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Crossing over between closely linked markers spanning the centromere of chromosome 3 in *Drosophila melanogaster**†

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SUMMARY

Recombination in a short genetic interval spanning the proximal region of chromosome 3 was studied in the regions *st-in-ri-eg*^{2}-*Ki-p*^{p}. Crossover frequencies in this region varied considerably in different genetic backgrounds; however, in all genotypes, the following observations were made: (1) an excess of multiple recombinant chromosomes indicative of high negative interference, was detected; (2) among the multiple recombinants, a positive correlation of simultaneous exchange in the most proximal and shortest adjacent genetic intervals was noted; (3) several classes of reciprocal products were not equally recovered. Three possible explanations for these results are: pre-meiotic exchange, chromatid interference and gene conversion.

I. INTRODUCTION

The proximal heterochromatin of the major chromosomes of *Drosophila melanogaster* has perennially aroused the interest of geneticists. Experiments have indicated that crossing over in heterochromatin is unlikely in this organism (Baker, 1958; Roberts, 1965), although exceptions have been reported for the *bobbed* locus (Ritossa, Atwood & Spiegelman, 1966).

The study of crossing over in proximal regions of chromosomes of Drosophila has yielded puzzling results. For example, it has been observed that while the centric region makes up 20% of the mitotic length of chromosome 3 cytologically, it constitutes only 1% of the total genetic length (Dobzhansky, 1930; Painter, 1935). This early evidence suggested that little or no crossing over occurs in heterochromatin. Beadle (1932) explored this problem by studying recombination in females heterozygous for translocations between chromosomes 3 and 4. He found that crossing over between markers decreased upon their displacement to a more proximal position, thereby suggesting that some sort of inhibitory effect of the centromere normally exists. More recently, Thompson (1963*a*, *b*) proposed the

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possibility that exchange pairing of the centromeres was unique in that initial attraction could be followed by subsequent localized centromeric repulsion just prior to exchange. Accordingly, this would account for the observed decrease in crossing over between loci adjacent to the centromere.

Another noteworthy property of proximal crossing over is that recombinagenic agents preferentially tend to increase exchange in or adjacent to proximal heterochromatin (Plough, 1917; Muller, 1926; Schultz & Redfield, 1951; Suzuki & Parry, 1964). Early studies of crossing over in *Drosophila* showed that concurrent exchange within closely linked regions in different arms of the same chromosome was independent (Graubard, 1934; Stevens, 1936). This has also been confirmed for Neurospora (Bole-Gowda, Perkins & Strickland, 1962) and for yeast (Hawthorne & Mortimer, 1960) and it contrasts with positive interference for exchange in adjacent intervals of the same chromosome (Morgan, Sturtevant & Bridges, 1925). However, Morgan *et al.* (1925) found coincidence values of 1·3 for crossing over near the centromere of chromosome 3 of *Drosophila*, an observation suggesting that negative interference exists in this region.

Recently, the correct location of the centromere of chromosome 3, relative to the position of loci known to be tightly linked, has been ascertained. Thus, *radius incompletus* and *inturned* have been assigned to the left arm (Arajävi & Hannah-Alava, 1969); *Kinked* to the right of the centromere (Merriam & Garcia-Bellido, 1969); and *eagle* to the left arm and *Deformed* to the right (Holm *et al.* 1969). The unequivocal left and right localization of these genes adds a new dimension to any work with recombination in these regions. Consequently, an intensive study of crossing over in the centrically-adjacent intervals of chromosome 3 was initiated in order to further characterize proximal exchange events.

2. MATERIALS AND METHODS

Recombination was measured in the proximal region of chromosome 3 using the following markers (for a complete description, consult Lindsley & Grell, 1968): *st*, *scarlet* (44·0); *in*, *inturned* (47·0); *ri*, *radius incompletus* (47·0); eg^2, *eagle-2* (47·3); *Ki*, *Kinked* (47·6); and p^p, *pink peach* (48·0). Fig. 1 is a schematic representation of the map positions of the markers along the chromosome (Lindsley & Grell, 1968). The centric blocks of heterochromatin are believed to be immediately flanked by *eagle* (Holm *et al.* 1969), and *Kinked* (Merriam & Garcia-Bellido, 1969) on the left and right respectively. The *st-in* interval was designated as 1, *in-ri* as 2, *ri-*eg^2 as 3, eg^2-*Ki* as 4 and *Ki-*p^p as 5. Note that the centromere lies in interval 4.

