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Recombination Can Initiate and Terminate at a Large Number of Sites Within the *rosy* Locus of *Drosophila melanogaster*

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Manuscript received September 16, 1987
Accepted November 2, 1987

ABSTRACT

This report presents the results of a recombination experiment designed to question the existence of special sites for the initiation or termination of a recombination heteroduplex within the region of the *rosy* locus. Intragenic recombination events were monitored between two physically separated *rosy* mutant alleles ry^{301} and ry^{2} utilizing DNA restriction site polymorphisms as genetic markers. Both ry^{301} and ry^{2} are known from previous studies to be associated with gene conversion frequencies an order of magnitude lower than single site mutations. The mutations are associated with large, well defined insertions located as internal sites within the locus in prior intragenic mapping studies. On the molecular map, they represent large insertions approximately 2.7 kb apart in the second and third exons, respectively, of the XDH coding region. The present study monitors intragenic recombination in a mutant heterozygous genotype in which DNA homology is disrupted by these large discontinuities, greater than the region of DNA homology and flanking both sides of the locus. If initiation/or termination requires separate sites at either end of the locus, then intragenic recombination within the *rosy* locus of the heterozygote should be eliminated. Contrary to expectation, significant recombination between these sites is seen.

CLASSICAL genetic theory postulates that heterozygous chromosomal rearrangements inhibit meiotic recombination for some distance beyond the rearrangement breakpoints by disrupting the intimate pairing necessary for homologous exchange to occur. This notion derives from extensive classical recombination data as well as cytological observations in higher eukaryotes such as Drosophila and maize. The notion that synapsis is disrupted in the vicinity of the breakpoints of chromosomal rearrangements leads to the prediction that if one analysed intragenic recombination, intragenic deletions and insertions would similarly inhibit exchange in adjacent regions of the same gene. However, in prokaryotes, recombination occurs readily between nucleotide substitution mutations and non-overlapping deletion mutations within the same gene. Similarly, in *Drosophila melanogaster*, intragenic deletions and insertions within the *rosy* locus do not appear to disrupt the recovery of crossovers between *rosy* point mutations and the boundary of the insertion or deletion heterozygosity. The only dramatic effects of *rosy* locus insertion/deletions are that large insertion/deletions (of several hundred nucleotides or larger) convert less readily than do point mutations within the locus (Chovnick, Ballantyne and Holm 1971; Gelbart *et al.* 1974; Gelbart, McCarron and Chovnick 1976; Clark *et al.* 1986).

Despite many years of extensive investigation, the mechanism(s) underlying meiotic or general recombination remain as a major unsolved problem area of genetics and molecular biology. Most of the genetic data upon which contemporary models have been developed (*e.g.*, Meselson and Radding 1975; Szostak *et al.* 1983) derive from studies in several fungal systems and prokaryotes (see reviews, *Cold Spring Harbor Symposia in Quantitative Biology* 1978; 1984). In higher metazoa, the only substantial body of data has come from recombination studies involving the *rosy* locus of *Drosophila melanogaster* (see review in Hilliker and Chovnick 1981). As noted (*op. cit.*), intragenic recombination in Drosophila appears to be consistent with the fungal data, and in particular, with the view that general recombination involves conversion (*i.e.*, a nonreciprocal transfer of information) in the immediate region of each exchange event, and that a fraction of these events are seen as crossovers for flanking outside markers. Operationally, in random strand mutant heteroallele recombination experiments, we classify recombinants as either crossovers (for flanking markers), or conversions (noncrossovers for flanking markers).

Hastings (1987) has reviewed the current state of models of recombination and has presented further evidence in support of those models involving the formation of a heteroduplex intermediate. Gene conversion is seen as correction of the heteroduplex by excision of the region of mismatch followed by repair

synthesis, while postmeiotic segregation represents the failure of correction. Indeed, postmeiotic segregation has been seen in Drosophila only in the presence of an excision repair defective mutation (CARPENTER 1982, 1984).

