Chromosome Rearrangement by Ectopic Recombination in Drosophila melanogaster: Genome Structure and Evolution

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Manuscript received May 13, 1991
Accepted for publication August 13, 1991

ABSTRACT

Ectopic recombination between interspersed repeat sequences generates chromosomal rearrangements that have a major impact on genome structure. A survey of ectopic recombination in the region flanking the white locus of Drosophila melanogaster identified 25 transposon-mediated rearrangements from four parallel experiments. Eighteen of the 25 were generated from females carrying X chromosomes heterozygous for interspersed repeat sequences. The cytogenetic and molecular analyses of the rearrangements and the parental chromosomes show: (1) interchromosomal and intrachromosomal recombinants are generated in about equal numbers; (2) ectopic recombination appears to be a meiotic process that is stimulated by the interchromosomal effect to about the same degree as regular crossing over; (3) copies of the retrotransposon roo were involved in all of the interchromosomal exchanges; some copies were involved much more frequently than others in the target region; (4) homozygosis for interspersed repeat sequences and other sequence variations significantly reduced ectopic recombination.

Chromosomal rearrangement by ectopic pairing and recombination between interspersed repeat sequences presents a mechanism for dramatic reorganization of eukaryotic genomes. Reorganization by gene duplications or deletions, or by inversions or transpositions of various kinds offers raw material for evolutionary modifications and at the same time poses a threat to the stability of the genome. The insertion or excision of transposons at various sites in the genome account for a significant portion of mutations in Drosophila and other eukaryotes. However, possibly of equal or greater consequence are the changes in chromosome structure that result from the involvement of interspersed repeat sequences in unequal crossing over. Meiotic and mitotic exchange between ectopically paired repeat sequences that create chromosomal rearrangements have been studied in yeast (Roeder 1983; Petes and Hill 1988), and in Drosophila (Goldberg et al. 1988; Davis, Shen and Judd 1987; Engels and Preston 1984; and Lim 1988). Rearrangements associated with some recessive mutations cause human diseases. These include hypercholesterolemia (locus: low density lipoprotein receptor, Lehrman et al. 1987), hereditary angioedema (locus: C1 inhibitor; Stoppa-Lyonnet et al. 1990) and human steroid sulfatase deletions (locus: STS, Yen et al. 1990), indicating that such ectopic exchanges between dispersed repeat sequences also occur in the human germline.

Little is known about the quantitative contribution of ectopic exchanges between repeat sequences to the origin of chromosomal rearrangements. In Drosophila melanogaster there are some 40 families of transposable elements with copies of the elements of each family closely conserved. The average copy numbers of these families range from only a few to over 50. These elements comprise 10–12% of the genome. Surveys of the distributions of transposable elements in the euchromatic regions of the genome in populations of D. melanogaster (Charlesworth and Langley 1989) have shown that while the presence of an insert at any particular site is rare, the number of possible sites is so large that the average individual has multiple representatives of each family. Considering the number of interspersed repetitive elements, it is evident that, depending on the rate of ectopic recombination, the resulting chromosome aberrations could be a major source of mutations in germinal and somatic cells. The impact of these mutations on development and fitness of zygotes is for the most part unknown. It has been proposed, however, that the ectopic exchange process with its associated mutational effects is an important population genetic mechanism influencing the average copy numbers of these DNA parasites in natural populations of D. melanogaster (Langley et al. 1988).

To learn more about the role of ectopic exchange in the origin of chromosomal rearrangements, we have carried out a series of experiments designed to measure the amount and types of ectopic exchanges in a region of the X chromosome surrounding the

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white locus of D. melanogaster. Specifically we were interested in discovering (1) which repeated elements are involved and how frequently; (2) whether exchanges are meiotic or mitotic, interchromosomal or intrachromosomal; (3) whether homozygosis of genetic polymorphisms in a region influences ectopic exchanges in that region; and (4) how recombination frequencies are influenced by the interchromosomal effect. The interchromosomal effect is seen where heterozygous rearrangements are present in one or more of the major chromosome arms in D. melanogaster. Their presence results in an increase in crossing over of the other pair(s) of chromosomes, with the increase most marked in regions bordering centric heterochromatin and at the distal ends of chromosome arms. The mechanistic basis for the increased recombination is not well understood [see Lucchesi (1976) for review]. The answers to these questions will help in understanding the ectopic exchange process, and will provide avenues of investigation into its role in mutation processes and the containment of transposable element copy number.

**MATERIALS AND METHODS**

**Drosophila strains and crosses:** Two X chromosomes were selected for this study. One, carrying the mutant markers yellow-2 (y²), white-ric (w''), split (spl) and echinus (ec), was selected because of the useful markers flanking the white locus and because considerable molecular characterization of its white region had already been done [see Judd (1987) for a review]. The w'' mutation was determined to be a derivative of white-apricot (w') in which a copy of the transposon roo had inserted into a copy of copia occupying a site at coordinate 0.0 of the white locus map (Davis, Shen and Judd 1987). This copia insert originally caused the white-apricot mutation. The other X chromosome was extracted from the wild type Oregon-R strain carried in this laboratory. It was of unknown transposon content in the region of interest.

