Gene Conversion in Drosophila and the Effects of the Meiotic Mutants *mei-9* and *mei-218*

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ABSTRACT

Simple meiotic gene conversion tracts produced in wild-type females were compared with those from two meiotic mutants, *mei-9* and *mei-218*. The positions and lengths of conversion tracts were determined by denaturing gradient gels and DNA sequencing. Conversion tracts in wild type averaged 885 base pairs in length, were continuous, and displayed no obvious hot spots of initiation. Some unusual conversion events were found in the *mei-218* and *mei-9* samples, although most events were indistinguishable from wild-type tracts in their length and continuity.

GENE conversion is the result of nonreciprocal transfer of genetic information from one chromatid to another, and can occur in association with, or in the absence of crossing over. Conversion results in the 3:1 segregation of a marker in a meiotic tetrad, and to identify such segregation unambiguously requires analysis of all four members of the tetrad. Since this is not possible in Drosophila, simple conversion events are operationally identified as the class of intragenic recombinants without associated flanking marker exchange (CHOVNICK, BALLANTYNE and HOLM 1971), using flanking markers that are close enough to preclude the occurrence of double crossover events, yet far enough away to make co-conversion unlikely.

The *rosy* locus of Drosophila provides a suitable selective system for the recovery of meiotic recombination events (CHOVNICK, BALLANTYNE and HOLM 1971). The *rosy* gene encodes the enzyme xanthine dehydrogenase, and mutants in the gene are much more sensitive than wild type to purine added to the culture medium. This biochemical selection allows the recovery of intragenic recombination events, including both crossovers and simple conversions, which occur at a frequency of approximately 10^{-5}.

In a recent study (CURTIS *et al.* 1989), we compared two classes of gene conversion tracts in *rosy*, with and without associated crossing over. The lengths of simple gene conversion tracts were longer [826-base pair (bp) average for 12 events, see MATERIALS AND METHODS] than crossover-associated tracts (343-bp average for four events), but the two classes were indistinguishable in other respects. Most of these recombinants came from a single experiment, and it seemed important to examine a larger set of gene convertants obtained using different markers within the *rosy* locus and in different genetic backgrounds.

In a study on the effects of meiotic mutations on intragenic recombination, CARPENTER (1984) used the purine selection technique to isolate recombinants at *rosy* in a wild-type genetic background and in the presence of two meiotic mutations, *mei-9* and *mei-218*. These mutations each decrease by about 12-fold the frequency of meiotic crossing over in the genome without decreasing the frequency of simple gene conversion (CARPENTER 1982). *mei-9*, in addition, is defective in the somatic repair of DNA damage (BOYD, GOLINO and SETLOW 1976; BAKER, CARPENTER and RIPOLL 1978) and gives frequent postmeiotic segregation (ROMANS 1980a,b; HILLIKER and CHOVNICK 1981; CARPENTER 1982), as well as reverse crossover events (crossovers with reversed flanking marker configurations) (CARPENTER 1982). The *rosy* mutant alleles in this experiment, ry^{41} and ry^{502}, were induced on different wild-type background chromosomes, ry^{+0} and ry^{+5}. These wild-type alleles carry polymorphisms in the *rosy* gene which cause the xanthine dehydrogenase (XDH) protein products to migrate with different electrophoretic mobilities (MCCARRON *et al.* 1979). CARPENTER used this property to examine the co-conversion of mutant sites and electrophoretic determinant sites in the various convertants. She concluded that the average co-conversion tract length in *mei-218* appeared to be half as long as the average from wild type or *mei-9*. However this technique provided only an approximate estimate of tract lengths, because the exact sequences of the two alleles, including the locations of the *rosy* mutations and electrophoretic sites, were not known.

DNA sequence polymorphisms between the parental chromosomes can serve as unselected markers to define the parental identity of the DNA in recombinants. In the previous study (CURTIS *et al.* 1989), denaturing gradient gel electrophoresis and DNA sequencing were used to follow polymorphisms, and

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thus to map gene conversion tracts and crossover junctions. Some events were analyzed by both techniques, and the resolution of the denaturing gradient gel system in determining the endpoints of conversion tracts was comparable to that of DNA sequence analysis. We have now measured the physical lengths of the simple gene conversion tracts recovered by CARPENTER (1984). Results are presented from both the wild-type and mutant-derived events. The spectrum of events obtained is discussed in terms of models for recombination and the roles of the *mei-218* and *mei-9* genes in this process.

MATERIALS AND METHODS

Isolation of recombinants: The isolation of the recombinant chromosomes and their genetic characterization has been described (CARPENTER 1984). The *mei-9* alleles used were $mei\text{-}9^a$ and $mei\text{-}9^b$, either homozygous or in transheterozygous combination. Because the results obtained from the three combinations were genetically indistinguishable (CARPENTER 1984), the specific allelic sources of the *mei-9* events are not distinguished here.