The present report is focused upon the notion that recombination may be polar in that it may initiate or terminate at fixed sites in the DNA. This notion developed first from studies of conversion in fungal systems which exhibited allele conversion frequency gradients (*i.e.*, polarity) paralleling map order (LISSOUBA *et al.* 1962; MURRAY 1963). It is assumed generally, that recombination initiates from nicks in the DNA, followed by limited nuclease digestion and eventual repair replication. In the event of replication copying from a nonsister DNA strand in a heterozygote, a region of heteroduplex mismatch may result. Polarity of mismatch correction (*i.e.*, conversion) has been taken to imply the existence of special fixed sites in the DNA for initiation or termination of the heteroduplex. A molecular model for such special sites in eukaryotic recombination is provided by the Chi sites of lambda phage (STAHL 1979). Effort to examine the possibility of polarity of conversion frequency directly in the *rosy* system (HILLIKER and CHOVNICK 1981) was frustrated by limitations inherent to the Drosophila intragenic recombination system. We are unable to examine all of the products of a single meiotic event as in fungal tetrad analysis, and our large scale selective systems permit recovery only of wild-type recombinants. Nonetheless, the present study is able to question the possible existence of special sites for initiation or termination of a recombination heteroduplex in the vicinity of the *rosy* locus.

Most of the *rosy* locus mapping data is limited to a 5-kb segment of the gene corresponding to most of the XDH coding region (HILLIKER and CHOVNICK 1981; CLARK *et al.* 1986), for which the DNA sequence is known (KEITH *et al.* 1987; LEE *et al.* 1987). In order to test for the existence of special sites for initiation or termination of recombination in *D. melanogaster*, we have assayed for exchange between two *rosy* mutant alleles (ry^{301} and ry^{2}) that are physically separate and are associated with large, well defined insertions. These insertions occur within polypeptide coding regions of the gene and convert far less readily than do point mutations (CLARK *et al.* 1986; COTE *et al.* 1986). Moreover, both are internally located within the recombinational or genetic map of *rosy* locus mutations (CLARK, HILLIKER and CHOVNICK 1986; CLARK *et al.* 1986). If (1) special sites for the initiation or termination of a recombination heteroduplex exist on either side of the locus, and if (2) all crossovers involve a conversion event in the immediate region of each exchange, then we would expect that the reduced conversion seen with these insertion mutations would be paralleled by an elimination of crossing over between these mutant sites.

MATERIALS AND METHODS

Chromosomes, balancers and mutations: Several abbreviated designations are employed. These include *MKRS* = *Tp(3)MKRS, M(3)S34 kar* ry^{2} *Sb*; *P18* = *In(3L)P* + *In(3R)P18, Ubx* ry^{41} *kar*, e^{4}; ry^{301} is a 7.0-kb *calypso* insertion (CLARK *et al.* 1986; COTE *et al.* 1986) located in exon 2 of the rosy locus (LEE *et al.* 1987). ry^{2} is an 8.5-kb *B104* insertion located in exon 3 of the *rosy* locus, approximately 2.7 kb downstream of the ry^{301} insertion (COTE *et al.* 1986; LEE *et al.* 1987). *Df(3R)*ry^{36} is a *rosy* region deficiency that completely lacks the 8.1-kb *Sal*I fragment that includes the entire *rosy* locus as well as adjacent non-*rosy* DNA (CLARK and CHOVNICK 1986). Additional information concerning these chromosomes and mutations may be found in LINDSLEY and GRELL (1968), and HILLIKER *et al.* (1980).

Probes: Plasmid constructs employed to generate *rosy* locus probes may be found in CLARK and CHOVNICK (1986). Plasmid DNA was isolated by standard ethidium bromide, cesium chloride equilibrium centrifugation following sodium dodecyl sulfate (SDS) lysis of chloramphenicol amplified, plasmid bearing bacteria. (MANIATIS, FRITSCH and SAMBROOK 1982). The 1.4-kb *Bgl*II fragment utilized as a probe was liberated from the plasmid by appropriate endonuclease digestion. Liberated fragments were separated by agarose gel electrophoresis and were recovered from the gel by electrophoresis onto ion exchange paper (NA 45 Schleicher & Schuell, Keene, New Hampshire). The bound DNA was recovered by elution from the ion exchange paper and ethanol precipitation (DRETZEN *et al.* 1981). ^{32}P-labeled DNA was prepared utilizing the method of RIGBY *et al.* (1977).