Three different studies of crossing over were performed: Experiment I, one hundred *st in ri* eg^2 *Ki* p^p/+ + + + + + females were test-crossed for 5 consecutive 3-day broods and their progeny scored. Experiment II, crossing over was similarly measured in 213 *st in ri* eg^2 + +/+ + + + *Ki* p^p females; 108 of these were studied for five 3-day broods (Expt. II*a*) and 105 for one 3-day brood (Expt. II*b*). Experiment III, crossing over was measured in 25 *C(1)M3*/*Y*;*st in ri*

eg^2 + +/+ + + + $Ki\ p^p$ females for five 3-day broods. Expt. III females were tested in order to determine any interchromosomal effects of the inversions contained in each arm of the compound X (Lucchesi & Suzuki, 1968).

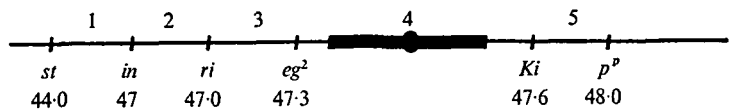

Fig. 1. Schematic representation of proximal region of the third chromosome showing the genetic markers used. Published map positions are given below the symbols with numerically designated crossover intervals indicated above the line.

All females tested were mated individually (within 40 h of eclosion at 22 ± 0·5 °C) with 2 or 3 males homozygous for *st in ri* eg^2 $Ki\ p^p$ e^s. The third chromosomes of all females were isogenized prior to use in order to minimize the presence of lethals in the stocks. However, the other chromosomes were not made coisogenic and the $Ki\ p^p$ chromosome of Expt. II*b* females was of a different origin from that of Expt. II*a* females. Progeny in each vial were scored until the eighteenth day after the parents had been introduced.

RESULTS

A summary of the numbers of progeny examined and the crossover values for the region studied (including published map distances, Lindsley & Grell, 1968) is given in Table 1. Data for females carrying normal rod X's (columns 2 and 3, Table 1) reveal that recombination was consistently higher in Expt. I than in Expt. II ($\chi^2 = 108{\cdot}9$, $P = 0{\cdot}05$). These differences probably reflect random differences in genetic backgrounds in the two series. The insertion of *C(1)M3* into test females (columns 4 and 5, Table 1) noticeably augmented recombination, thereby reconfirming its interchromosomal effects on crossing over near proximal heterochromatin. These effects were more prominent for the distal-most intervals; for example, recombination in region 1 and 5 increased 3- and 4-fold, respectively.

Table 2 summarizes the number of different crossover chromosomes recovered. A total of 3603 single, 85 double and 20 triple crossover chromosomes was scored. The most frequent class of doubles occurring in Expts. I and II involved regions 1 and 5 (nearly a third of the total) and 3 and 4 (more than a third of the total). Other doubles frequently recovered were 3, 5 and 1, 4. Although double crossovers in those regions known to be on the same side of the centromere (1, 2 and 3) were never recovered, 1, 2, 4 and, 1, 3, 4 triple crossovers, which included two of these regions, were encountered.

With respect to double crossover chromosomes involving the two distal-most regions (1 and 5) lying on opposite sides of the centromere, these data appear similar to those arising from earlier work in *Drosophila* (Graubard, 1934; Stevens, 1936), in *Neurospora* (Bole-Gowda *et al.* 1962) and yeast (Hawthorne & Mortimer,

Table 1. *Crossover frequencies in the* st *to* p^p *interval in chromosome 3*

Genetic region	Reference values	Experiment number I	Experiment number IIa rod X	Experiment number III C(1)M3	Ratio III/IIa
1	3·0	3·99	1·77	5·39	3·05
2	0·06	0·25	0·20	0·30	1·50
3	0·30	0·21	0·08	0·17	2·13
4	0·30	0·43	0·22	0·64	2·90
5	0·40	0·65	0·25	1·11	4·44
Number of fertile females	—	92	108	25	—
Number of progeny	—	36948	33139	4063	—

Table 2. *Type and numbers of recombinant chromosomes recovered*

Region	Experiment number I	IIa	IIb	III
Singles				
1	1393	574	217	547
2	71	63	12	13
3	27	17	4	30
4	102	50	24	58
5	183	64	41	114
Totals	1775	768	298	762
Doubles				
1, 2	0	0	0	0
1, 3	0	0	0	0
1, 4	3	2	0	1
1, 5	14	8	1	2
2, 3	0	0	0	0
2, 4	2	0	0	1
2, 5	4	0	0	1
3, 4	22	6	1	4
3, 5	5	2	2	1
4, 5	0	3	0	0
Totals	50	21	4	10
Triples				
1, 2, 3	0	0	0	0
1, 2, 4	3	0	0	0
1, 2, 5	0	0	0	0
1, 3, 4	2	2	0	0
1, 3, 5	0	0	0	0
1, 4, 5	4	4	2	2
2, 3, 4	0	0	0	0
2, 3, 5	0	0	0	0
2, 4, 5	0	0	0	0
3, 4, 5	1	0	0	0
Totals	10	6	2	2

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1960), suggesting that positive interference does not extend to regions in different arms of the chromosomes.