DNA isolation: Genomic DNA was prepared from flies carrying a recombinant chromosome over $Df(3R)ry^{36}$, which removes the entire *rosy* region (COTE *et al.* 1986). DNA was prepared as described (BENDER, SPIERER and HOGNESS 1983) with the addition of phenol/chloroform and chloroform extraction steps.

Denaturing gradient gel electrophoresis: Procedures were as described (CURTIS *et al.* 1989; GRAY *et al.* 1991), with the addition of an acid depurination step to enhance the electrophoretic transfer of DNA fragments: the gels were treated for 10 min in 0.25 N HCl prior to alkali denaturation. Three different ranges of denaturant were used, depending on the regions of the *rosy* gene to be examined: 20–80%, 25–85% and 40–90%. Blots were probed with random-primed, isolated fragment probes (SAMBROOK, FRITSCH and MANIATIS 1989) from the *rosy* region. Blots with bound probe were kept moist so that probes could be stripped off and the blots reprobed. Probes were removed by boiling blots for 15 min in 0.01 × SSPE, 0.5% SDS. Ten or more cycles of reprobing did not result in any noticeable loss of signal.

Several small probes specific for single fragments were used to identify bands unambiguously on gradient blots. Two adjacent fragments were incorrectly ordered in our previous study (CURTIS *et al.* 1989) (fragments identified as C and D in Figure 3). We have corrected the lengths assigned to the affected simple gene conversion events (CURTIS *et al.* 1989, Table 1), and these are: #28: 3246 bp, #29: 1209 bp, #31: 1599 bp and #32: 832 bp. In addition, the tract length for event #30 has been more precisely determined by DNA sequencing. The conversion included the ry^{406} mutant site and three adjacent polymorphisms, giving a median tract length of 281 bp. These corrections yield a mean length of 1266 ± 814 bp and a harmonic mean length of 826 bp, instead of 1208 ± 790 bp and 752 bp as previously reported for these 12 events. Although the numbering of these events (#28–32) overlaps with the numbering of new events presented in Figure 1 of this report, the previously reported events are independent and should not be confused with the results presented here.

Some ry^{41} conversion events extended beyond the end of the sequenced *rosy* *Hin*dIII fragment. Endpoints of these events could nevertheless be determined based on shifts in certain restriction fragments on gradient gels. The restriction map of the *snake* region was derived from the DNA sequence provided by ROB DELOTTO (unpublished results). This map was confirmed for the ry^{41} and ry^{502} alleles by restriction mapping with standard genomic southern blots. Endpoints of conversion tracts in this region are taken as the ends of shifted restriction fragments, and thus the resolution of the endpoints is not as good as in regions where the exact locations of polymorphisms are known from the sequence. This uncertainty probably inflates the length measurement for the affected events, and in particular for event #45.

Polymerase chain reaction: Polymerase chain reaction (PCR) amplifications of various segments of the *rosy* gene were carried out on some of the recombinant and background chromosomes. Genomic DNA was isolated from stocks hemizygous for the desired *rosy* allele, as described above. Two pairs of primers were used which hybridize to opposite strands of the sequence at a spacing of 2.1 or 2.6 kb (see Figure 1). Reactions were done according to a protocol suggested by STAN TABOR. The reaction mix included: 0.25 μg genomic DNA, 5 mM $MgCl_2$, 5 mM isocitrate, 20 mM MOPS, pH 8.1, 300 μM each dNTP, 2.5 pmol (0.1 μM) each primer, 0.01 mg/ml gelatin, 2.5 units Amplitaq enzyme (U.S. Biochemical) and water to a final volume of 50 μl. The reactions were overlaid with 50 μl paraffin oil and cycled on a Perkin Elmer thermocycler for 30 cycles (94°, 1′; 55°, 1′; 72°, 3′). After amplification, the reaction products were treated with Klenow enzyme to fill in any incomplete duplex.

DNA sequencing: In order to sequence across the gene conversion tracts in some convertants, and across the ry^{41} and ry^{502} mutations, we cloned subfragments of the PCR generated DNA fragments. The PCR amplified products were digested with either *Eco*RI and *Nru*I, or *Nde*I and *Hin*dIII to give fragments of 1909 and 2078 bp, respectively. These fragments were gel isolated and cloned into pEMBL vectors (DENTE, CESARANI and CORTESE 1983). Plasmid DNAs were obtained from 1.5-ml cultures by simple akaline lysis (SAMBROOK, FRITSCH and MANIATIS 1989) and sequenced directly. Several isolates of each clone were prepared independently and pooled for the sequencing reactions to average out any PCR-generated sequence errors. Sequencing was performed using the Sequenase protocol (U.S. Biochemical) with T7 DNA polymerase kindly provided by STAN TABOR. Sequencing was done on one strand using oligonucleotide primers spaced at 200–400-bp intervals. The sequences of the 7.3-kb *Hin*dIII fragments from the ry^{+0} and ry^{+5} background alleles were also determined from four overlapping PCR generated fragments (ry^{+0}), or from a full length *Hin*dIII clone derived from a bacteriophage lambda library (ry^{+5}). Parts of these sequences are published (LEE *et al.* 1987; CURTIS *et al.* 1989), and the full sequences will be presented elsewhere.