Polytene chromosome squashes of both parental strains were subsequently probed in situ to determine the copy numbers and relative positions of roo and copia in the X chromosomes (Table 1). We chose these two probes because both transposons were positioned within the white locus of w'' and because roo is generally present in high copy number (Figure 1).

Stocks of each parental type were established from a single male of each type, by crossing to FM7 females, then backcrossing F1 daughters to the parental male to establish cultures that were essentially isogenic for the X chromosomes.

**Heterozygous autosomal rearrangements,** SM1, Cy/+ and TM3, Ubx''/+ of the second and third chromosomes respectively, were introduced into the y² w'' spl ec strain and maintained by selection. When males of this strain were mated to Ore-R females (Figure 2), two of the four resulting classes of F1 females were selected as virgins. Females of both classes were heterozygous for the two X chromosomes; one class carried rearranged autosomes (cross A), while the other had autosomes of standard configuration (cross B). The autosomal rearrangements are known to be effective in increasing crossing over in the region of the white locus by a factor of 3 to 5 (Judd 1959). Both classes of females were crossed to males of genotype z1 w6h25 spl sn1, and offspring were examined for individuals exhibiting exceptional eye color, i.e., other than the wild type of Ore-R and the orange-brown of w''/w''h25.

Females of the genotype y² w'' spl ec; SM1/+; TM3/+ (cross C) and Ore-R; SM1/+; TM3/+ (cross D), both of which were homozygous for the X chromosomes, were also crossed to z1 w6h25 spl sn1 males and the offspring were scored for exceptional eye-color phenotypes. These crosses may be viewed as controls for the heterozygous genotypes in crosses A and B; however, they were included to test for a possible effect of homozygosity per se on ectopic pairing and exchange. The X chromosomes of the females in crosses C and D were homozygous for both the interspersed repeat sequence differences and any nucleotide sequence differences between them. Either type of genetic variant may have different consequences in heterozygous or homozygous condition. Unfortunately, exchange of flanking markers cannot be scored in crosses C and D and the construction of new marker arrangements would likely have upset the patterns of sequence variation.

The design of the four crosses allows deletions or breakpoints within the white locus to be easily recognized by their bleached white phenotype. Duplications for the wild-type white locus should produce an orange mottle phenotype in females because of the interaction of the white duplication with the heterozygous z1 mutation. However, that phenotype is similar to the w''/w''h25 phenotype and possibly would not be recognized. Deletions or breakpoints within the xeste locus would be expected to produce an orange colored eye that would be easily recognized as an exception in cross D but recognized with difficulty in other crosses because of its similarity to the w''/w''h25 phenotype.

**Molecular analysis:** DNA for analysis was isolated from 0.5 to 2 g of adult flies and purified on a cesium chloride gradient as described in Bingham, Lewis and Rubin (1981). Genomic libraries were constructed from DNA from parental and deficiency strains as described previously (Shrimp-
rootontaining plasmid was hybridized in somes from each of the parental strains used in the study. (A) Ore-TON, MONTGOMERY and LANGLEY 1986), with the following change: ligations were packaged using  Gigapack  Gold (Stra-}

standard techniques (MANIATIS,  FRITSCH and SAMBROOK 1982), for each. Transfer was either by the capillary technique to polytene  chromosomes and analysis: A num-

Cytological analysis and in situ hybridizations: Salivary gland dissection and squashes for polytene chromosome cytology were performed essentially as described by PARDUE and GALL (1975).

DNA used for in situ hybridizations to polytene chromosomes was labelled with Biotin-16dUTP from Boehringer-Mannheim (LANCER, WALDROP and WARD 1981) by the random-primed technique, using conditions and reagents included with the Boehringer-Mannheim Random-Primed DNA Labeling Kit. Slide preparation of chromosomes was as described in PARDUE and GALL (1975) with modifications by J. Lim (JOHNSON-SCHLITZ and LIM 1987). The conditions for the hybridization, washes and detection were previously described (MONTGOMERY, CHARLESWORTH and LANGLEY 1987).

RESULTS

A summary of the results from four experiments designed to detect rearrangements in the region of the white locus is given in Figure 2. Mutations that survive as heterozygotes and that modify the expression of the white or zeste loci will usually be recognized and recovered by this screen. The viabilities of deficien-
cies are such that usually only those with both breakpoints in sections 2 through 5 on the polytene chromosome map (flanking the white locus at 3C2) are likely to survive and produce offspring with excep-
tional eye color phenotypes. Duplications for the region, through generally having good viability, are not easily recognized by this screen because they have phenotypes identical or similar to parental pheno-
types.