Of the 20 triple crossover chromosomes scored, 12 involved exchanges in regions 1, 4 and 5. Furthermore, all of the triples recovered had a crossover in the most proximal interval (eg^2 to *Ki*).

Table 3. *Coefficients of coincidence computed from all multiples recovered*

	Experiment number		
Intervals	I	II*a*	III
1, 2	0·81	—	—
1, 3	0·64	4·25	—
1, 4	1·90	6·21	1·43
1, 5	1·90	8·17	1·23
2, 3	—	—	—
2, 4	12·50	—	—
2, 5	6·75	—	—
3, 4	75·20	137·16	22·36
3, 5	11·90	30·00	49·20
4, 5	4·83	38·40	6·93

Coefficients of coincidence were calculated for doubles in all three experiments (excluding Expt. II*b*). In all cases but 1, 2 and 1, 3 doubles of Expt. I, these values exceeded unity (Table 3). Extremely high values for 3, 4 and 4, 5 exchanges (and 3, 5 exchanges for Expt. III) indicate a very high negative interference in these intervals. Therefore, in spite of very tight linkage between *st* and p^p, the recovery of multiple crossover chromosomes greatly exceeds conventional expectations. It is noteworthy that while single exchanges were increased in all intervals by *C(1)M3*, a concomitant increase in the occurrence of multiple exchanges (except for 3, 5 doubles) as attested to by coincidence values, did not occur.

Previous workers have suggested that some rare multiple exchange chromosomes could, in fact, result from successive single crossover events. Thus, a mitotic crossover in a gonial cell could be followed by a meiotic exchange to produce an apparent double crossover chromosome (Whittinghill, 1955; Suzuki, Baillie & Parry, 1966). Such a Two-Step model predicts that the gonial exchange could be amplified through mitotic divisions, thereby generating doubles amidst a cluster of single crossovers (Suzuki *et al.* 1966). The progeny of individual females yielding double exchanges (but not triples) were examined for evidence of clustering of single crossovers in the regions where the doubles had occurred. A sample of 5 such females (Expt. I) is given in Table 4 (along with mean values for females yielding no multiple exchange progeny). No noticeable clusters of singles appeared to accompany doubles for the regions in question.

If multiple recombinant chromosomes are generated by a Two-Step mechanism, then crossover values for multiple-producing females would be expected to be higher than the values from females producing no multiples. These subsets of data were significantly different (Table 5) and in both experiments, crossover values

for regions 3 and 4 were higher in those females producing crossovers. However, when the crossover data of the nine females of Expt. I that had produced triple recombinant progeny (within the *st* to p^p interval) were examined, in each case the distribution of crossover types followed a Poisson distribution (Table 6).

Table 4. *Interval-specific examination of data of females producing double recombinant chromosomes (Experiment I)*

Female	Type of double	Number of doubles	Singles occurring in either interval	Total number of progeny
J	1, 5	1	23 (16·8)*	391 (360)
K	3, 4	2	2 (1·3)	388 (360)
L	2, 4	1	3 (1·6)	465 (360)
M	3, 5	2	2 (2·6)	466 (360)
N	1, 4	1	13 (15·6)	429 (360)

* Numbers in parentheses represent mean value of comparable data for 52 non-multiple females.

Table 5. *Crossover values in females producing multiples compared to those in females showing none*

	Female type			
	Multiples		No multiples	
	Expt. I	Expt. II*a*	Expt. I	Expt. II*a*
Number of females	42	24	52	85
Number of progeny	19284	7111	15378	26029
Crossover values Intervals				
1	4·09	1·65	3·71	1·76
2	0·28	0·22	0·17	0·18
3	0·26	0·21	0·07	0·05
4	0·53	0·36	0·24	0·15
5	0·56	0·40	0·59	0·20

Significant difference for χ^2 was indicated for a subset comparison of both experiments at $P = 0{\cdot}01$.

