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Further observations on intragenic recombination in *Drosophila melanogaster*

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SUMMARY

This report examines several issues bearing upon intragenic recombination in higher eukaryotes. The fine structure data accumulated in our analysis of the genetic organization of the rosy locus in *Drosophila melanogaster*. Firstly, we confirm that a conversion event has a markedly less than 50% probability of resulting in flanking marker exchange, a finding consistent with more recent analyses of the available *Saccharomyces* data (e.g. Fogel *et al.* 1978). As reported earlier, co-conversion of recombinationally separable sites within the *rosy* locus occurs (McCarron, Gelbart & Chovnick, 1974). In this report, we demonstrate that the frequency of co-conversion is inversely proportional to the distance between co-converting sites. As in fungi, real conversion frequency differences are observed among *rosy* mutant alleles, and the data suggest that there may be a relationship between allele conversion frequency and map position. Unlike *Neurospora* and *Saccharomyces*, only one flanking marker exchange class is recovered from any given mutant heteroallele recombination experiment. In this respect, the *Drosophila* system resembles *Aspergillus*. As in *Neurospora* and *Saccharomyces*, *rosy* locus intragenic recombinants associated with flanking marker exchange exhibit interference with crossing over in adjacent regions, while no interference is seen among recombinants exhibiting parental flanking markers. Finally, experimental results are discussed which demonstrate the occurrence of postmeiotic segregation in *Drosophila*. These analogies between *Drosophila* and fungi provide further evidence in support of the notion that eukaryotes share common molecular mechanism(s) of meiotic recombination.

1. INTRODUCTION

Analysis of intragenic recombination in *Drosophila melanogaster* has demonstrated a series of parallels between crossing over and gene conversion. We use the term conversion with reference to those intragenic recombinants exhibiting parental

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flanking markers, and believe that these are products of non-reciprocal events, as substantiated by earlier half-tetrad studies (Chovnick *et al.* 1970; Ballantyne & Chovnick, 1971). These parallels are that: (1) Conversion is seen in mutant heterozygotes, and not in homozygotes. (2) Conversion occurs in meiosis in females, but not in males. (3) Like crossing over, conversion requires effective pairing during meiosis of the region being monitored. This is inferred from the observations: (*a*) Conversion is suppressed by heterozygous rearrangements with breaks in the immediate region of interest. (*b*) Conversion occurs with undiminished frequency within a heterozygous paracentric inversion with breakpoints at some distance from the region under study. (4) Conversion frequency is subject to the inter-chromosomal effect (see review, Lucchesi & Suzuki, 1968), and may be increased by the presence of heterologous rearrangements. (5) Like crossing over, the conversion process yields products which, at the molecular level, are truly recombinant derivatives of the parental genetic information. These parallels are reviewed in Chovnick, Ballantyne & Holm (1971) with more recent observations described in Chovnick (1973) and McCarron *et al.* (1974).

From half-tetrad data involving very tightly linked, but recombinationally separable mutant heteroalleles, we have inferred that all recombinants (flanking marker crossovers as well as those exhibiting parental flanking markers) result from non-reciprocal events (Smith, Finnerty & Chovnick, 1970). In contrast, examination of the products of recombination events involving more distant sites reveals that reciprocal exchange events do occur among the flanking marker crossover class (Chovnick, 1961; Chovnick *et al.* 1970; Ballantyne & Chovnick, 1971). Finally, half-tetrad analysis of the *bithorax* region mutants (where map distances between separable mutants are as large or greater than the total length of the *rosy* locus) reveals that all recombination events are flanking marker crossovers, and reciprocal exchange events as well (Lewis, 1967).

Taken together, all of these observations are consistent with the view that all recombination involves conversion (i.e. a non-reciprocal transfer of information) in the immediate region of the exchange event. Thus, as the length of the interval being monitored increases (distance between the mutant heteroalleles in the cross), the greater is the likelihood that the conversion event will be resolved as a reciprocal exchange for the flanking outside markers.