Analysis of exceptional offspring from cross A: A total of 23 individuals having nonparental eye colors were recovered from cross A. One of these proved to be the result of X chromosome nondisjunction (line 11) and two others (lines 5 and 8) failed to produce offspring. The 20 exceptional progeny that were analyzed from cross A arose independently, i.e., from

**FIGURE 1.**—Sites of hybridization of the transposable element, roe, to Oregon-R and y2 w+ spl ec X chromosomes. A biotinylated roe-containing plasmid was hybridized in situ to polytene chromosomes from each of the parental strains used in the study. (A) Oregon-R by roe; (B) y2 w+ spl ec by roe. Arrows indicate the 3C region of the X chromosomes (magnification 500X).
**Heterozygous X Crosses**

**Oregon-R Females**  
(isogenic X chromosome)  

Select two classes of females

Cross A  
\[ y^2 \text{ wric spo ec} \]  
SM1  
TM3  
++ + + +  

and

Cross B  
\[ y^2 \text{ wric spo ec} \]  
+  
+  
+  

Chromosomes screened  
Exceptions recovered

100,398  
20

127,153  
3

Select offspring with non-parental eye color from each cross

**Homozygous X Crosses**

Cross C  
\[ y^2 \text{ wric spo ec} \]  
SM1  
TM3  
+  

Cross D  
\[ + \]  
SM1  
TM3  
+  

81,512  
6

Select offspring with non-parental eye color from each cross

**Figure 2.**—Mating schemes used to recover rearrangement-bearing chromosomes. The parental females for crosses A and B were sibling females selected from the initial cross. Deletions of the white locus or breakpoints in the white locus produce a bleached white eye color. Duplication of the white locus or deletion of the zeste locus produces an orange-mottle or solid orange eye color, respectively.

Different parents. The molecular analyses show that of the 20 lines, 17 are X chromosome deletions that remove all or part of the white locus; one is an inversion with breakpoints in the white locus and in 18F of the polytene chromosome map. The remaining two are spontaneous mutations of the white locus that are associated with insertions of non-white locus sequences into the gene.

The 17 deletion lines form eight different classes, with breakpoints that range from region 2F to 3D on the polytene chromosome map (Figure 4). The largest class, containing five lines (1, 9, 10, 21 and 23) is a deletion extending from coordinates -190 to +41 on the white locus map. A copy of the transposon roo is found at the breakpoint junction of all five lines (Figure 5) and all show an exchange of flanking markers, strongly suggesting that they originated from an interchromosomal exchange between a copy of roo located at -190 in the Oregon-R chromosome and one at +41 in the w" chromosome (Figure 6). This interpretation is supported by the molecular analyses of the two parental chromosomes that show a copy of roo at each of these sites.

Two other classes of two lines each (lines 14 and 16 and lines 6 and 7) also have a copy of roo at their breakpoints, and are also associated with exchange of flanking markers. The proximal breakpoint, common to both classes, is located in the roo at coordinate +41 of the w" chromosome. The two classes have different distal breakpoints, however. The distal breakpoint of lines 14 and 16 is distal to coordinate -225 with the deletion extending to +41 on the white locus map. Lines 6 and 7 are broken distal to coordinate +100 of the zeste locus map, with the deletion extending to +41 on the white locus map. They complement l(1)EC226 at chromosomal position 2F1, but fail to complement all loci between and including phl and w. As in the previously discussed class of five lines, these classes appear to have originated by ectopic pairing and exchange between a copy of roo distal to white locus in the Oregon-R chromosome and one proximal to white locus in the w" chromosome. Clones that would detect distal breakpoints for these two classes were not available.

Labeled roo DNA hybridizes in situ to three or four sites in region 2F-3A of the Oregon-R chromosome.
Ectopic Recombination in Drosophila

**FIGURE 3.** Molecular map of deficiency breakpoints spanning the *zeste* to *white* region. This map is a consensus map of the region from studies by M. GOLDBERG (personal communication), PIRROTTA, HADFIELD and PRETORIUS (1985), and MARIANI, PIRROTTA and MANET (1985). The molecular locations of loci other than *white* (w) and *zeste* (l) are indicated for reference and are approximate. Breakpoint regions for deficiencies recovered in this study are indicated by arrowheads. Dashed lines indicate the molecular region within which the breakpoint could be mapped. The cloned DNA sequences that detected breakpoints are shown as shaded bars. Map coordinates for the *white* region have, as the point of insertion of *copia* in the w" allele. For the *reste* region, 0 is the site of a *P* element insertion (MARIANI, PIRROTTA and MANET 1985). New insertions that occurred in this experiment at or near the *white* locus are also shown, and represent lines 12, 19, 32, 33 and 53. Breakpoints A, D, E, L and M occurred beyond the reach of available cloned DNA, and are therefore not localized molecularly. Breakpoints by line numbers (in parentheses) are as follows: A (6, 7, 41-46), B (2), C (4, 13, 15), D (18), E (14, 16), F (1, 9, 10, 21, 23), G (22), H (3), I (17), J (51), K (41-46), L (2, 3, 4, 13, 15, 17, 22, 51), M (18). Genetic loci are as follows: l(1)Ec226, polehole (phl), giant (gt), technical knock out (tko), and *zeste-white* lethals (zw 10, 13, 2 and 9).

Within the limits of resolution of the *in situ* hybridization, two of these sites correspond to the observed distal breakpoints in these two classes of deficiencies.

More than half of the deletions, 9 of 17, thus involve the copy of *roo* at coordinate +41 in the *w"* chromosome in interchromosomal asymmetrical exchange with another *roo* at one of three other positions in the Oregon-R chromosome (Figure 6). The eight remaining deletions from cross A (lines 2, 3, 4, 13, 15, 17, 18 and 22), all appear to have had their origins as intrachromosomal exchange products.