DNA extraction and Southern analysis: DNA was extracted from adult flies following the method of CLARK *et al.* (1984). Procedures for Southern analysis are described in RUSHLOW, BENDER and CHOVNICK (1984) and CLARK *et al.* (1984).

Selective system mating system: Selective system matings were carried out following a purine selection protocol suitable for discriminating between XDH^{-} and wild-type levels of activity (CHOVNICK 1973).

The experimental system: Mutant heteroallele females, ry^{301}/ry^{2}, were crossed in large scale fine structure mapping experiments to tester males *P18/MKRS*, and their progeny were reared on purine supplemented selective medium. Figure 1 illustrates this mating and indicates the relative positions within the *rosy* locus DNA of the ry^{301}, *calypso* insert, the ry^{2}, *B104* insert, as well as the distribution of heterozygous restriction sites that play a role in the analysis of the recombinants. Surviving ry^{+} exceptions, occuring as rare single individuals among the matings, were crossed to the tester stock to establish lines from which DNA is extracted and the *rosy* DNA analysed.

RESULTS AND DISCUSSION

Large scale matings were carried out following the protocol described above. Thirteen ry^{+} individuals survived the purine selective crosses in a total of 1.27 $\times 10^{6}$ sampled progeny. The survivors arose as rare, single individuals of either sex, scattered at random among the matings that comprised the experiment. Following the protocol of Figure 1, all 13 exceptional

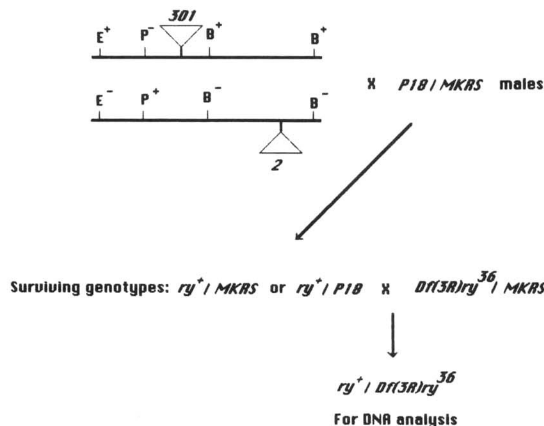

FIGURE 1.—Mating scheme employed in the recombination experiment. The relative positions within the *rosy* locus DNA of the ry^{301}, *calypso* insert, the ry^2, *B104* insert, as well as the distribution of heterozygous restriction sites that serve as unselected markers are indicated. E^+—*Eco*RII site present, E^-—*Eco*RII site absent, B^+—*Bgl*II site present, B^-—*Bgl*II site absent, P^+-*Pvu*II site present, P^-—*Pvu*II site absent.