In recent years our attention has been directed to questions dealing with gene organization and regulation in *Drosophila melanogaster* (reviewed in Chovnick *et al.* 1980). In these studies, we have accumulated a large body of fine structure data, some of which is pertinent to the analysis of intragenic recombination. The present report considers these data, and their relevance to our understanding of the nature of intragenic recombination in higher eukaryotes. Experiments specifically involve the *rosy* locus in *Drosophila melanogaster*, and all of the data reviewed in the present report have come from random strand fine structure studies.

2. MATERIALS AND METHODS

(i) *The genetic system*

Figure 1 presents a genetic map of the *rosy* region of chromosome 3 noting the position of the *rosy* locus and closely linked genetic markers used in this investigation. Previous reports have described this experimental system as well as our genetic and biochemical nomenclature (Chovnick *et al.* 1976; Chovnick, Gelbart & McCarron, 1977). The *rosy* locus (*ry:* 3–52·0) consists of a single structural element coding for a peptide of 150000 daltons which, as a homodimer, functions as the enzyme xanthine dehydrogenase (XDH) (Edwards, Candido & Chovnick, 1977). Moreover, compelling evidence has been presented for the existence of a *cis*-acting control element located adjacent to the left (centromere proximal) side of the XDH structural element (McCarron *et al.* 1979).

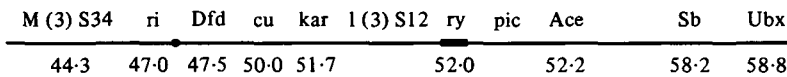

Fig. 1. A genetic map of the proximal region of chromosome 3. Map positions of various mutants used in this investigation are indicated. Mutants not described in Lindsley & Grell (1968) are discussed in Chovnick *et al.* (1976) and Hilliker *et al.* (1980).

Figure 2 summarizes our present map of sites within the XDH coding element of the *rosy* locus. Figure 2A presents a map of XDH^-, non-complementing, rosy eye colour mutant sites. Estimation of the boundaries of the XDH coding element is provided by the maps of three classes of unambiguous coding element site variants presented in Fig. 2B (XDH^-, allele-complementing, rosy eye colour mutant sites), Figure 2C (electrophoretic mobility sites) and Fig. 2D (purine sensitive 'leaky' structural mutant sites). The left boundary is set by the leftmost allele-complementing site mutant, ry^{606}. On the basis of comparative recombination data and the failure of large-scale tests with ry^{606} to produce recombinants, the non-complementing site mutant, ry^{23}, must also mark the left border. At the right end of the map, several electrophoretic sites and the complementing mutants, ry^2 and $ry^{L\cdot19}$, identify the right boundary of the coding element, with no known XDH variants beyond them.

In addition to the sites that map inside the XDH structural element (Fig. 2), two separable sites have been identified that map to a region located between the left boundary of the XDH structural element, and *l(3)S12*, a mutant site in the functionally independent genetic unit immediately to the left of *rosy* (Fig. 3). These sites, designated by the superscripts *i1005* and *i409*, are associated with *cis*-acting variation in number of molecules of XDH monomers available for dimer formation. We have been unable to associate this variation in any systematic manner with structural features of the XDH molecules, and we have eliminated the possibility of their association with tandem duplications of the XDH structural element