Lines 4 and 15 have similar but not identical breakpoints, all of which appear to involve repetitive sequences in the Oregon-R chromosome. In the region of their distal breakpoints both lines lack DNA sequences between +18 and +20 of the *zeste* locus map (detected by clone λ17-8-53) but contain sequences detected by a probe extending from +30 to +43 (clone λT1-1). The region between coordinates +20 and +30 on the *zeste* map is known to contain repetitive sequences (MARIANI, PIRROTTA and MANET 1985). We have evidence for insertional variation in this region, but we have not identified a previously described transposon located there in the Ore-R chromosome. The proximal breakpoints in lines 4 and 15 are proximal to coordinate +120 (w map) in a region that lies between the roughest (*rst*) and *Notch* (*N*) loci. Clones that would allow exact determination of the breakpoints are not available. This circumstantial evidence indicates a close association between breakpoint and repeat sequences.

Three other deletions of the Ore-R chromosome, lines 2, 3 and 22, also have their proximal breakpoints in the region between *rst* and *N*. We are unable to determine whether the proximal breakpoints are all the same. However, each of the three has a unique distal break.

Line 2 is broken between coordinates +55 and +61.5 of the *zeste* map in a region of repetitive DNA sequences that is very near if not identical to the distal break of Df(1)62g18 (JUDD, SHEN and KAUFMAN 1972; MARIANI, PIRROTTA and MANET 1985) (Figures 3 and 7).
different from the breakpoint in line 3 discussed above.

The one remaining deletion from cross A, line 18, has breakpoints distal to -225 of the \( w^{+c} \) chromosome and proximal to the \( N \) locus. It is an intrachromosomal deletion of the \( y^{2} w^{+c} spl ec \) chromosome, having a yellow-2, white, Notch, echinus, recessive lethal phenotype. Cloned DNA that would allow precise location of breakpoints was not available. Complementation mapping locates the distal break between \( l(1)zw^{1} \) and \( l(1)zw^{8} \), cytological position 3A4,5 on the polytene chromosome map. Line 18 is not rescued by \( w^{Y} \) (2D1.2; 3D3.4), but it is rescued by \( Dp(1;3)w49a \) (3A9.B2; 3E2.3) and it complements \( diminutive \) (dm, approximate location 3D3.6). These cytogenetic results suggest that the proximal breakpoint is between 3D3.4 and 3E2.3, which agrees with our cytological observations that place the break at 3D4.5.

Line 20 is an inversion that occurred in the \( y^{2} w^{+c} spl ec \) chromosome. It has a white eye-color phenotype in addition to those of the flanking markers. Cytological examination of the polytene chromosomes shows breakpoints at 3C and 18F. In the \( w^{+c} \) chromosome, \( copia \), with a copy of \( roo \) inserted 2.95 kb from its 3' end, is inserted at coordinate 0.0 of \( \text{white} \). In situ analysis shows that there is also a copy of \( roo \) at 18F in that chromosome. Molecular analysis of the \( \text{white} \) locus of Line 20 shows a restriction pattern like that of \( w^{+c} \) for endonucleases that cut in \( \text{white} \) locus and in either of the transposons. This is consistent with the inversion being formed by pairing and exchange between two copies of \( roo \) in reverse orientation at 3C and 18F. The BamHI restriction pattern for Line 20 \( \text{white} \) locus definitively rules out the involvement of \( copia \) as the site of exchange in the formation of the inversion. BamHI cuts in \( roo \) but not in \( copia \), thus such a digest of line 20 DNA would differ from the \( w^{+c} \) pattern if \( copia \) were the site of the breakpoint (Figure 8).

The two remaining white-eyed offspring from cross A, lines 12 and 19, show restriction patterns consistent with the insertion of non-\( \text{white} \) locus, presumably transposon, sequences into the \( \text{white} \) locus of the \( w^{+c} \) chromosome. Line 12 has approximately 2 kb inserted between coordinate -5 and the EcoRI site in the 3' end of \( copia \). Line 19 has about 5 kb inserted between the 5' \( SalI \) site in \( roo \) and coordinate +2 of \( \text{white} \) (data not shown).

**Analysis of exceptional offspring from cross B:**

Three exceptional individuals of independent origin were recovered from cross B. Line 51 is a deletion of the 5' region of the \( \text{white} \) locus in the \( w^{+c} \) chromosome from +0.0 to proximal of +120. In addition to the flanking mutant markers, this line shows a \( \text{white}, \text{roughest} \) phenotype. The restriction map of this line is best interpreted as having the 3' portion of \( copia \) and

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**Figure 4:** The cytological limits of the deficiencies recovered in these experiments are shown by open bars. Stippled areas indicate that the breakpoint has not been located molecularly.

<table>
<thead>
<tr>
<th>Lines 1, 9, 10, 21, 23</th>
<th>Lines 14 &amp; 16</th>
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<tbody>
<tr>
<td>Line 17</td>
<td>Line 3</td>
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<tr>
<td>Line 22</td>
<td>Line 51</td>
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<tr>
<td>Line 2</td>
<td>Lines 4, 13, 15</td>
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<tr>
<td>Line 18</td>
<td>Lines 41-46</td>
</tr>
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Line 3 has a distal break at -30 (\( w \) map) and a proximal break in the \( rst \) to \( N \) region, creating a small deletion that is hemizygous viable with a \( \text{white}, \text{roughest} \) phenotype. The molecular analysis shows that the Ore-R chromosome has a 10 kb insertion between \( \text{coordinates} -50 \) and -65 (\( w \) map), making it a deletion of at least 20 kb larger than in line 3. Restriction maps of this region of several wild-type strains show considerable insertional polymorphism and a cluster of EcoRI restriction sites that may indicate tandem repeats (Pirrotta, Hadfield and Pretorius 1983). The line 22 deletion does not extend distally to remove \( l(1)zw^{9} \), which means that this locus, most proximal to \( w \), must lie distal to coordinate -50.