RESULTS

The 7.3-kb *Hin*dIII fragments of the ry^{+0} and ry^{+5} alleles were sequenced to identify polymorphisms, and the positions at which the sequences differ are shown in Figure 1. As described by FISCHER and LERMAN (1982), one or more nucleotide substitutions in otherwise identical DNA fragments can alter the melting temperature, and thus the mobility, of the fragments on denaturing gradient gels. To identify the ry^{41} and ry^{502} mutations, we first localized them to unique fragments relative to their parental alleles, ry^{+0} and ry^{+5}, using denaturing gradient gels. We then used the

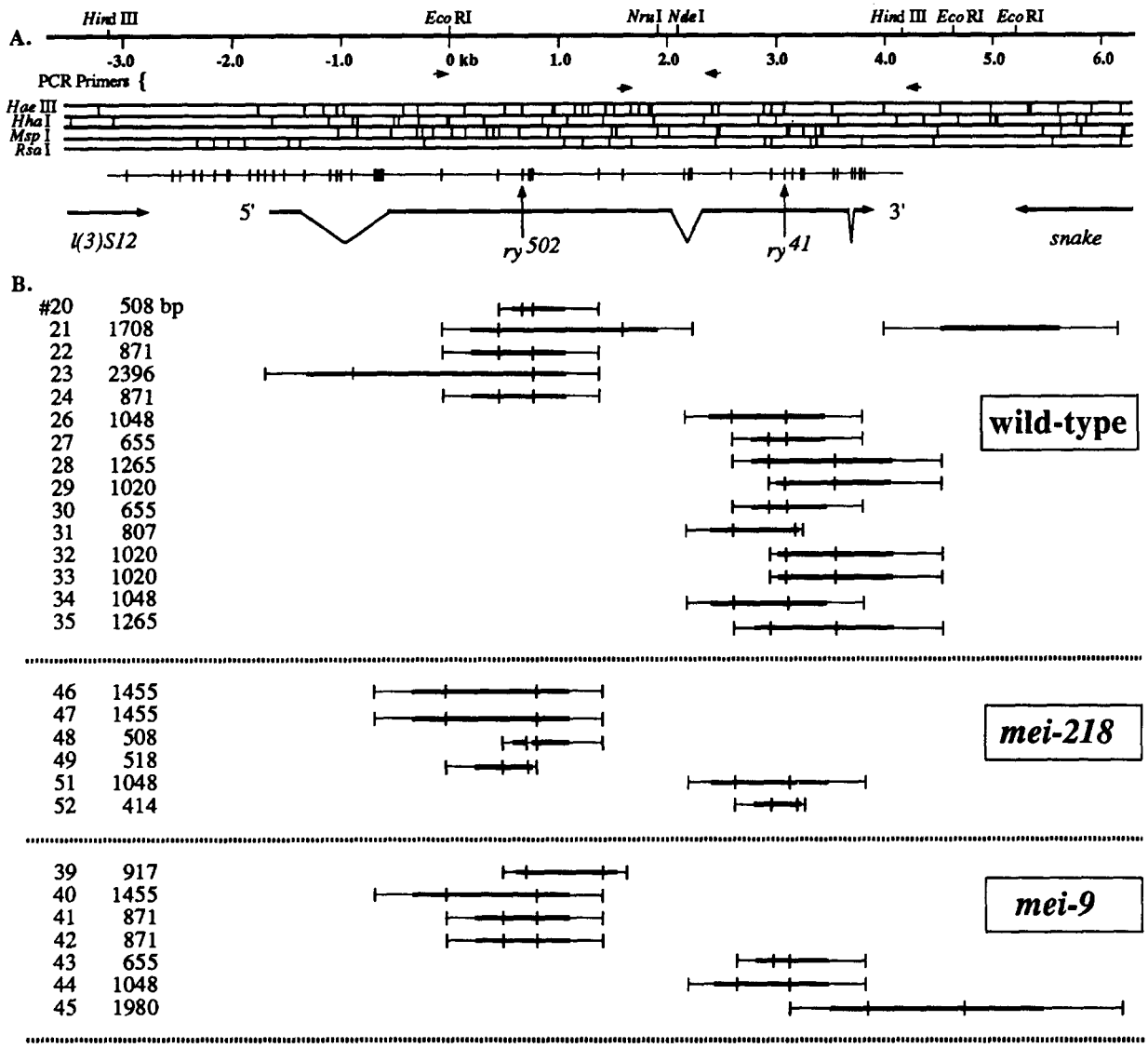

FIGURE 1.—Location and extents of conversion tracts in the *rosy* gene. A (from top to bottom): 1, A partial restriction map of the *rosy* region; not all sites are shown for each enzyme. 2, Arrowheads indicate the locations of primers used for PCR amplifications. 3, The restriction maps for the four enzymes used in the gradient gel analysis, *Hae*III, *Hha*I, *Msp*I and *Rsa*I. 4, Hash marks on the line below the restriction maps indicate the positions of all sites within the sequenced *Hin*dIII fragments that were polymorphic between ry^{502} and ry^{41}. Arrows point out the sites of the two mutations. 5, The location of the *l(3)S12*, *rosy* and *snake* transcripts relative to the DNA map. The *l(3)S12* (RILEY 1989; D. CURTIS, unpublished data) and *snake* (DELOTTO and SPIERER 1986) positions are approximate. B: For each conversion event, the thick bar indicates the length and position of the conversion tract. In #48 the broken bar indicates the discontinuity in the tract (see also Figure 3B). Each endpoint of the thick bars has an uncertainty represented by the thin bars terminated with hash marks. In these regions, either there were no polymorphisms to score, or there were several polymorphisms which could not be distinguished on gradient gels. Thus, the minimum length of a conversion tract is the distance between the internal hash marks; all polymorphisms between the internal hash marks were included in the conversion tract. The maximum length of a conversion tract is the distance between the outer hash marks; no polymorphisms outside of the outer hash marks were converted. The thick bar is drawn to represent the median length, halfway between the minimum and maximum possible length. The columns of numbers on the left give the arbitrary numerical designation of each recombinant chromosome (left column) and the median length of the conversion tract in base pairs (bp, right column). The length given for #21 refers to the tract at left, which crosses the ry^{502} mutation (see text).

PCR to amplify DNA containing the mutations, cloned fragments of this DNA, and sequenced across the mutations. The locations of the two sites are shown in Figure 1. In ry^{502}, three bases (bp 683–685) are deleted and replaced by an adenosine residue, resulting in a frameshift. In ry^{41}, three bases (bp 3095–3097) are deleted, removing a single glycine residue from the predicted XDH polypeptide (see KEITH *et al.* 1987 for sequence coordinates). Including the two mutant sites, the ry^{502} and ry^{41} alleles differ at a total of 50 positions in the 7.3-kb *Hin*dIII fragment.