A tetrad analysis, as inferred from single strand recovery (Weinstein, 1936), was initiated with a view to distinguishing between a meiotic and a gonial origin of the triple exchanges (Table 7). The Two-Step production of rare multiple exchange chromosomes was inferred from an insufficient number of double exchange chromosomes predicted from a tetrad analysis of the multiple exchange chromosomes (Suzuki *et al.* 1966). In every case examined in the present tests (Expts. I, II*a* and II*b*), the number of triple exchange tetrads (E3) was equivalent to or exceeded that of the double exchange tetrads (E2). This would lend support to a Two-Step explanation of the origin of the multiples. Comparison of the minimum expected numbers of doubles (i.e. doubles generated by E3) with the actual numbers recovered (generated by both E2 and E3) reveals (Table 8) that in a few

cases (1, 2; 1, 3 and 4, 5), the observed were appreciably less than the expected, while in most of the remaining classes the observed exceeded or approximated the expected.

Table 6. *Analysis of females amongst whose progeny triple recombinant chromosomes were detected (Experiment I)*

Female	Types of exchange (*st* to p^p) 0	1	2	3	*Chi-square values
A	431	28	0	1	0·002
B	438	35	1	1	0·329
C	460	27	0	1	0·060
D	482	25	0	1	0·081
E	423	15	1	1	0·046
F	163	10	0	2	2·200
G	467	23	0	1	0·427
H	401	29	2	1	2·280
I	295	21	1	1	1·333

0, no exchange; 1, 1 exchange; 2, 2 exchanges; 3, 3 exchanges.
* In each case recombination was found to approximate a Poisson distribution at $P = 0{\cdot}05$.

Table 7. *Tetrad analysis of the crossover data (inferred from recovery of single strands)*

Tetrad distribution	Experiment number I	II*a*	II*b*
Triple exchange	80	48	16
Double exchange	80	12	16
Single exchange	3 410	1 536	734
No exchange	32 378	31 543	10 088
Total tetrad sample	36 948	33 139	10 854

Table 9 shows a summary of the reciprocal crossover classes recovered from Expts. I and II*a* females, along with the numbers of each class obtained. In several cases (particularly for the more proximal intervals), these classes do not appear to be equally represented, despite the apparent lack of any obvious selective advantage for non-mutant alleles.

In order to rule out high revertibility of the markers studied as a contributive factor to some of the multiple exchange chromosomes, revertants were screened for in homozygous stocks, and none was found among $1{\cdot}1 \times 10^4$ *st in ri* eg^2 or $2{\cdot}0 \times 10^4$ *st in ri* eg^2 *Ki* p^p e^s chromosomes.

4. DISCUSSION

Although genetically small, the region studied in these experiments represents a large portion of the physical length of chromosome 3. Unexpectedly, these experiments have revealed non-classical recombinant events in the formation of many

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TABLE 8. *Comparison of observed with expected (from meiotic triple exchange tetrads) double exchanges*

Classes of doubles	Experiment number: I Expected	I Observed	IIa Expected	IIa Observed	IIb Expected	IIb Observed
1, 2	3	0	0	0	0	0
1, 3	2	0	2	0	0	0
1, 4	9	3	6	2	2	1
1, 5	4	14	4	8	2	2
2, 3	0	0	0	0	0	0
2, 4	3	2	0	0	0	1
2, 5	0	4	0	0	0	1
3, 4	3	22	2	6	0	4
3, 5	1	5	0	2	0	1
4, 5	5	0	4	3	2	0

Table 9. *Types and numbers of recombinant chromosomes recovered*

Region	Experiment I Genotype	Number recovered	*R*	Experiment IIa Genotype	Number recovered	*R*
Singles						
1	*st*	726	1:1	*st Ki* p^p	302	1:1
	in ri eg^2 *Ki* p^p	667		*in ri* eg^2	272	
2	*st in*	56	3·5:1	*st in Ki* p^p	39	1·5:1
	ri eg^2 *Ki* p^p	15		*ri* eg^2	24	
3	*st in ri*	16	1·5:1	*st in ri Ki* p^p	5	2·5:1
	eg^2 *Ki* p^p	11		eg^2	12	
4	*st in ri* eg^2	58	1·5:1	*st in ri* eg^2 *Ki* p^p	31	1·5:1
	Ki p^p	43		+ + + + + +	19	
5	*st in ri* eg^2 *Ki*	75	1·5:1	*st in ri* eg^2 p^p	19	2·5:1
	p^p	108		*Ki*	45	
Doubles						
1, 4	*st Ki* p^p	3	3:0	*st*	2	2:0
	in ri eg^2	0		*in ri* eg^2 *Ki* p^p	0	
1, 5	*st* p^p	7	1:1	*st Ki*	0	8:0
	in ri eg^2 *Ki*	7		*in ri* eg^2 p^p	8	
2, 4	*st in Ki* p^p	1	1:1	*st in*	0	—
	ri eg^2	1		*ri* eg^2 *Ki* p^p	0	
2, 5	*st in* p^p	2	1:1	*st in Ki*	0	—
	ri eg^2 *Ki*	2		*ri* eg^2 p^p	0	
3, 4	*st in ri Ki* p^p	19	6:1	*st in ri*	6	6:0
	eg^2	3		eg^2 *Ki* p^p	0	
3, 5	*st in ri* p^p	1	4:1	*st in ri Ki*	2	2:0
	eg^2 *Ki*	4		eg^2 p^p	0	
4, 5	*st in ri* eg^2 p^p	0	—	*st in ri* eg^2 *Ki*	3	3:0
	Ki	0		p^p	0	