Two lines, 13 and 17, are deletions that occurred in the \( y^{2} w^{+c} spl ec \) chromosome. Line 13 is a large deletion extending from between +20 and +30 on the \( z \) map to between \( rst \) and \( N \) proximally. Note that the distal breakpoint is in the same region as in lines 4 and 15, and the proximal breakpoint is indistinguishable from those seen in lines 2, 3, 4, 15, 18 and 22. Line 17 has its distal break at about coordinate -15 of the \( \text{white} \) locus map, in the same region as but

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**Legend:**

- \( \text{white} \)
- \( \text{roughest} \)
- \( \text{diminutive} \) (dm, approximate location 3D3.6)
at least 1 kb of roo at the breakpoint but with all sequences of white locus proximal to 0.0 deleted (data not shown). The most logical explanation is that the deletion resulted from ectopic pairing and exchange between roo elements at coordinates 0.0 and proximal to +120. It should be noted that the proximal breakpoint is in the same region as seven of the eight intrachromosomal deletions found in cross A.

Line 52 is derived from the \( y^2 \) \( w^{cy} \) spl ec chromosome. It has a white phenotype in addition to the flanking markers, and is viable as a hemizygote. The restriction patterns from five different endonucleases showed no changes from the \( w^{cy} \) pattern. This strongly suggests that line 52 carries a mutation created by a change in one or a few nucleotides in the white locus (data not shown).

Line 53, which has a white eyed, hemizygous viable phenotype, appears to be a mutation in the Ore-R chromosome. Molecular analysis shows that there is an insertion of about 6 kb of DNA into the white locus...
between coordinates -0.67 and +1.6 (data not shown). Quite likely the mutation was caused by the insertion of a transposon into that region of the gene.

**Analysis of exceptional offspring from cross C:**
Three white eyed individuals of independent origin appeared from cross C, involving females homozygous for \( y^2 w^{ne} spl ec \) X chromosomes and heterozygous for the autosomal inversions SM1 and TM3. All three lines are hemizygous viable. Line 31 shows restriction patterns like those of \( w^{ne} \), suggesting that it is a spontaneous mutation involving one or a few nucleotides in the white locus. Lines 32 and 33 are both associated...
with the insertion of non-white locus sequences into the locus. Line 32 has about 4 kb of DNA inserted between coordinate -0.67 and the most 3' SalI site in roo. Similarly, line 33 has an insert of about 6.5 kb between the BamHI site in roo and the BamHI site at +1.38 (data for lines 32 and 33 not shown). It is entirely possible that in both lines 32 and 33 the insertion sites were in the copia-roo complex at 0.0 in the white locus. It is of significance that none of the exceptions recovered from cross C resulted from ectopic pairing and exchange between transposons.

**Analysis of exceptional offspring from cross D:**

Six individuals with zeste eye color were recovered from cross D. All six (lines 41, 42, 43, 44, 45 and 46) are deletions that extend from 2F4-5 to 3A8-9. They fail to complement phi, gt, tk0, z, l(l)zw1, l(l)zw8, l(l)zw4 and l(l)zw10, but complement l(l)zw2. Molecular analysis shows that the distal breakpoint is distal to +100 on the z map. The proximal breakpoint is at -190 on the w map, identical to the distal breakpoints found in lines 1, 9, 10, 21, and 23 from cross A (Figure 5). These deletions have the transposon roo at their breakpoints. Unlike the cross A deletions, which were generated via ectopic interchromosomal exchanges between the roo element at -190 in the Oregon-R chromosome and a roo in the w'w' chromosome at coordinate +41, the deletions from cross D extend distally from the roo at coordinate -190 to another copy in Ore-R distal to zeste. Because the females used in cross D were homozygous for the X, we are unable to determine whether the exchange was inter- or intrachromosomal.

Two of the cross D lines arose from subcultures of one mating group and two from another; the remaining two occurred in different mating groups. Each mating group consisted of four to six parental females. It is possible that the deficiencies in all six cross D lines have a common origin as a preexisting change in the Ore-R strain. Thus, it is unclear whether these deletions should be scored as a single event or as having arisen during the experiment as six separate events.

**Interchromosomal effect:** Map distances were determined for the y-w and the w-spl intervals for crosses A and B to measure the effect of the autosomal rearrangements on crossover frequencies in the X chromosomes. For the y-w interval the values were 5.4 (95% confidence interval: 4.5–6.3) and 0.7 (95% confidence interval: 0.5–1.0) map units for crosses A and B, respectively. The standard map distance for this interval is 1.5 m.u. The w-spl interval values from cross A and B were 2.4 (95% confidence interval: 1.8–3.1) and 1.1 (95% confidence interval: 0.8–1.5) m.u., respectively, with the standard being 1.5 m.u. For the whole interval, y to spl, the observed map distances were 7.8 (95% confidence interval: 6.7–8.9) vs. 1.8 (95% confidence interval: 1.4–2.3) in crosses A and B, respectively. Thus there was at least a 2.9-fold increase (6.7/2.3) associated with inversion heterozygosity in the autosomes.