We then compared the parental alleles ry^{502} and ry^{41} with the recombinants on genomic denaturing gradient gel blots. All of the surviving gene convertant stocks from CARPENTER (1984) were analyzed, including 15 events isolated from wild-type background, 6 from *mei-218*, and 7 from *mei-9*. Using gradient gels, it was not always possible to distinguish short conver-

sion tracts which crossed a few closely spaced polymorphisms from conversions of a single site. In addition, event #48 was unusual in that it had a unique pattern of polymorphisms on gradient gels and also created a unique XDH polypeptide electromorph (CARPENTER 1984). To resolve the lengths and structures of the shortest tracts, we PCR amplified, cloned, and sequenced the regions in question for two conversion events from *mei-218*, #48 and #52. We also sequenced the shortest event derived from wild-type in our earlier study (labeled #30 in CURTIS *et al.* 1989).

Wild-type events: The locations and extents of the conversion tracts are illustrated in Figure 1. With the exception of the event #21 (see DISCUSSION), all of the wild-type conversion tracts appear continuous; there are no interrupted or patchy tracts apparent at this level of resolution. Because a large fraction of the polymorphisms cause shifts in bands on the denaturing gradient gels, continuity as judged by gradient gels should correspond in most cases to sequence level continuity.

The data provide no evidence for preferred sites of initiation for conversion events. Although some clustering of conversion tract endpoints seems at first apparent, for example at the 3′ side of the ry^{502} conversions, this is probably an artifact of two features of the data. The first is that there are no polymorphisms in some regions of the sequence, for example between +777 and +1388, which might otherwise subdivide the endpoints. The second feature is the relative uniformity of conversion tract lengths and the obligate centering of the tracts over the mutant lesions. The apparent excess of conversions of ry^{41} relative to ry^{502} recovered in wild-type background is not significant because some of the recombinants originally isolated have been lost and were not examined here, and the original ratio of conversions of the two mutations was nearly unity (CARPENTER 1984).

The lengths of the conversion tracts are listed in Figure 1, and the distribution of lengths is presented in histogram form in Figure 2. The mean length of the 15 wild-type events determined here is 1077 ± 467 bp (±SD). This result is consistent with the average calculated from our previous sample of 12 events (CURTIS *et al.* 1989). The shortest of these previously reported events, #30, has now been sequenced. This information, plus other refinements of the earlier results (see Materials and Methods), yield an average for these previous 12 events of 1266 ± 814 bp. Thus the results from two separate experiments using different markers and different genetic backgrounds agree on the average simple conversion tract length at the *rosy* locus. Pooling the 27 wild-type events gives an average length of 1161 ± 638 bp, with a standard error of the mean of 123 bp.