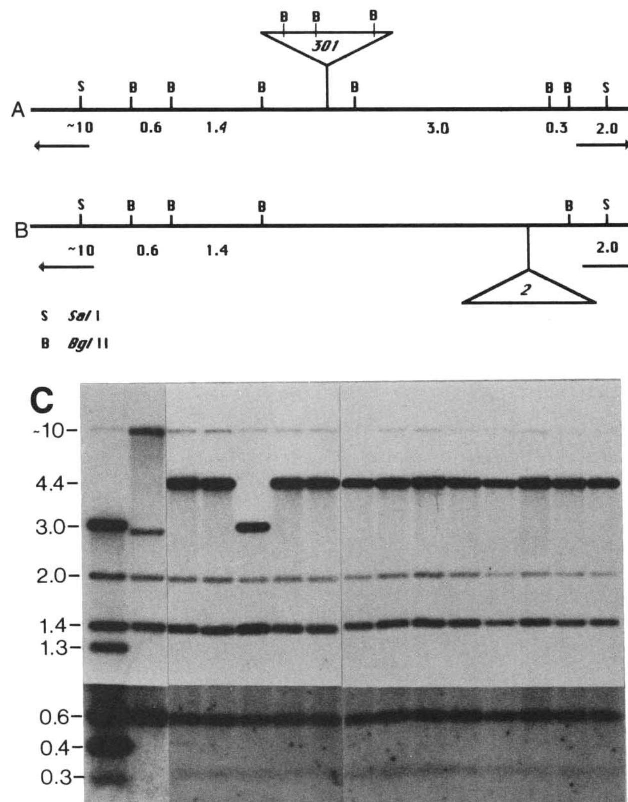

FIGURE 2.—*Bgl*II restriction analysis of ry^{301} and ry^2 bearing chromosomes and ry^+ recombinants. (A) *Bgl*II sites present in the *rosy* region of the ry^{301} bearing chromosome. The sites within the *calypso* insert of ry^{301} are taken from COTE *et al.* 1986. (B) *Bgl*II sites on the ry^2 bearing chromosome. The *Sal*I sites denote the limits of the 8.1-kb *Sal*I fragment. Intervals (in kb) between the *Bgl*II sites are indicated except for those sections involving the inserts. (C) Southern blot of *Bgl*II digested genomic DNA probed with the 8.1-kb *Sal*I *rosy* fragment which covers the entire region. Genomic digests were loaded as follows: Lane 1, ry^{301}; lane 2, ry^2; lanes 3–15 ry^+ recombinants, the single ry^{301} to ry^+ conversion is presented in lane 5 while the other lanes display crossovers. The autoradiogram of lanes 1 and 2 presents a single x-ray exposure with an intensifying screen being employed over the lower portion of the filter to darken the low molecular weight bands without overexposing the larger bands. The autoradiogram presented in lanes 3–15 is a composite of two gels and two x-ray exposures; a long exposure was employed to detect the low molecular weight bands, while a shorter exposure used for the upper portion of the gel.

ry^+ bearing chromosomes were examined for the restriction site markers (Figure 1) in DNA extracts of flies heterozygous for a complete deletion of the *rosy* locus, $Df(3R)ry^{36}$. Southern analyses of *Bgl*II, *Eco*RII and *Pvu*II digests are presented in Figures 2, 3 and 4, respectively, and the recombination data are summarized in Figure 5.

Figure 2A illustrates the *Bgl*II sites present in the *rosy* region of the ry^{301} bearing chromosome. The sites within the *calypso* insert of ry^{301} are taken from COTE *et al.* (1986). Figure 2B presents the *Bgl*II sites on the ry^2 bearing chromosome, and Figure 2C presents a Southern blot of *Bgl*II digested genomic DNA extracts probed with the 8.1-kb *Sal*I *rosy* fragment which covers the entire region. The extracts of the parental ry^{301} and ry^2 are included as well as each of the 13 recombinants. Points to be noted are: (1) both of the parental lanes and all recombinants exhibit *Bgl*II fragments of ~10 kb, 2.0 kb, 1.4 kb and 0.6 kb (Figure 2C). These reflect identical *Bgl*II sites within the *rosy* region DNA of both ry^{301} and ry^2 (Figure 2, A and B). (2) The rightmost heterozygous marker in the cross is seen as a 0.3-kb fragment present in the parental ry^{301} lane and absent in the ry^2 lane. All 13 recombinants possess this fragment indicating that they are B^+ for the rightmost, heterozygous flanking marker in the cross (Figures 1 and 5). (3) The internal *Bgl*II marker present immediately to the right of the ry^{301} insert and absent on the ry^2 DNA is responsible for an intense band at 3.0 kb in the ry^{301} lane and in one of the recombinants (Figure 2C lane 5). (4) The ry^{301} lane exhibits two unique bands (0.4 kb and 1.3 kb) reflecting *Bgl*II sites within the *calypso* insert which produce fragments carrying *rosy* DNA. Similarly, the *B104* insert of ry^2 (Figure 2C lane 2) produces one fragment that intensifies the ~10-kb band, and a second fragment just under 3.0 kb.

We next consider the leftmost heterozygous flanking marker in the cross, the *Eco*RII site located to the left of ry^{301}, and absent in the ry^2 DNA. This heterozygosity is located within the 1.4-kb *Bgl*II DNA segment common to both the ry^{301} and ry^2 DNA (Figures 2 and 3). Figure 3, A and B, illustrates the distribution of *Eco*RII sites in the immediate region of the 1.4-kb *Bgl*II fragment, and illustrate the fragments to be expected in an *Eco*RII digest probed with the 1.4-kb *Bgl*II fragment. Figure 3C demonstrates that the ry^2 DNA produces 1 fragment of 2

