations broken in the third chromosome centric heterochromatin. For *ru h st* p^p *ss e/T(Y;3)A85* females, seven progeny were recovered from double crossovers in regions immediately flanking the translocation breakpoint, whereas only 0.34 progeny are expected in the absence of interference. In an analagous experiment with *T(Y;3)G101*, 0.49 double-exchange progeny were expected and none were observed.

In further experiments, recombination was examined in the vicinity of the breakpoints of *T(Y;3)J139* and *T(Y;3)B155* (Table 3). Negative chromosome interference was once again observed when the third chromosome translocation breakpoint was scored as a marker. It seems unlikely that such translocation breakpoints are subject to conversion. Most molecular models of conversion (see CATCHESIDE 1977) would require that the two different DNA helices forming the portions of chromosome 3 on either side of the breakpoint simultaneously denature and that a strand from the structurally normal chromosome base pair across the gap; subsequent degradation and repair synthesis would then yield an intact, nontranslocated DNA helix. In the absence of any independent evidence supporting the occurrence of this type of conversion event, we will assume that translocation breakpoints are not subject to conversion.

It is interesting to note that the translocation breakpoints cause no dramatic decrease in the rate of proximal recombination, which is more than 4% between *ri* and p^p in both experiments. These females should be subject to the interchromosomal effect of *C(1)M3*, and the results are similar to those from females carrying this compound chromosome and *Df(3R)29c76*. While translocation breakpoints in the middle of chromosome arms greatly inhibit recombination in their vicinity, those breakpoints near the tips and bases have little inhibitory effect (ROBERTS 1976). Moreover, BROWN (1940) and THOMPSON (1964) have observed that in the presence of translocations with proximal breakpoints, recombination near autosomal centromeres is normal or increased relative to control values.

We have argued that high coincidence values near the chromosome 3 centromere cannot be explained solely by the two-step mechanism, by negative chromatid interference, or by gene conversion. While any of these mechanisms could still contribute to high coincidence values, we favor the interpretation that multiple exchanges actually occur more frequently than would be predicted from the total frequency of exchange. The basis for this genetic behavior is presently unclear; but, as suggested by SINCLAIR (1975), negative interference may in some way be characteristic of regions in the genome where the rate of recombination is low. Although one of the most basic premises of recombinational mapping is that there is a constant probability of exchange per unit physical length of chromosome, it has been pointed out many times that this premise is not true in *Drosophila melanogaster*. First, little or no exchange occurs in the centric heterochromatin of the *X* chromosome (BAKER 1958; ROBERTS 1965) or probably of the major autosomes. Moreover, exchanges are not equally likely along the euchromatic portions of the major chromosomes. LINDSLEY and SANDLER (1977) have recently reexamined this phenomenon by calculating probabilities of exchange per unit physical length (termed "coefficients of exchange") for all chromosomes. Both major autosomes have low coefficients of exchange proximally, with this parameter increasing more-or-less symmetrically in the two arms to reach an approximately maximal value from the midpoints of the arms to the tips. The *X* chromosome shows a maximal exchange rate in the middle, with a rather severe distal reduction of exchange and a

reduction near the centric heterochromatin that is not nearly as marked as in the autosomes. Chromosome *4* normally does not undergo crossing over.

The physical and biochemical factors that act to constrain exchange near centromeres and/or enhance its frequency in the middle of chromsome arms are not known. However, it has been noted in a number of experimental contexts that perturbations of normal meiotic conditions tend to alter the distribution of exchanges so that differences in the probability of exchange per unit physical distance are reduced. Thus, although in some cases the overall frequency of recombination is lower, there is a proportional increase of exchanges in centric regions or in chromosome *4* relative to medial chromosomal intervals. Further, in several cases where multiple exchanges can be recognized, increased frequency of exchange is accompanied by negative chromosome interference.