R, Ratio of complement classes.

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of the chromosomes recovered. Thus, interference as monitored by coefficients of coincidence for these intervals is high and negative. Similar reports exist (although not for centromeric regions) for many other organisms; for example; yeast (Lindegren, 1955; Leupold, 1958), *Aspergillus* (Calef, 1957; Pritchard, 1960), Neurospora (Mitchell, 1955*a*, *b*), barley (Søgaard, 1974) and maize (Salamini & Lorenzoni, 1970), as well as for *Drosophila* (Sturtevant, 1951; Hexter, 1958; Green, 1959, 1960).

In single burst studies of bacteriophage, the lack of reciprocal recombinants concomitant with high negative interference for short genetic intervals (Chase & Doerman, 1958) prompted workers to posit the existence of short localized regions of pairing, within which recombination is highly probable. Investigators of *Aspergillus* (Calef, 1957; Pritchard, 1960) used this hypothesis to explain coincidence values exceeding 100 for exchange between tightly linked loci (less than 0·1 map units). De Serres (1950) discovered a similar phenomenon in his studies of recombination between two closely linked but functionally distinct groups of *ad-3* mutants of Neurospora, as did Søgaard (1974) at the eciferum loci of barley. Søgaard (1974) discounted any explanation invoking localized pairing for his work because of the relatively large interlocus intervals examined.

Earlier work in *Drosophila* included intragenic studies of recombination at the *white* locus (Green, 1959, 1960). Here, in some cases, exceptional chromosomes could be accounted for by assuming true double exchange was occurring at a frequency lower than that of single intragenic crossing over. However, in crossover studies involving different *white-apricot* pseudoalleles, while no singles were recovered, four exceptions appeared which could be explained by gene conversion. Sturtevant (1951) unexpectedly recovered high numbers of double crossovers between markers while mapping the fourth chromosome in triploid females, a system which may be more analogous to the experiments reported here.

The work presented here is concerned solely with intergenic proximal recombination in *Drosophila*. Previously, it had appeared that exchange within genetically short regions in this organism was generally accompanied by high positive interference (Morgan *et al.* 1925). Exceptions to this rule have more recently prompted workers to seek alternative possibilities, such as the occurrence of successive gonial and meiotic exchanges to produce rare multiples (Whittinghill, 1955; Suzuki *et al.* 1966). Some evidence does appear to support this idea, namely, the dearth of double relative to single exchange tetrads and the higher levels of recombination in those females producing multiple exchange progeny. However, examination of the data for individual females failed to show the clustering phenomenon that would be predicted for the females generating multiples. Furthermore, the types of double crossover chromosome encountered were not dissimilar from those that would be predicted as arising from the different types of meiotic triple exchange tetrads and in most cases, the numbers of the recovered doubles exceeded those of the expected (see Table 8). Nevertheless, this model must be reckoned with as a possible contributive factor to the results of these experiments.