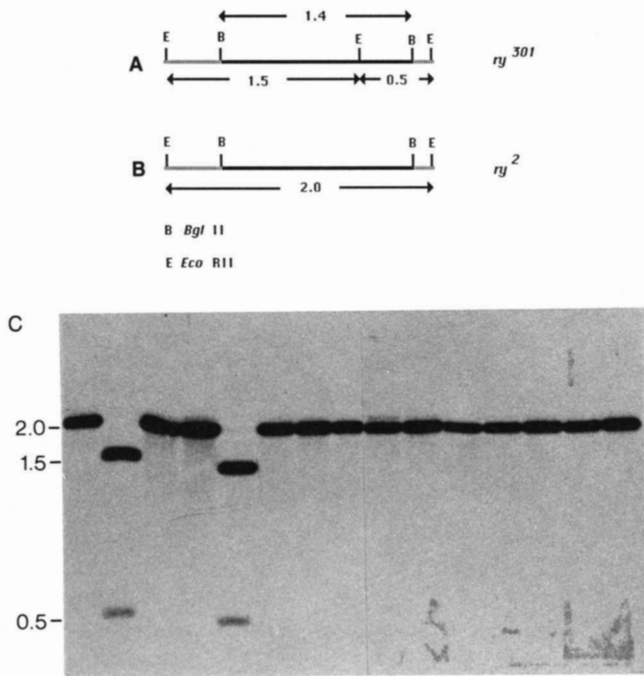

FIGURE 3.—*Eco*RII restriction analysis of ry^{301} and ry^2 bearing chromosomes and ry^+ recombinants. (A and B) Distribution of *Eco*RII sites in the immediate region of the 1.4-kb *Bgl*II fragment in ry^{301} and ry^2, respectively. Also depicted are the fragments expected in a whole genome Southern analysis of *Eco*RII digested DNA probed with the 1.4-kb *Bgl*II fragment (indicated by solid line). (C) Southern blot of *Eco*RII digested genomic DNA probed with the 1.4-kb *Bgl*II fragment. Genomic digests were loaded as follows: Lane 1, ry^2; lane 2, ry^{301}; lanes 3–15 ry^+ recombinants, the single ry^{301} to ry^+ conversion is presented in lane 5 while the other lanes display crossovers. This autoradiogram is a composite of two gels.

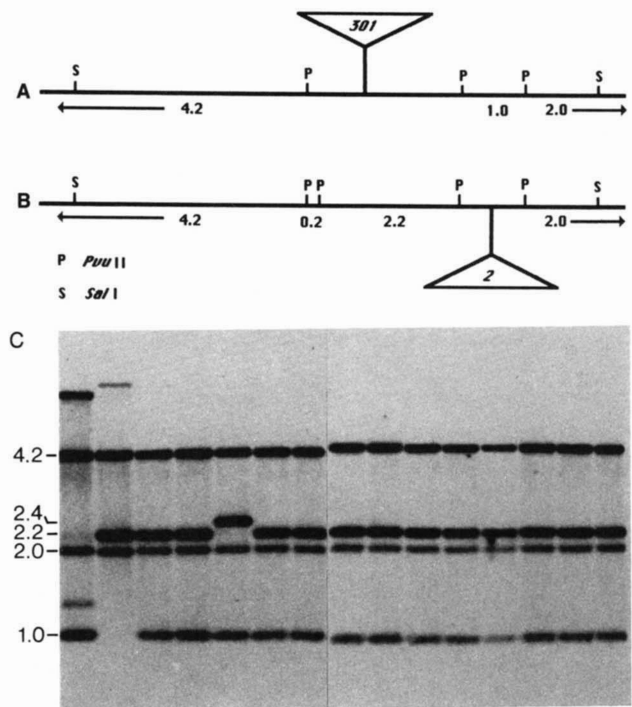

FIGURE 4.—*Pvu*II restriction analysis of ry^{301} and ry^2 bearing chromosomes and ry^+ recombinants. (A and B) Distribution of *Pvu*II sites present in the *rosy* region indicating the relative positions of ry^{301} and ry^2, respectively. The *Sal*I sites denote the limits of the 8.1-kb *Sal*I fragment. Intervals (in kb) between the *Pvu*II sites are indicated except for those sections involving the inserts. (C) Southern blot of *Pvu*II digested genomic DNA extracts probed with the 8.1-kb *Sal*I *rosy* fragment which covers the entire region. Genomic digests were loaded as follows: Lane 1, ry^{301}; lane 2, ry^2; lanes 3–15 ry^+ recombinants, the single ry^{301} to ry^+ conversion is presented in lane 5 while the other lanes display crossovers. This autoradiogram is a composite of two gels.