In cross A, twenty individuals among a total of 100,398 offspring had nonparental eye colors; eighteen of these appeared to be transposon-associated rearrangements. (18/100, 398 = 1.8 × 10⁻⁴; 95% confidence interval: 1.1 × 10⁻⁴ to 2.2 × 10⁻⁴). Cross B produced only three nonparental eye color flies among 127,153 offspring. One of them, line 51, appears to have been generated as an ectopic exchange involving the copia-roo transposon complex within white (1/127, 153 = i × 10⁻³; 95% confidence inter-
val: $2 \times 10^{-7}$ to $4 \times 10^{-5}$). Thus there is at least a 2.75-fold increase in the frequency of transposon-associated rearrangements when comparing crosses A and B, which differ only in the heterozygosity for autosomal inversions. This is comparable to the magnitude of the increase in normal crossing over. There was no increase observed in the other classes of exceptions (insertions or point mutations) in crosses A and B.

Effect of homozygosity of the X chromosome: In crosses C and D, the interchromosomal effect on classical crossing over cannot be measured because X chromosomes were homozygous. However, the numbers of exceptional eye-color offspring generated by crosses C and D were clearly lower than from cross A, yet parental females used in all three cases were heterozygous for autosomal inversions. None of the exceptions from cross C involve exchanges between repeated sequences. The exception(s) from cross D do involve repeat sequences, but they all may have been derived from a single preexisting mutation. The results of these two crosses indicate that homozygosity of the two X chromosomes significantly reduces the yield of exceptions (especially those associated with repeat sequences).

DISCUSSION

We have surveyed about four of the numbered sections of the polytene chromosome map or about 0.5% of the euchromatic genome sequences for ectopic exchange events. In relating the observed frequencies of ectopic recombination to the entire genome of natural or laboratory populations of *D. melanogaster*, it should be noted that the screen employed is sensitive primarily to deletion events. Most duplications and a large majority of inversions will not be detected. We have no evidence concerning ectopic exchanges between heterologs that would produce translocations. If such occur our screen would not detect them unless one break was within the *white* locus. Clearly the frequencies of ectopic exchanges we observed are likely to represent less than half the real values.

It might appear that the choice of *w*<sup>ec</sup>, which is associated with the insertion of *copia* and *roo* into the *white* locus, as one of the chromosomes in this study may have biased the screen toward a higher number of exchanges in the target area. However, the *copia-roo* complex was involved in only two cases, the inversion in line 21 from cross A and the deletion in line 51 of cross B. The *copia-roo* complex did appear to be the target of new transposon insertions in lines 12, 19, 52 and 53.

Of more significance is the exceptionally frequent involvement of *roo* in the ectopic exchanges observed. The numbers of *roo* elements on the two X chromosomes used in the study (26 in Oregon-R and 21 in *w*<sup>ec</sup>) are approximately twice the average number observed in natural populations (MONTGOMERY and LANGLEY 1983; MONTGOMERY, CHARLESWORTH and LANGLEY 1987; CHARLESWORTH and LAPID 1989). It is possible that the level of ectopic exchange in natural populations is somewhat lower and that the increase in copy number in these laboratory stocks reflects relaxation of various selective processes including those associated with ectopic exchange.

Interchromosomal effect: Except for the six copies of the deficiencies from cross D (lines 41–46), 18 of the 19 transposon-associated rearrangements recovered in the four experiments reported here were from cross A females that were heterozygous for the X chromosomes and for the autosomal rearrangements *SM1* and *TM3*. One was generated from cross B, utilizing female sibs that had standard autosomal configurations, which indicates that the interchromosomal effect enhances the ectopic pairing and exchange involving transposons. In view of the overall increase in crossing over in the *y*-spl region seen in cross A compared to that in cross B, a case can be made for transposon involvement being stimulated to about the same degree as crossing over in general. This strongly supports the concept that the process generating the rearrangements recovered here is meiotic and responsive to the interchromosomal effect. Only in cross D, which yielded the remaining six (or one) transposon-associated rearrangements, is there any suggestion of recovery of a cluster of exceptional offspring that would indicate a premeiotic origin. Because the six exceptional individuals from cross D, though apparently identical deletions, were recovered from four separate lines, we consider it unlikely that the deletions were premeiotic in origin. However, it is possible that all descended from a single ectopic exchange that occurred in the Ore-R strain prior to the selection of the parental females used in cross D. If this is the case, the fertility and/or viability of females heterozygous for this deletion would have to be quite low to account for the small numbers recovered (2, 2, 1 and 1) from the four separate mating groups with four to six females each.