As previously mentioned, work in several organisms has indicated that exchange across the centromere is marked by lack of chromosome interference, but that some

negative interference may occur proximally on chromosome 3 of Drosophila (Morgan *et al.* 1925). It is noteworthy that Strickland (1961) and Bole-Gowda *et al.* (1962) provided evidence in *Neurospora* for chromatid interference, particularly with respect to centromeric crossing over. Hawthorne & Mortimer (1960) mentioned a similar situation for yeast. Howe (1956) and Stadler (1956) had repudiated earlier claims that the phenomenon had been demonstrated in *Neurospora*. One report claiming positive chromatid interference in *Drosophila* (Bonnier & Nordenskiold, 1937) was subsequently refuted by Welshons (1955), who did find evidence for the existence of negative chromatid interference (i.e. an excess of two- over four-strand doubles), particularly for exchange between short genetic intervals on attached *X* chromosomes. Baldwin & Chovnick (1967) in their study of exchange in compound third chromosomes, failed to detect chromatid interference. Davis (1974), using the meiotic mutant *mei-s332* to recover half tetrads, corroborated the latter two investigations. However, since none of these experiments pursued a study of proximal exchange, particularly across the centromere, chromatid interference cannot be entirely eliminated as a possibility here. In the present study, the occurrence of negative chromatid interference could provide a viable explanation of these data since an excess of two-strand doubles would generate more double crossover chromatids relative to singles, thereby inflating coincidence values.

The demonstration of conversion in *Drosophila* has previously been strictly limited to intragenic exchange. Recombination and conversion may be manifestations of the same homologous exchange event, according to previous findings at *maroon-like* (Smith, Finnerty & Chovnick, 1970) and yeast (Hurst, Fogel & Mortimer, 1972) which indicated that half of the conversion events were associated with exchange of flanking markers.

In this present work, conversion provides another explanation for the frequent production of multiple recombinant chromosomes. For example, simple conversion of eg^2 to its wild-type allele and vice versa, would result in apparent 3, 4 double crossovers. Triples involving 3, 4 exchange may be explained by the conversion of *eagle* accompanied by exchange of either of the most distal markers. Extending this logic, 1, 4, 5 triples could result from conversion of *Ki* or Ki^+ with a crossover in region 1, while 1, 2, 4 triples could be generated by conversion of *ri* or ri^+ and an exchange in 4. It must be emphasized that the erstwhile failure to detect intergenic exchange events resembling conversion in *Drosophila*, is almost certainly related to the effects of high positive interference and that the absence of interference across the centromere might permit the materialization of such phenomena. Indeed, it appears that the main prerequisite for the high frequency of rare multiples in other genetic systems has proven to be the utilization of tightly linked markers (Calef, 1957; Søgaard, 1974) and conversion was offered as a possible contributor. Crossover frequencies in this present work indicate that the region *in* to *Ki* is particularly small, genetically.

It is noteworthy that with an attached *X* chromosome, the coincidence values were decreased (in most cases), thereby suggesting fewer multiples relative to single crossovers. Previous demonstration of intrinsically (Schultz & Redfield,

1951) and extinsically (Suzuki & Parry, 1964) mediated recombinagenesis in *Drosophila*, were marked by decreases in positive interference (more frequent relative occurrence of multiples) for adjacent regions. Therefore, the present data are not inconsistent with the suggestion that conversion may be involved here as a contributive factor to the appearance of multiple recombinant chromosomes, since one would expect recombinagenic agents to effect a similar increase in the occurrence of true multiples as well as of singles. M. M. Green (personal communication) reports similar results for his work with this region.

Other support for the conversion-based explanation for the multiples may be provided by the inequities apparent in the reciprocal crossover classes (Table 9). Remember also that spontaneous reversion of these loci was not observed.

The main argument against a conversion-centred model is that previously this phenomenon was limited to euchromatic, intragenic exchange (Chovnick, Ballentyne & Holm, 1971) and that the exchange intervals examined here are very large cytologically. However, since the basis for the genetic shortness of the intervals is unknown, considerations of exchange here obviously merit an approach from new perspectives. This is emphasized by recent discoveries which have localized repetitive satellite DNA of *Drosophila* to constitutive heterochromatin (Gall, Cohen & Polan, 1971; Peacock *et al.* 1973). Certainly, this structural organization may confer unique properties of pairing and crossing over on centromeric intervals. The latter idea may also be germane to any argument involving chromatid interference.

One approach that may aid in distinguishing between the different possibilities is the utilization of females carrying third chromosome pericentric inversions which include all of the loci used in this study. Scrutiny of recombination in such heterozygotes would select for even-numbered and against odd-numbered crossovers, since the latter would not survive owing to extensive duplications and deficiencies. Comparisons of control frequencies of multiples with the progeny of these females should provide information as to the origin of the multiple chromosomes with respect to the possibilities mentioned.

It may also be possible to make use of meiotic mutants which affect nondisjunction but have no effects on recombination, in order to capture half tetrads and thus test for reciprocality and chromatid interference for proximal crossing over on this chromosome (Davis, 1974). However, this approach would be a formidable project given the low levels of recombination in these regions.