Since the events we have examined were recovered by selection for a functional *rosy* gene, they do not represent a random sampling of conversion events. Long events would not be recovered if they included both the ry^{41} and ry^{502} mutant sites, which are 2414 bp apart, and if patchy repair of heteroduplex mismatches did not occur. However, long conversion tracts extending to the left of ry^{502}or to the right of ry^{41} could have been recovered. Since only one event of sufficient length to include both sites was found (#23), the mutations were probably spaced distantly enough that few if any long events were lost due to co-conversion. A more significant source of experimental bias is likely to be against recovery of very short events: only conversion tracts which cross one of the mutations (and result in the conversion of ry^- to ry^+) will be recovered, and the longer the tract, the more likely it is to cross a ry^- marker. We have therefore applied a correction to the data to cancel

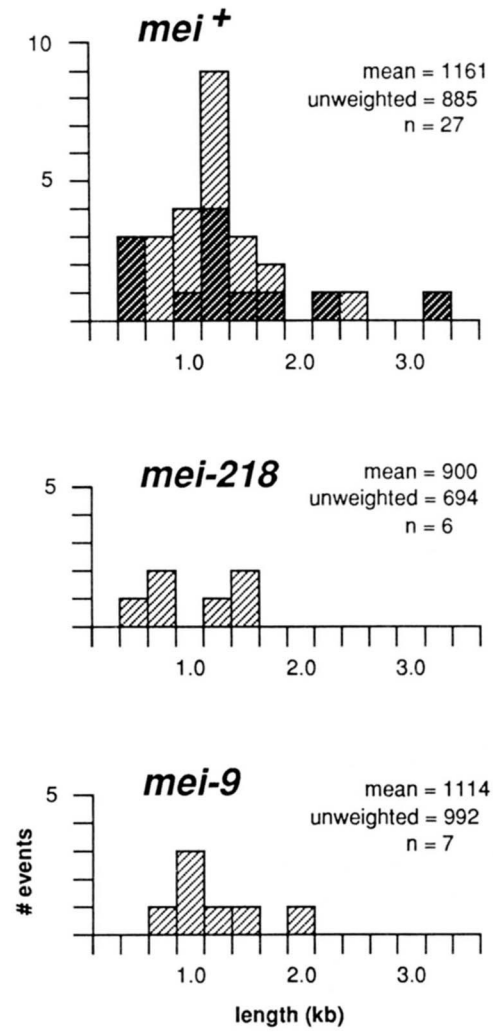

FIGURE 2.—The distribution of lengths of conversion tracts recovered in wild-type, *mei-218* and*mei-9* genetic backgrounds. The wild-type (*mei*$^+$) data include the 15 events presented here (light shading) and the 12 events reported in CURTIS *et al.* (1989) (dark shading). Histogram bins include events from 1–250 bp, 251–500 bp, 501–750 bp, 751–1000 bp, etc. Beside the histograms are listed the experimental mean length (mean), the unweighted, harmonic mean (unweighted), and the sample size (*n*) for each category.

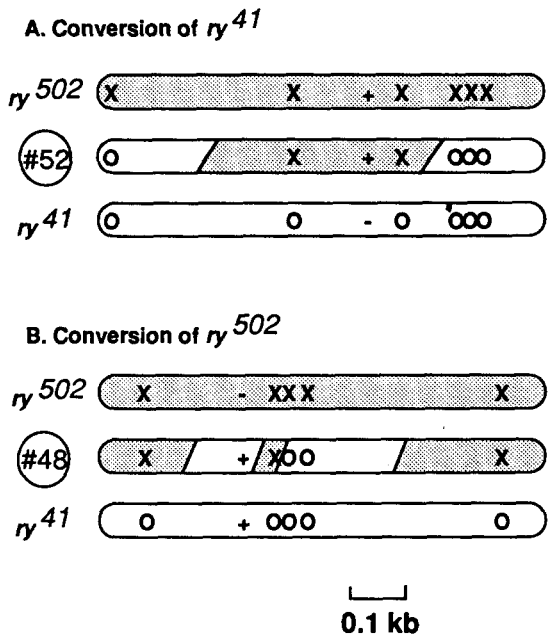

FIGURE 3.—Schematic representation of the sequence analysis of two *mei-218* conversion tracts. Sequence polymorphisms are represented by Xs and Os and are drawn to scale as they occur on the parental chromosomes. The − and + symbols indicate the sites of the mutations and their wild-type alleles. Shading indicates DNA of ry^{502} parental origin and no shading indicates ry^{41} origin. Different segments of the gene are represented in A and B.

out the experimental bias against short events. The correction is accomplished by taking the harmonic mean of the lengths. The harmonic mean X is calculated from the experimental median lengths w_i, with N = number of events in the sample, by the formula $X = N/\Sigma 1/w_i$. This calculation involves no assumption of the actual distribution of simple conversion tract lengths; it merely converts a weighted average to an unweighted average. The unweighted mean length of the 27 events is 885 bp. We believe this more closely represents the actual average conversion tract length.