kb, while the ry^{301} DNA produces two fragments, one of 1.5 kb and the other of 0.5 kb. Figure 3C presents a blot which indicates, as well, the *Eco*RII digestion results for all recombinants. Of interest is that 12 of the recombinants are E^- like the ry^2 parental DNA. Since all of the recombinants carry the B^+ flanking marker at the right end of the region like the ry^{301} parental DNA (Figure 2C), then the 12 E^- recombinants are crossovers for the flanking outside markers (Figure 5). The single E^+ recombinant, which is also B^+ for the right end flanking marker, thus carries both of the ry^{301} parental outside flanking markers, and is classified as a conversion of ry^{301} to ry^+ (Figure 5).

Consider next the 13 recombinant lines of Figure 2C. Twelve of the recombinants possess a 4.4-kb *Bgl*II fragment which indicates that the crossovers were all located in the interval between the *Bgl*II site to the right of the ry^{301} insert (Figure 2A), and to the left of the ry^2 insert (Figure 2B). The remaining recombinant is the one that possesses the parental outside flanking markers and has been classified as a conversion. This recombinant does not produce a 4.4-kb fragment, but rather has a 3.0-kb fragment like the ry^{301} parent. Hence, we conclude that the

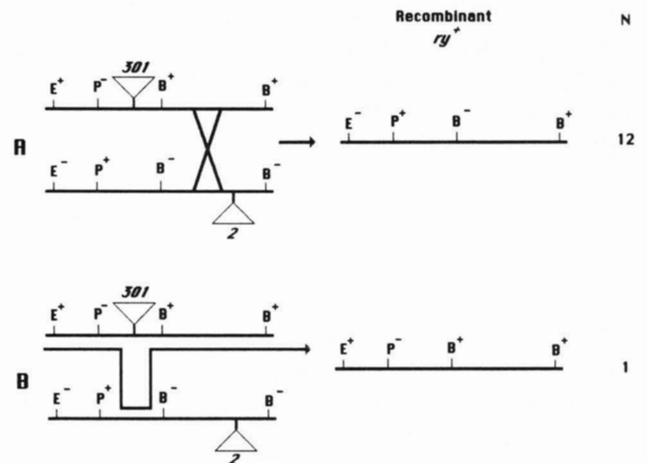

FIGURE 5.—Summary of recombination analysis. (A) The ry^{301}/ry^2 female heterozyogote and the ry^+ recombinant resulting from a crossover event between ry^{301} and ry^2. (B) The ry^{301}/ry^2 female heterozyogote and the ry^+ recombinant that results from a conversion of ry^{301} to ry^+. The relative positions within the *rosy* locus DNA of the ry^{301}, *calypso* insert, the ry^2, *B104* insert, as well as the distribution of heterozygous restriction sites that serve as unselected markers are indicated. E^+-*Eco*RII site present, E^--*Eco*RII site absent, B^+-*Bgl*II site present, B^--*Bgl*II site absent, P^+-*Pvu*II site present P^--*Pvu*II site absent. The number of recombinants in each class is indicated.

TABLE 1

Recombination map distance *vs.* kilobase distance in mutant heteroallele recombination tests involving site mutations and large insertion mutations

Mutant heteroallele recombination test	No. of ry$^+$ crossovers	N ($\times 10^{-6}$)	Map distance cM($\times 10^3$)	Distance estimate[a] kb ($\times 10^{-3}$)	cM/kb ($\times 10^6$)
ry^{606}/ry^{5205} [b]	16	0.26	12.3	4.0	3.08
ry^{606}/ry^{3} [c]	46	2.53	3.64	2.7	1.35
ry^{301}/ry^{2}	12	1.27	1.89	2.7	0.70

[a] Estimates from data presented in LEE *et al.* (1987); KEITH *et al.* (1987).
[b] Data from CLARK, HILLIKER and CHOVNICK (1986).
[c] Data from CLARK *et al.* (1986).