Finally, another experiment which may provide more information about centric exchange could involve actually testing for eg^2 or eg^1 conversion in multiply-marked heterozygous females and a comparison of this with known frequencies if the former does indeed occur.

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REFERENCES

ARAJÄRVI, P. & HANNAH-ALAVA, A. (1969). Cytogenetic mapping of *in* and *ri*. *Drosophila Information Service* **44**, 73.

BAKER, W. K. (1958). Crossing over in heterochromatin. *American Naturalist* **92**, 59–60.

BALDWIN, M. & CHOVNICK, A. (1967). Autosomal half-tetrad analysis in *Drosophila melanogaster*. *Genetics* **55**, 277–293.

BEADLE, G. W. (1932). A possible influence of the spindle fiber on crossing over in Drosophila. *Proceedings of the National Academy of Sciences, U.S.A.* **18**, 160–165.

BOLE-GOWDA, B. N., PERKINS, B. N. & STRICKLAND, W. M. (1962). Crossing over and interference in the centromere region of linkage group I of *Neurospora*. *Genetics* **47**, 1243–1252.

BONNIER, G. & NORDENSKIÖLD, M. (1937). Studies in *Drosophila melanogaster*. with attached-X's. I. Crossing over values. Frequencies of reciprocal and non-reciprocal exchanges. Chromatid interference. *Hereditas* **23**, 257–278.

CALEF, E. (1957). Effect on linkage maps of selection of crossovers between closely linked markers. *Heredity* **11**, 265–279.

CHASE, M. & DOERMANN, A. H. (1958). High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics* **43**, 332–352.

CHOVNICK, A., BALLENTYNE, G. H. & HOLM, D. G. (1971). Studies on gene conversion and its relationship to linked exchange in *Drosophila melanogaster*. *Genetics* **69**, 179–209.

DAVIS, B. K. (1974). Chromatid interference in *Drosophila melanogaster*. *Genetics* **77**, 16S.

DE SERRES, F. J. (1958). Recombination and interference in the ad-3 region of *Neurospora crassa*. *Cold Spring Harbor Symposia on Quantitative Biology* **23**, 111–118.

DOBZHANSKY, T. (1930). Cytological map of the second chromosome of *Drosophila melanogaster*. *Biologische Zentralblatt* **50**, 671–685.

GALL, J. G., COHEN, E. H. & POLAN, M. L. (1971). Repetitive DNA sequences in Drosophila. *Chromosoma (Berlin)* **33**, 319–344.

GREEN, M. M. (1959). Double crossing over or gene conversion at the white locus in *Drosophila melanogaster*? *Genetics* **45**, 15–18.

GREEN, M. M. (1960). Apparent double crossing over in a short genetic interval in *Drosophila melanogaster*. *Nature, London* **126**, 990–991.

GRAUBARD, M. A. (1934). Temperature effect on interference and crossing over. *Genetics* **19**, 83–94.

HAWTHORNE, D. C. & MORTIMER, R. K. (1960). Chromosome mapping in *Saccharomyces*: centromere linked genes. *Genetics* **45**, 1085–1110.

HEXTER, W. M. (1958). On the nature of the garnet locus in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, U.S.A.* **44**, 768–771.

HOLM, D. G., BALDWIN, M., DUCK, P. & CHOVNICK, A. (1969). The use of compound autosomes to determine the relative centromeric position of chromosome 3. *Drosophila Information Service* **44**, 112.

HOWE, H. B. (1956). Crossing over and nuclear passing in *Neurospora crassa*. *Genetics* **41**, 610–622.

HURST, D. D., FOGEL, S. & MORTIMER, R. K. (1972). Conversion-associated recombination in yeast. *Proceedings of the National Academy of Sciences, U.S.A.* **69**, 101–105.

LEUPOLD, U. (1958). Studies on recombination in *Schizosaccharomyces pombe*. *Cold Spring Harbor Symposia on Quantitative Biology* **23**, 161–170.

LINDEGREN, C. C. (1955). Non-Mendelian segregation in a single tetrad of *Saccharomyces* ascribed to gene conversion. *Science* **121**, 605–607.

LINDSLEY, D. L. & GRELL, E. H. (1968). Genetic Variations of *Drosophila melanogaster*. *Carnegie Institution of Washington Publication No.* 627.

LUCCHESI, J. C. & SUZUKI, D. T. (1968). The interchromosomal control of recombination. *Annual Review of Genetics* **2**, 53–86.

MERRIAM, J. R. & GARCIA-BELLIDO, A. (1969). Linkage Data, *D. melanogaster*. *Drosophila Information Service* **44**, 51.