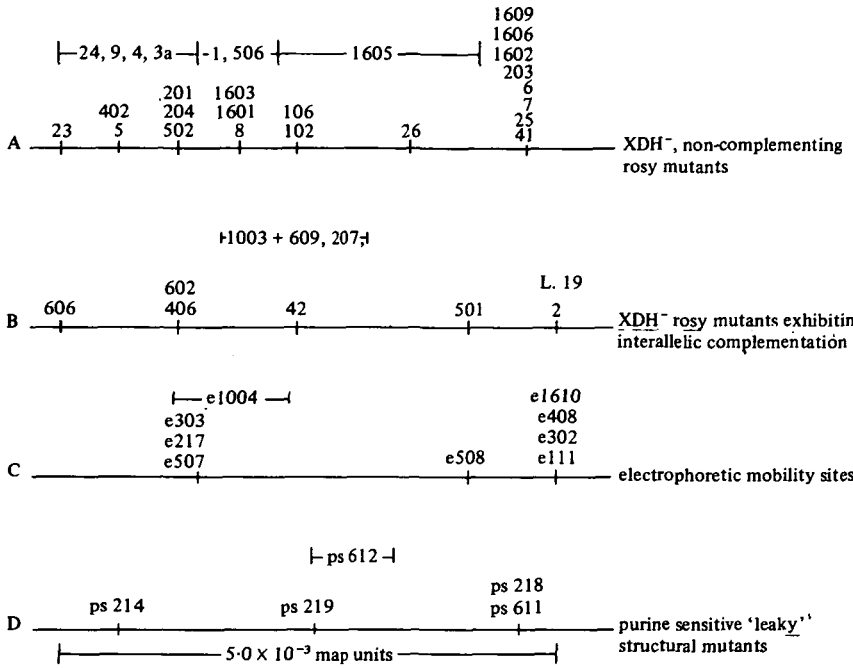

Fig. 2. Genetic fine structure maps of *rosy* locus sites. Map locations of unambiguous structural element variants (B, C, and D) are positioned relative to map of XDH⁻ non-complementing mutants (A). From McCarron *et al.* (1979).

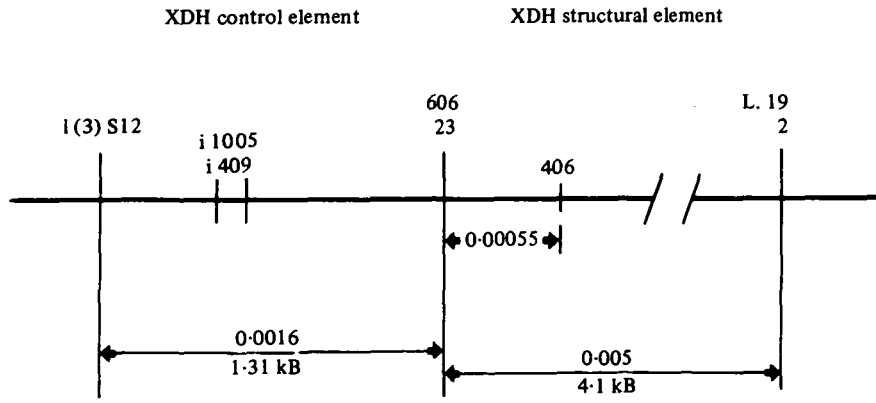

Fig. 3. The *rosy* locus. Size estimates of the structural and control elements. From McCarron *et al.* (1979).

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(Chovnick *et al.* 1976; McCarron *et al.* 1979). Wild-type alleles associated with normal activity levels are *i1005N*, *i409N* in genetic constitution, while very much lower than normal activity is *i1005L*, *i409N*. Very much higher than normal activity is *i1005N*, *i409H*. The phenotype associated with the double variant, *i1005L*, *i409H* is yet to be confirmed. (*L*, *N* and *H* refer to *rosy* locus variants associated with low, normal and high XDH activity levels respectively.)