As first observed by PLOUGH (1917), females placed at temperature extremes display an altered frequency and distribution of exchanges. More recently, GRELL and DAY (1974) have carefully examined the effects of elevated temperatures on recombination. The effects of heat shock vary with the stage treated and the chromosomal interval being examined. When affected by temperature, most regions show an enhancement of recombination frequency, and the increase is most dramatic in regions near centromeres and in chromosome *4*. GRELL and DAY (1974) also examined the effect of heat shock on coincidence values. Alterations of coincidence values varied depending on the stage treated and the intervals studied. One might expect that an increase in the recombinational length of a chromosome segment would be accompanied by a decrease in interference, but there was no correlation of the effect of heat on interference with its effect on crossover frequencies. For the *X* chromosome, the largest heat-induced increases in the coefficient of coincidence were observed in the most proximal and distal pairs of adjacent regions. Whereas the control coincidence value for the most proximal pair of intervals was zero, heat treatment increased coincidence to more than one. In a more recent study, GRELL (1978) also examined exchange near the chromosome *2* centromere. An analysis of these data (her Table 1) shows that heat-induced recombination in the intervals examined (*Bl* to *lt* and *lt* to *stw*) is likewise characterized by negative chromosome interference.

A number of other experiments involving a perturbation of normal meiotic conditions are associated with a relative increase in exchange in regions normally showing low coefficients of exchange. This phenomenon has been observed in triploid females for both distal and proximal regions of the *X* chromosome (see SCHULTZ and REDFIELD 1951) and for the proximal regions of chromosomes *2* and *3* (REDFIELD 1930, 1932). The interpretation of coincidence values in triploids is difficult, however. A more clearcut example is crossing over in chromosomes *4* of diplo-4 triploid females (STURTEVANT 1951). Three percent crossing over was observed between the most widely separated markers (*ci* and *sv*), and exchange was characterized by negative chromosome interference.

A similar pattern of increase is observed in the case of interchromosomal effect caused by inversions in other chromosomes (*cf.*, SCHULTZ and REDFIELD 1951). Unfortunately, with respect to the regions close to centromeres in which we would like to examine coincidence, the earlier studies did not utilize appropriate markers or the data were not presented in a suitable form. Both GREEN (1975) and SINCLAIR (1975) examined the impact of interchromosomal effect on coincidence values in proximal chromosome 3. In neither case was the increase in crossing over accompanied by an increase in multiple exchanges; in fact, there may be some trend toward a reduction in multiple exchanges. However, the data of SUZUKI and PARRY (1962) suggest an increase in negative interference when recombination frequencies in this region are increased by interchromosomal effect.

A number of meiotic mutants cause an increase in crossing over in regions normally characterized by low coefficients of exchange relative to those with high coefficients, although the total rate of recombination is often diminished (see BAKER and HALL 1976). These data do not comment on the nature of interference in regions immediately adjacent to centromeres, however, as markers were not appropriately chosen or insufficient exchange occurred in the region of interest.

In spite of the many studies on crossing over in Drosophila performed over the years, we have a paucity of data examining the regions of interest in an interpretable manner. Because the studies examining the regions immediately flanking centric heterochromatin have not simultaneously followed the overall distribution of exchanges, we do not know if the negative chromosome interference observed is due in part to a redistribution of exchanges from more medial to more proximal or (possibly in the case of the *X* chromosome) more distal regions. GRELL and DAY (1974) suggested such a redistribution as a mechanism explaining the differential regional effects of heat shock. On the other hand, the results of GREEN (1975) and SINCLAIR (1975) indicate that negative chromosome interference is a normal characteristic of the proximal third chromosome, and does not result from the local increase in exchange due to interchromosomal effect. Thus, it may be that negative chromosome interference is a normal characteristic of all regions with a very low coefficient of exchange. One way to visualize this phenomenon is based on a modification of the "effective pairing" model of PRITCHARD (1960), which was devised to account for negative chromosome interference observed in very short regions. In its original form, this model suggested that a given region is infrequently available for exchange ("effectively paired"), but that when it is available, crossing over is characterized by multiple exchanges. Thus, in a very different context, we suggest that when regions with very low coefficients of exchange do undergo crossing over, the state of pairing of these regions generates negative interference.

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