MITCHELL, M. B. (1955*a*). Aberrant recombination of pyridoxin mutants of *Neurospora*. *Proceedings of the National Academy of Sciences, U.S.A.* **41**, 215–220.

MITCHELL, M. B. (1955*b*). Further evidence of aberrant recombination in *Neurospora*. *Proceedings of the National Academy of Sciences, U.S.A.* **41**, 935–937.

MORGAN, T. H., STURTEVANT, A. H. & BRIDGES, C. B. (1925). The genetics of *Drosophila*. *Bibliographia Genetica* **2**, 1–262.

MULLER, H. J. (1926). The regionally differential effect of X-rays on crossing over in autosomes of *Drosophila*. *Genetics* **10**, 470–507.

PAINTER, T. S. (1935). The morphology of the third chromosome in the salivary gland of *Drosophila melanogaster* and a new cytological map of this element. *Genetics* **20**, 301–326.

PEACOCK, W. J., BRUTLAG, D., GOLDRING, E., APPELS, R., HINTON, C. S. & LINDSLEY, D. L. (1973). The organization of highly repetitive DNA sequences in *Drosophila melanogaster* chromosomes. *Cold Spring Harbor Symposia on Quantitative Biology* **28**, 405–416.

PLOUGH, H. H. (1917). The effect of temperature on crossing over in *Drosophila*. *Journal of Experimental Zoology* **24**, 147–209.

PRITCHARD, R. H. (1960). Localized negative interference and its bearing on models of gene recombination. *Genetical Research* **1**, 1–24.

ROBERTS, P. A. (1965). Difference in the behavior of eu- and heterochromatin: crossing over. *Nature, London* **205**, 725–726.

RITOSSA, F. M., ATWOOD, K. C. & SPIEGELMAN, S. (1966). A molecular explanation of the *bobbed* mutants of *Drosophila* as partial deficiencies of 'ribosomal' DNA. *Genetics* **54**, 819–834.

SALAMINI, F. & LORENZONI, C. (1970). Genetical analysis of glossy mutants of maize. III. Intracistronic recombination and high negative interference at the gl_1 locus. *Molecular and General Genetics* **108**, 225–232.

SCHULTZ, J. & REDFIELD, H. (1951). Interchromosomal effect on crossing over in *Drosophila*. *Cold Spring Harbor Symposia on Quantitative Biology* **16**, 175–197.

SMITH, P. D., FINNERTY, V. G. & CHOVNICK, A. (1970).Gene conversion in Drosophila: non-reciprocal events at the maroon-like cistron. *Nature, London* **228**, 442–444.

SØGAARD, B. (1974). The localization of eciferum loci in barley. III. Three point test of genes on chromosome I in barley. *Hereditas* **76**, 41–47.

STADLER, D. R. (1956). Double crossing over in *Neurospora*. *Genetics* **41**, 623–630.

STEVENS, W. L. (1936). The analysis of interference. *Journal of Genetics* **32**, 51–64.

STRICKLAND, W. N. (1961). Tetrad analysis of short chromosome regions of *Neurospora crassa*. *Genetics* **46**, 1125–1141.

STURTEVANT, A. H. (1951). A map of the fourth chromosome in *Drosophila melanogaster* based on crossing over in triploid females. *Proceedings of the National Academy of Sciences, U.S.A.* **37**, 405–407.

SUZUKI, D. T., BAILLIE, D. & PARRY, D. (1966). The origin of multiple crossover chromatids in short genetic intervals in *Drosophila melanogaster*. *Genetics* **54**, 1359–1370.

SUZUKI, D. T. & PARRY, D. M. (1964). Crossing over near the centromere of chromosome 3 in *Drosophila melanogaster* females. *Genetics* **50**, 1427–1432.

THOMPSON, P. E. (1963*a*). Centric pairing and crossing over in *Drosophila melanogaster*. *Genetics* **48**, 697–701.

THOMPSON, P. E. (1963*b*). Evidence on the basis of the centromere effect in the large autosomes of *Drosophila melanogaster*. *Genetics* **49**, 761–769.

WEINSTEIN, A. (1936). The theory of multiple-strand crossing over. *Genetics* **21**, 155–199.

WELSHONS, W. J. (1955). A comparative study of crossing over in attached-X chromosomes of *Drosophila melanogaster*. *Genetics* **40**, 918–936.

WHITTINGHILL, M. (1955). Crossover variability and induced crossing over. *Journal of Cellular and Comparative Physiology* **45**, 189–220.