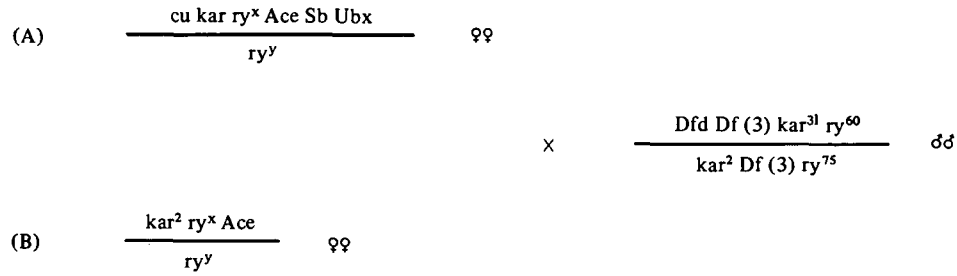

Fig. 4. Typical 'random strand' crosses for fine structure analysis of the *rosy* locus. Females, heterozygous for *rosy* mutant heteroalleles and flanking markers are crossed to tester males. The latter carry the XDH^{-} *rosy* site mutant, *ry*^{60}, on one chromosome, as well as *Df(3)kar*^{31} (*DF87C 2/3 – 87C9/D1*), which is missing *kar* as well as several vital genes. The other paternal chromosome has the *rosy* deletion, *Df(3)ry*^{75} (*Df87D1/2 – 87D14/E1*). This chromosome is *Ace*$^{+}$, but is missing other vital genes immediately flanking *rosy*.

(ii) *Selective system matings*

The experiments involve two general series of matings of *rosy* heteroygous females to tester males as indicated in Fig. 4. For most experiments, involving XDH^{-} – rosy eye colour mutants, progeny are reared on purine supplemented medium following a protocol (Chovnick, 1973) which effectively kills before eclosion all individuals lacking wild-type levels of XDH activity. In a few experiments, slight variations on this protocol were followed (McCarron *et al.* 1979). Further information on the mutants and rearrangements employed may be found eleswhere (Lindsley & Grell, 1968; Gelbart *et al.* 1974; Gelbart, McCarron & Chovnick, 1976; Gelbart & Chovnick, 1979; Hilliker *et al.* 1980).

(iii) *Genetic tests of exceptional progeny*

Surviving individuals of the selective system crosses (Fig. 4), all of which are *ry*$^{+}$ in phenotype, may be classified immediately with respect to the markers *kar* and *Sb*. In crosses where one of the *rosy* alleles is associated with a wild-type eye color, lowered XDH activity and susceptibility to purine killing, all surviving exceptional progeny are retested for purine sensitivity, in order to screen out possible 'leakers' (i.e., non-recombinants with low XDH activity levels which

escaped the purine selection regime). All exceptional progeny were crossed singly to *kar*^{2} *Df(3R)ry*^{75}/*Tp(3)MKRS*, *M(3)S34 kar ry*^{2}*Sb* flies. Progeny phenotypes confirm the diagnoses with respect to *kar*, *ry*, and *Sb*. The progeny were further tested in two ways: (1) males carrying the *ry*$^+$ exceptional chromosome were crossed to an appropriate *Ace* tester stock to diagnose the presence or absence of the *Ace* marker, and (2) in many of the crosses *ry*$^+$/*Df(3R)ry*^{75} progeny were used to determine XDH electrophoretic mobility employing the procedures outlined in McCarron *et al.* (1979).

(iv) *Classification of recombinants*

Recombinant chromosomes were classified as either crossovers or conversions. Conversions are *ry*$^+$ exceptional chromosomes not exhibiting flanking marker exchange (*kar-Ace*). Crossovers are *ry*$^+$ exceptional chromosomes exhibiting exchange between *kar* and *Ace*. In the series A crosses (Fig. 4), conversions and crossovers were also assayed for coincident recombination in the *Ace* to *Sb* interval.

For the greater part, conversion and crossover frequencies are expressed as uncorrected proportions. In such cases, to convert crossover frequency to map distance (in standard map units) one merely multiplies crossover frequency by 200.

Table 1. *Frequency* ($\times 10^6$) *of* ry$^+$ *recombinants resulting from the indicated heteroallele tests*

Heteroallele pair	Analysis of *ry*$^+$ chromosomes			
	Crossovers	Conv-*ry*^{5}	Conv-*ry*^{y}	*N* ($\times 10^{-6}$)
5/402	—	0·75	—	1·34
5/8	—	2·7	9·3	0·75
5/502	3·2	2·7	7·3	2·2
5/506	3·3	4·4	—	0·9
5/1	4·4	7·3	2·9	0·68
5/203	12·7	4·0	4·0	1·5
5/42	12·9	9·0	3·9	0·78
5/2	17·5	6·2	0·6	1·6
5/501	17·9	7·1	4·8	0·84
5/41	29·8	6·0	22·4	1·34