***mei-218* events:** The locations of the six *mei-218* events are shown in Figure 1, and the two sequenced *mei-218* events are diagrammed in more detail in Figure 3. #52 is a continuous conversion of the ry^{41} mutation and two adjacent polymorphisms. #48 provides the only example we have seen of a patchy event. In this event, the converted ry^{502} site is separated from two other converted polymorphisms by an unconverted marker. #48 is treated arbitrarily as a single conversion tract with respect to its overall length (see DISCUSSION). The average length of the six *mei-218* events is 900 ± 485 bp, and the unweighted mean is 694 bp. There is no significant difference between the lengths of these tracts and those recovered in a wild-type background (Figure 2).

***mei-9* events:** The only unusual *mei-9* event is the one labeled #45. It is anomolous in that it does not cross the ry^{41} mutation. This recombinant chromosome is phenotypically ry^- and it was recovered as the only chromosome transmitted through the germ line from a phenotypically ry^+, mosaic animal. It represents one of the two products of a postmeiotic segregation event (see DISCUSSION). The remaining events isolated in the *mei-9* background appear indistinguishable from the wild-type sample in their length, distribution, and continuity (Figures 1 and 2). The seven events together average 1114 ± 454 bp in length, and the unweighted average is 992 bp. Three crossover events from *mei-9* were also examined (data not shown). Conversion of sites contiguous to the crossover junction could not be detected without examining the other chromatid which participated in the exchange. However, no patchy conversions of sites separated from the crossover events were detected.

DISCUSSION

Our results show that a typical simple gene conversion tract in the Drosophila *rosy* locus is about 885 bp long. Comparable measurements of meiotic conversion tracts have been made in one other organism, the yeast *Saccharomyces cerevisiae*, where tracts average from 1 to 3 kb, depending on the genetic interval monitored (BORTS and HABER 1987, 1989; JUDD and PETES 1988; SYMINGTON and PETES 1988).

Gradients of conversion frequency as a function of map position have been seen at some loci in fungi (reviewed in WHITEHOUSE 1982). The rates of conversion can differ by as much as 20-fold across a locus, as seen at the yeast *ARG4* gene (NICOLAS *et al.* 1989) or as little as 2-fold, as seen at the Ascobolus *b2* gene (KALOGEROPOULOS and ROSSIGNOL 1988). A shallow gradient has been suggested at *rosy* (HILLIKER and CHOVNICK 1981), in which mutations near the center of the locus convert less frequently than markers at the ends. This is at most a 2-fold effect, however, and CLARK, HILLIKER and CHOVNICK (1988) have argued that recombination events do not initiate exclusively at the ends of the locus. The scattering of conversion endpoints across the locus which is seen in the sample presented here and in the data of CURTIS *et al.* (1989) argue that there is little polarity of conversion frequencies at *rosy*.

The data indicate that conversion events at *rosy* are normally continuous. An apparent exception is the chromosome labeled #21, isolated as a conversion of ry^{502}, which has two separate conversion tracts. We propose that the second tract on the right is an independent, unselected event that occurred while this stock was being maintained. The stock was balanced over P18, a rearranged chromosome carrying the ry^{41} allele. The rearrangements on the balancer prevent the recovery of crossover chromatids, but only slightly depress the level of gene conversion in the rosy interval (CHOVNICK 1973). We have examined 470 kb of DNA in the course of these experiments. These stocks were maintained for 8 years. Assuming 12 generations/year at 18° and a frequency of 2×10^{-5} conver-