The involvement of *roo*: Sixteen of the 25 transposon-associated rearrangements recovered from the four experiments involved copies of *roo*. The data in Table 1 show that the Oregon-R X chromosome contained 26 copies of *roo* and the *y*<sup>2</sup> *w*<sup>ec</sup> *spl ec* X chromosome had 21. The region flanking the *white* locus over which deletions extending into that gene would be viable as heterozygotes is about four numbered sections, 2 to 5, on the polytene chromosome map. The Ore-R chromosome had eight copies of *roo* in that region and the *y*<sup>2</sup> *w*<sup>ec</sup> *spl ec* chromosome had five, including the copy inserted into *copia* at coordinate
0.0 within the *white* locus. Clearly this rather large number of copies in the target region offered considerable opportunity for *roo* to be involved in rearrangements that could be recognized by our genetic screen. It is important to note that the *copy of roo* at +41 was involved in rearrangements much more often than other *roo* elements in the same region. A similar finding was reported (LIM 1988) in studies of interchromosomal exchanges involving the *hobo* element. One copy of *hobo* was a participant in every *hobo*-mediated rearrangement scored.

**Interchromosomal exchanges:** Another notable aspect is that every one of the interchromosomal exchanges recovered from cross A involved copies of *roo*, one of which was at +41 in the *w*^tr^ chromosome. These exchanges total nine in number; five of one class and two each in two other classes. Another *roo* site at −190 of the *white* locus map in the Ore-R chromosome was the corresponding copy involved in the formation of the class of five from cross A above and in all six of the exceptional offspring recovered in cross D. Because females homozygous for the Ore-R X chromosome were used in cross D, it is not possible to determine whether these 6 exceptions were from inter- or intrachromosomal exchanges.

**Intrachromosomal exchanges:** The observation that approximately half of the ectopic recombinants were intrachromosomal is of particular interest. This is likely to be an underestimate of this fraction because inversions can be generated by ectopic exchange only as intramolecular events, and only those inversions that disrupt the *white* locus would be recognized by the genetic screen employed.

The intrachromosomal exchanges utilized a diverse array of repetitive sequence sites. Each line appears to have a unique combination of breakpoints, although lines 2, 3, 4, 13, 15, 17 and 22 could possibly share the same breakpoint in the region proximal to coordinate +120 on the *white* locus map. This region lies beyond any available cloned sequences, therefore, we have not been able to map breakpoints with precision.

We have been unsuccessful in attempts to clone the breakpoints of most of the intrachromosomal deletions. This strongly suggests that those regions contain repetitive sequences that are unstable in cloned constructs. Available restriction maps of the regions of breakpoints show insertional variation, and the available clones covering some of these regions contain repetitive DNA (M. Goldberg, personal communication; Pirrotta, Hadfield and Pretorius 1983; Mariani, Pirrotta and Manet 1985). This supports our evidence that all of these breakpoints are in or proximate to repetitive sequences. We, therefore, favor the view that ectopic pairing and exchange between interspersed repeat sequences is the mechanism of their origins. We interpret these results as evidence that both inter- and intrachromosomal ectopic exchange respond to the interchromosomal effect, indicating that they are primarily meiotic in origin.

The diversity of types of intrachromosomal deletions and the complete lack of evidence of clusters lend additional weight to the view that the intrachromosomal rearrangements are meiotic in origin. It is interesting to compare these results with those reported by Engels and Preston (1984) and LIM (1988), who found predominantly (if not exclusively) premeiotic ectopic intrachromosomal exchange associated with *P* and *hobo* elements, respectively. The premeiotic nature and transposon-specificity of the events in their experiments may reflect the dysgenic properties of the stocks used and the elements involved.

**Heterozygosity per se:** Possibly the most important point to be drawn from the four experiments comes from comparisons of the frequencies and types of rearrangements from heterozygous and homozygous X chromosomes. Crosses A, C and D are equivalent in autosomal heterozygosity for *SM1* and *TM3*. The expectation was that crossing over in the target region would be stimulated equally by the interchromosomal effect in all three crosses, and that transposon-associated exchanges would be influenced accordingly. We consider it significant that neither the array nor frequencies of rearrangements expected from crosses C and D, based on what was generated from cross A, were realized. Although the interchromosomal exchange classes generated in cross A would not be duplicated in either cross C or D, it was expected that rearrangements resulting from pairing and exchange between other combinations of transposons would appear. Frequencies might be expected to be somewhat different, depending on the numbers and orientations of transposon copies. However, the numbers of exceptional offspring from both cross C and D were significantly below those recovered in cross A.

A significant point is that the intrachromosomal exchange classes seen in cross A were completely absent from crosses C and D. They would be expected to occur as they did in cross A unless homozygosity, *per se*, reduces the ectopic pairing frequency. These results focus attention on how interspersed repeat sequences become involved in ectopic pairing. It is clear that repeat sequences can search over considerable distances for a pairing partner. How this search is initiated is not clear. However, homozygosity for interspersed repeat sequences, and/or other types of sequence variation, reduces the frequency of ectopic recombination. It is probable that homozygous elements will pair with their allelic counterpart and thus be unavailable for involvement in ectopic pairing. Exchange between homozygous elements would go...
undetected. The result would be that fewer exceptions would be generated in crosses C and D.

Presumably ectopic pairing involves the establishment of a heteroduplex region, an early step prerequisite to recombination. However, our results show that ectopic exchange is not limited to interchromosomal pairing partners. The formation of intrachromosomal heteroduplexes raises interesting questions about the regulation of the recombination process, particularly with regard to sister chromatid exchanges, which go undetected genetically. Our observations are consistent with the idea that heterozygous sequence variation has the potential to modify the way that heteroduplex regions form during the recombination process.