DNA segment of the conversion of ry^{301} to ry^+ did not extend to the *Bgl*II site immediately to the right of the *calypso* insert on the ry^{301} chromosome. The converted DNA exhibits this ry^{301} parental B$^+$ marker (Figures 1 and 5).

By virtue of the *Pvu*II heterozygosity immediately to the left of the *calypso* insert of ry^{301} (Figure 1), we are able to further limit the extent of this conversion event. Figure 4, A and B, presents the distribution of *Pvu*II sites present in the *rosy* region indicating the relative positions of ry^{301} and ry^2 respectively. Intervals between the *Pvu*II sites are indicated except for those sections involving the inserts. Consider the 13 recombinant lanes on the *Pvu*II blot (Figure 4C). The 12 lanes of DNA from the crossovers exhibit identical Southern analyses with fragments of 1.0 kb, 2.0 kb, 2.2 kb, and 4.2 kb, further confirming that the crossovers occurred between the ry^{301} and ry^2 insertions. The DNA from the conversion is missing the 2.2-kb band, but exhibits one at 2.4 kb indicating that it is P$^-$ for the *Pvu*II site immediately to the left of the ry^{301} insert, like the ry^{301} parental DNA. Hence the conversion event of ry^{301} to ry^+ essentially removed the *calypso* insert and did not extend sufficiently into the *rosy* DNA to include the immediate markers flanking the insert, a distance of approximately 400 bases. The missing 2.2-kb and 1.0-kb bands in the *Pvu*II digests of ry^{301} and ry^2 (Figure 4C) indicate the locations of insertion. The new bands in each case reflect the presence of *Pvu*II sites within the inserts.

The recombination analysis is summarized in Figure 5. From 1.27×10^6 progeny screened, 13 ry^+ recombinant chromosomes were recovered. Utilizing heterozygosity for restriction sites as genetic markers, 12 are clearly crossovers (yielding a map distance of 0.00189 cM), while only one recombinant is a conversion of ry^{301} to ry^+. The conversion frequencies are consistent with our prior observations on these mutant alleles (CLARK *et al.* 1986) when tested against site mutations ($ry^{301} = 0$ in 5.16×10^6 progeny sampled; $ry^2 = 2$ in 6.25×10^6 progeny). The surprising result is the recovery of significant numbers of crossovers within the 2.7-kb region of homology flanked by large heterozygous, non-*rosy* DNA insertions. Thus, for the DNA segment under investigation, only 14.8% of that DNA is homologous [2.7/(7.0 + 2.7 + 8.5)]. Clearly, the present data exclude the possibility of a single site on either side of the locus for initiation or termination of heteroduplexes. Rather, recombination may begin at a large number of sites within the *rosy* locus, and produce heteroduplexes that may terminate at a large number of sites. Those heteroduplexes that straddle one or the other insertion would lead, largely to apparent failure of recombination. On such a model, some reduction of crossovers due to the large heterozygous insertions should occur. That this, in fact, is the case, may be seen from an examination of the data summarized in Table 1. Row 1 (Table 1) summarizes recombination involving the allele complementing site mutations ry^{606}, located near the 5′ end of exon 2 and ry^{5205} which is close to the 3′ end of the gene. Considering this to be baseline normal crossing over in terms of cM/kb, we note that reduced exchange is observed in row 2 (Table 1) for the cross involving ry^{606} and ry^3 which is associated with an insertion of the 8.0-kb transposable element, *B104* located in intron 3 (COTE *et al.* 1986; LEE *et al.* 1987). However, when both mutant alleles are associated with large insertions as in the present study (row 3, Table 1), even more dramatic reduction of crossovers is seen.

The authors are pleased to acknowledge research support from the American Cancer Society (NP 491 and NP 568) to S.H.C., a grant from the Natural Science and Engineering Research Council of Canada to A.J.H., and research grant, GM-09886, from the U.S. Public Health Science to A.C.

LITERATURE CITED

CARPENTER, A. T. C., 1982 Mismatch repair, gene conversion, and crossing over in two recombination defective mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 5961–5965.

CARPENTER, A. T. C., 1984 Meiotic roles of crossing-over and of gene conversion. Cold Spring Harbor Symp. Quant. Biol. **49:** 23–29.