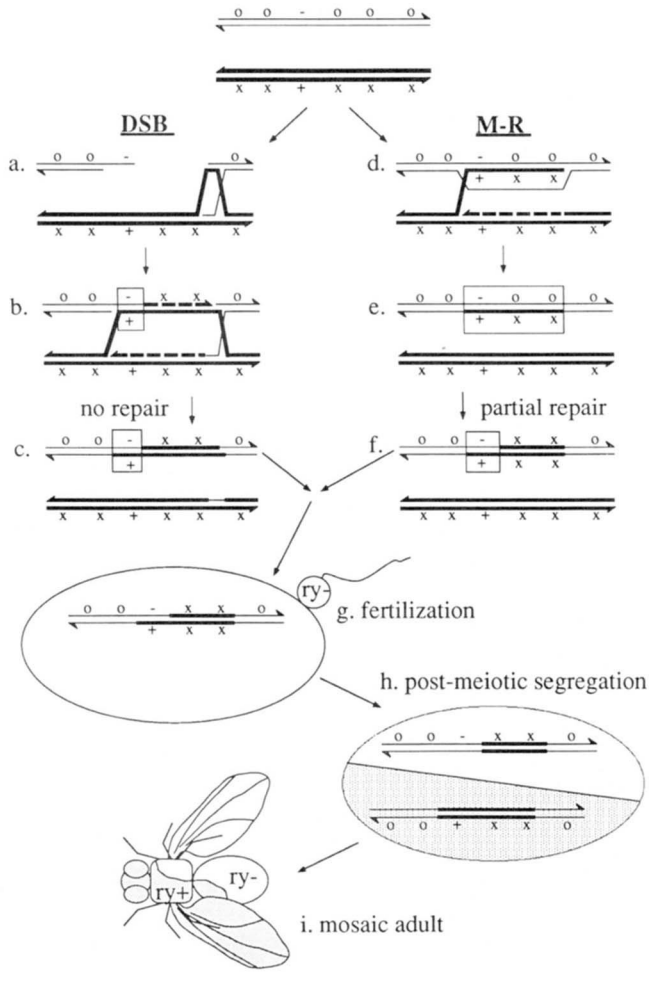

FIGURE 4.—Two possible explanations of the recombination event that gave rise to the chromosome #45. In the left column, recombination is initiated by a double strand break (DSB, SZOSTAK *et al.* 1983) and in the right column by a single strand nick as envisioned in the Meselson-Radding model (M-R, MESELSON and RADDING 1975). Each line represents a single strand of DNA, and the polarity of a strand is indicated with an arrowhead at the 3′ end. The symbols *X*, O, +, − are as described in Figure 3 but are not drawn to any scale. *DSB*: **a**, A double strand break is formed and enlarged to a gap by exonucleases, leaving 3′ overhanging ends. One of these invades the homologous duplex forming a D-loop. **b**, The D-loop enlarges as the invading 3′ end primes DNA synthesis. The D-loop anneals to the remaining overhanging 3′ end, forming heteroduplex DNA containing the ry^{41} mutation (inside box). Repair synthesis fills in the gap using the D-loop as template. **c**, The two junctions are resolved as a noncrossover by cutting and ligation. The upper chromatid in the figure is incorporated into the female gamete. *M-R*: In the alternate model, a single strand nick is formed. **d**, the resulting 3′ end primes DNA synthesis, and the displaced strand invades the homologous duplex to form a D-loop. **e**, The D-loop is degraded, leaving a segment of heteroduplex DNA (inside box), and the junction is resolved as a noncrossover. **f**, The heteroduplex is partially repaired as a conversion toward the donor sequences, but repair does not include the ry^{41} mutation site. The upper chromatid is equivalent to the upper chromatid in (**c**). generated in the DSB pathway, and is passed into the female gamete. **g**, The egg carrying a heteroduplex at *rosy* is fertilized by a sperm carrying a mutant *rosy* allele. **h**, The heteroduplex at *rosy* is resolved by DNA replication at the first zygotic division. The embryo develops into a mosaic of ry^+ and ry^- cells. Although the distribution of ry^+ tissue is random, the non-autonomy of *rosy* expression allows individuals with at least some wild-type fat body and malpighian tubule tissue to survive the purine selection. **i**, In this case the mosaic adult was entirely ry^- in the germ line and transmitted only one type of *rosy* allele with the structure of #45 (as drawn in the white sector of the egg in **h**).

sions/kb/generation at *rosy* (HILLIKER and CHOVNICK 1981), then we expect a probability of (470)(8)(12)(2 × 10^{-5}) = 0.9 for finding an unselected conversion tract in the sample. Thus, the left conversion tract in #21 was probably selected for in the original experiment, whereas the right tract might have occurred spontaneously while in stock. The second event is quite average in its length and continuity but has not been included in the calculation of average tract length.

A large number of regulatory, structural and enzymatic functions are undoubtedly required to carry out genetic exchange. Many mutants have been isolated in Drosophila which are defective in various of these functions (reviewed in BAKER *et al.* 1976b). *mei-218* is a candidate for a meiosis-specific recombination function, since it has no known somatic phenotype, but reduces meiotic crossing over to 8% of normal and abolishes the nonrandom distribution of crossover events. The frequency of recovery of simple gene conversions in the presence of *mei-218* is not reduced, however and is in fact twofold higher than controls (CARPENTER 1982, 1984). *mei-218* also reduces the number and changes the morphology of late recombination nodules, meiotic structures hypothesized to be recombination "machines" (CARPENTER 1975, 1979, 1988; VON WETTSTEIN, RASMUSSEN and HOLM 1984). CARPENTER reported that conversion tracts in *mei-218* were shorter by a factor of two than those from wild type or *mei-9*. However, we have examined all six *mei-218* events examined by CARPENTER, scoring more polymorphic markers than was possible using protein electrophoresis. The results indicate that *mei-218* events are in fact similar in length to wild-type and *mei-9* events. Since the sample size is small, we cannot exclude subtle effects of the *mei-218* mutation on conversion tract length.