**Comparison with yeast:** The ectopic exchange process has been studied extensively in microorganisms (PETES and HILL 1988). In Saccharomyces cerevisiae, exchange with engineered ectopic copies occurs at rates comparable to those with the homologous allele. Those results indicate that heterozygosity has little effect on ectopic exchange. Studies with artificial constructs show that heterozygous insertions are no more likely to be involved in ectopic exchange than when they are homozygous. We see no obvious reason for this discrepancy with our results.

Recent results indicate that the number or positions of available ectopic copies of a gene have little influence on the proportion of ectopic conversions or on the overall frequency of exchange (HABER et al. 1991). These authors suggest that the limiting step in recombination is the activation of a locus to be involved in exchange. The recombinationally activated allele can then pair with any one of the several copies throughout the genome. They also note that there is some hinderance to the extension of this simple model of meiotic pairing and exchange to higher eukaryotes having abundant middle repetitive dispersed DNA sequences.

In Drosophila, the region over which a sequence may search for an exchange partner appears to be quite wide within a chromosome as evidenced by recovery of a long inversion. It is possible, however, that the search may be restricted by mechanisms such as pairing site positions (HAWLEY 1980). Our results show considerable variation in the propensity of elements at different sites (even in the same family) to participate in ectopic exchange. This variation could reflect differences in chromosomal positions relative to pairing sites or inherent differences in levels of involvement in recombination among transposon insertions.

**Potential impact of translocations and other undetected types of rearrangements:** Our data indicate that inter- and intrachromosomal ectopic pairing and exchange can occur between widely separated sites in chromosomes. It should be noted, however, that these experiments were not designed to detect a large fraction of exchanges between repeat sequences in homologs. Also, recombination between nonhomologous chromosomes, an event that would have produced translocations, is not detected. Ectopic exchange has been shown to give rise to translocations in various fungi (PETES and HILL 1988). Our data do not allow assessment of the role transposons might have in the formation of translocations in Drosophila. If, however, transposons are important in the generation of translocations (as well as deletions, duplications and inversions), any estimate of the impact of transposons on fitness based on the results reported here, would have to be revised upward.

**Population genetics:** Ectopic exchange and its subsequent chromosomal rearrangements have been proposed as a general mechanism that contains copy numbers of transposable elements (LANGLEY et al. 1988). Unlike the situation with other parasites, the mechanism(s) that limits the growth of genomic parasites are obscure (CHARLESWORTH and LANGLEY 1986; CHARLESWORTH and LANGLEY 1989).

The results of our study here have three important implications for evaluating the possible role of ectopic exchange as a population genetic mechanism in the containment of copy number. (1) Different sites may have different propensities for involvement in ectopic exchange. Such a possibility has not been incorporated into the modelling. (2) The significance of the interchromosomal effect on ectopic exchange, normal exchange and disjunction is not clear. The fact that inversion heterozygosity is common in some species, while absent in others, should be considered in the interpretation of the differences in the distribution of transposable elements in various species. (3) Our observations of little or no ectopic exchange in the homozygous X chromosomes suggest that once an insertion reaches high frequency, the selection associated with ectopic exchange would favor the insertion-bearing chromosome (because of the high proportion as homozygotes). Thus the dynamics of such insertions would be quite different in small populations (in which they might drift into high frequency) compared with large populations (in which they would remain rare and thus heterozygous).

The allelic frequencies of middle repetitive insertions in natural populations are not known for many species. It is tempting, however, to view the contrast between D. melanogaster (insertions usually very rare) and humans (insertions usually fixed) in light of the differences in their population sizes, genetic drift, and the effect of heterozygosity on ectopic exchange.

One curious result from the surveys in natural populations of the densities of transposable elements in the D. melanogaster euchromatic genome, was their...
accumulation in the centromere-proximal regions where regular exchange is reduced (Charlesworth and Langley 1989; Charlesworth and Lapid 1989). However, no increase in density of elements was observed near the telomeres where regular crossing over is also reduced. It will be important to determine if ectopic exchange is not reduced in the telomeric region. The available evidence indicates that the interchromosomal effect is greatest near the telomere (Lucchesi 1976) which would suggest, in light of our results, that ectopic exchange may be quite high throughout the telomeric region when there is inversion heterozygosity in the other chromosomes, a common state in nature.

Our results demonstrate that ectopic exchange must be considered as a major source of spontaneous mutation in Drosophila. The underlying mechanisms may be investigated utilizing meiotic mutants. The role of heterozygosity and the interchromosomal effect deserve further attention. Finally these results raise new questions about the impact of ectopic exchange and naturally occurring polymorphisms (base-pair, insertion, and karyotypic) on the stability and evolution of genomes.

We thank Mike Goldberg, Vince Pirrotta, Mike Young and Linda Ambrosio for providing cloned DNA used in the analyses of rearrangements; Walter Eanes and Cedric Wesley for identifying a repetitive sequence by in situ hybridization; Mike Goldberg, Mike Resnick and Bob Voelker for helpful comments and discussion; and Jan Capps, Cindy Baker and Delores Hovey for excellent technical assistance.

LITERATURE CITED


Communicating editor: M. J. SIMMONS