CHOVNICK, A., 1973 Gene conversion and transfer of genetic information within the inverted region of inversion heterozygotes. Genetics **75:** 123–131.

CHOVNICK, A., G. H. BALLANTYNE and D. G. HOLM, 1971 Studies of gene conversion and its relationship to linked exchange in *Drosophila melanogaster*. Genetics **69:** 179–209.

CLARK, S. H., and A. CHOVNICK, 1986 Studies of normal and position-affected expression of *rosy* region genes in *Drosophila melanogaster*. Genetics **112:** 819–840.

CLARK, S. H., A. J. HILLIKER and A. CHOVNICK, 1986 Genetic analysis of the right (3′) end of the rosy locus in *Drosophila melanogaster*. Genet. Res. **47:** 109–116.

CLARK, S. H., S. B. DANIELS, C. A. RUSHLOW, A. J. HILLIKER and A. CHOVNICK, 1984 Tissue-specific and pretranslational character of variants of the rosy locus control element in *Drosophila melanogaster*. Genetics **108:** 953–968.

CLARK, S. H., M. MCCARRON, C. LOVE and A. CHOVNICK, 1986 On the identification of the *rosy* locus DNA in *Drosophila melanogaster*: intragenic recombination mapping of mutations associated with insertions and deletions. Genetics **112:** 755–767.

COTE, B., W. BENDER, D. CURTIS and A. CHOVNICK, 1986 Molecular mapping of the *rosy* locus in *Drosophila melanogaster*. Genetics **112:** 769–783.

DRETZEN, G., M. BELLARD, P. SASSONE-CORSI and P. CHAMBON, 1981 A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112:** 295–298.

GELBART, W., M. MCCARRON and A. CHOVNICK, 1976 Extension of the limits of the XDH structural element in *Drosophila melanogaster*. Genetics **84:** 211–232.

GELBART, W., M. MCCARRON, J. PANDEY and A. CHOVNICK, 1974 Genetic limits of the xanthine dehydrogenase structural element in *Drosophila melanogaster*. Genetics **78:** 869–886.

HASTINGS, P. J., 1987 Meiotic recombination interrepted as heteroduplex correction. In: *Meiosis*, Edited by P. B. MOENS. Academic Press, London.

HILLIKER, A. J., and A. CHOVNICK, 1981 Further observations on intragenic recombination in *Drosophila melanogaster*. Genet. Res. **38:** 281–296.

HILLIKER, A. J., S. H. CLARK, A. CHOVNICK and W. M. GELBART, 1980 Cytogenetic analysis of the chromosomal region immediately adjacent to the rosy locus in *Drosophila melanogaster*. Genetics **95:** 95–110.

KEITH, T. P., M. A. RILEY, M. KREITMAN, R. C. LEWONTIN, D. CURTIS and G. CHAMBERS, 1987 Sequence of the structural gene for xanthine dehydrogenase (*rosy* locus) in *Drosophila melanogaster*. Genetics **116:** 67–73.

LEE, C. S., D. CURTIS, M. MCCARRON, C. LOVE, M. GRAY, W. BENDER and A. CHOVNICK, 1987 Mutations affecting expression of *rosy* locus in *Drosophila melanogaster*. Genetics **116:** 55–66.

LINDSLEY D. L., and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LISSOUBA, P., J. MOUSSEAU, G. RIZET, J.-L. ROSSIGNOL, 1962 Fine structure of genes in the ascomycete *Ascobolus immersus*. Adv. Genet. **11:** 343–380.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MESELSON, M., and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

MURRAY, N. E. 1963 Polarized recombination and fine structure within the *me-2* gene of *Neurospora crassa*. Genetics **48:** 1163–1183.

RIGBY, P. W. J., M. DIEKMANN, C. RHODES and P. BERG, 1977 Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:** 237–251.

RUSHLOW, C., W. BENDER and A. CHOVNICK, 1984 Studies on the mechanism of heterochromatic position effect at the rosy locus in *Drosophila melanogaster*. Genetics **108:** 603–615.

STAHL, F. W., 1979 *Genetic Recombination. Thinking about It in Phage and Fungi*. W. H. Freeman, San Francisco.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

Communicating editor: W. M. GELBART