The *mei-218* sample did include the only clearly discontinuous conversion event seen, #48. It is not possible to determine whether this was the result of independent mismatch repair within an initial single heteroduplex interval, or the result of two separate, nearby conversion events. Independent, or incomplete repair of mismatches is not detected in wild-type events at *rosy*, either molecularly, as shown here, or genetically (CHOVNICK, BALLANTYNE and HOLM 1971). Incomplete repair would also be surprising in the case of *mei-218*, since the mutation causes no known somatic DNA repair defects and no post-meiotic segregation, and thus would not be expected to interfere with the normal processing of heteroduplex DNA intermediates. However, the proximity of

the converted sites suggests that they result from a single concerted event. The other five conversion tracts in the *mei-218* sample are normal and continuous, so it does not appear that the *mei-218* mutation has a dramatic defect in the ability to repair heteroduplex mismatches.

mei-9, in contrast to *mei-218*, has pleiotropic effects on DNA metabolism (BAKER *et al.* 1976a, 1978; BOYD, GOLINO and SETLOW 1976; BOYD *et al.* 1987; GATTI 1979). *mei-9* mutants are hypersensitive to killing by X-rays, UV light, and chemical mutagens such as MMS and nitrogen mustard, and they show increased mitotic recombination and chromosome breakage. In meiosis, *mei-9* reduces crossing over to 8% of controls, although the distribution of crossovers, and the appearance and number of recombination nodules, are normal (CARPENTER 1979). The frequency of gene conversion in *mei-9*, in contrast, is elevated approximately twofold over controls (ROMANS 1980a; CARPENTER 1984, 1982). Although postmeiotic segregation is virtually never seen in wild type (CHOVNICK, BALLANTYNE and HOLM 1971), at least 60% of ry^+ recombinants derived from *mei-9* are somatic mosaics of ry^- and ry^+ tissue (CARPENTER 1982), indicating postmeiotic segregation of unrepaired heteroduplex. Inasmuch as the *mei-9* alleles used are likely not null alleles (CARPENTER 1982), and since the actual percentage of mosaics may be underestimated because of reduced survival on the purine selection (ROMANS 1980b), the *mei-9* locus may be absolutely required for heteroduplex repair.

We expected that the *mei-9* mutation might lead to patchy repair of mismatches, either by independent repair of mismatches throughout a heteroduplex tract, or by partial repair beginning from one end. However, the conversion tracts in the *mei-9* sample were normal in their length and continuity. The harmonic mean length of the *mei-9* events was 992 bp, as compared to 885 bp for wild type. Thus it appears that all mismatches in a heteroduplex tract are repaired, or none are. The twofold higher frequency of conversion events in *mei-9* might be accounted for by postmeiotic segregation. In wild type, half of the heteroduplex tracts generated should be restored to the parental mutant configuration by heteroduplex repair. In *mei-9*, some of these would persist as heteroduplex, allowing the recovery of mosaic animals from the selection. Failure to repair heteroduplex mismatches *per se* cannot be the direct cause of the reduced crossover frequency in *mei-9*, since *mei-9* gives the same frequency of *X* nondisjuncion (from nonexchange *X* chromosomes) with isogenic (BAKER and CARPENTER 1972) and heterozygous (CARPENTER and SANDLER 1973) *X* chromosomes. However, the $mei\text{-}9^+$ gene product might still play an essential role in the proper sequential assembly or function of a multimeric recombination machine, regardless of whether or not heteroduplex mismatches are encountered.

One conversion event from the *mei-9* group, #45, was a ry^- allele transmitted from a mosaic, ry^+/ry^- female. Two possibilities are diagrammed in Figure 4 for how this event might have occurred, as envisioned in two predominant models for recombination. The Meselson-Radding and double strand break models differ in the way in which events are initiated, in predictions for the types of co-conversion events that should occur, and in other details (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). In the event giving rise to #45, only one of the two DNA strands in the initial heteroduplex (the one represented by #45) could be analyzed. The heteroduplex had to include the ry^{41} site, but beyond that, the details of the structures are drawn to fit the models most simply. The same two models can also explain the "reverse crossover" exceptions isolated in *mei-9* (CARPENTER 1982), if the Holliday junction intermediates are resolved in the crossover configuration.

No information is available from wild-type Drosophila about the biochemical mechanism of initiation of recombination events, *i.e.*, single strand nicks or double strand breaks. Because the *mei-9* mutation apparently affects a late step in recombination, and not the mechanism of initiation, the postmeiotic segregant products in *mei-9* should reflect normal recombination intermediates. Partial repair of heteroduplex, as required in the Meselson-Radding alternative (Figure 4f), does not seem likely, because *mei-9* gave no clearly patchy events, and the lengths of the *mei-9* conversion tracts are not shorter than normal. Therefore, while the data are very limited, the double strand break model might better fit the observed *mei-9* events. An examination of both segregant products from several *mei-9* postmeiotic segregation events should better distinguish between these alternative mechanisms.

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