RESULTS

(i) *Map distance and proportion of recombinants associated with flanking marker exchange*

An earlier report (Chovnick *et al.* 1971) described the results of experiments which were designed to examine the relationship between the various intragenic recombination classes and the interval between the mutant heteroallele markers. Table 1 amplifies one of the several sets of data from this study. The *ry*$^+$ re-

Table 2. *Relationship between proportion of recombinants associated with parental flanking markers and interval between* rosy *heteroalleles under test*

ry^X/ry^Y	Crossovers	ry^X conversions	ry^Y conversions	N ($\times 10^{-6}$)	Crossover frequency ($\times 10^6$)	Parental flanking marker recombinants / Total recombinants
5/203	19	6	6	1·5	12·7	0·39
5/405	15	4	3	0·8	18·8	0·32
5/506	3	4	0	0·9	3·3	0·57
5/501	15	6	4	0·84	17·9	0·40
5/1	3	5	2	0·68	4·4	0·70
5/42	10	7	3	0·78	12·8	0·50
5/42	5	5	4	0·64	7·8	0·64
5/41	40	8	30	1·34	29·9	0·49
5/41	21	11	20	1·26	16·7	0·60
5/2	28	10	1	1·60	17·5	0·28
5/502	7	6	16	2·20	3·2	0·76
8/110	2	1	2	0·55	3·6	0·60
8/103	4	2	1	0·87	4·6	0·43
8/102	2	1	2	0·90	2·2	0·60
8/203	3	4	16	1·07	2·8	0·87
8/ps214	1	1	3	0·74	1·4	0·80
8/207	6	5	2	0·75	8·0	0·54
8/502	6	3	9	1·83	3·3	0·67
8/609	3	5	3	0·69	4·3	0·73
8/602	3	1	3	0·77	3·9	0·57
8/606	3	2	2	0·64	4·7	0·57
8/ps612	2	2	1	0·60	3·3	0·60
8/501	9	13	1	2·65	3·4	0·61
8/3	1	7	0	0·83	1·2	0·88
8/L.19	3	0	3	0·72	4·2	0·50
8/42	2	3	4	0·76	2·6	0·78
8/41	11	11	18	1·23	8·9	0·72
8/1401	3	2	9	1·76	4·7	0·79
26/106	3	5	1	0·58	5·2	0·67
26/502	30	12	12	1·12	26·8	0·44
26/42	1	8	5	0·70	1·4	0·93
26/41	3	3	10	0·71	4·2	0·81
41/110	17	19	16	1·91	8·9	0·67
41/106	5	23	6	2·46	2·0	0·85
41/103	22	9	13	1·10	20·0	0·50
41/102	16	12	12	1·56	10·3	0·60
41/201	8	10	3	0·74	10·8	0·62
41/204	6	6	8	0·68	8·8	0·70
41/205	4	5	6	0·66	6·1	0·73
41/301	36	18	0	2·53	14·2	0·33
41/402	13	8	8	1·11	11·7	0·55
41/502	24	9	7	1·48	16·2	0·40
41/ps214	22	7	7	0·95	23·2	0·39
41/1	11	10	2	0·82	13·4	0·52
41/42	5	18	7	1·31	3·8	0·83
42/502	9	5	18	1·54	5·8	0·72
106/203	6	5	20	2·04	2·9	0·81
106/501	1	0	2	2·30	0·4	0·67
ps214/506	4	6	0	1·59	2·5	0·60
ps214/406	1	1	3	1·99	0·5	0·80
406/60/6	4	1	6	0·98	4·0	0·64

combinants recovered from each cross invariably fall into three classes with respect to distribution of the flanking markers. The flanking marker crossovers provide for unambiguous positioning of mutant sites, and the remaining two classes possess the parental distribution of flanking markers, which we designate as conversions of one of the other mutant site. From data of this sort the following major features are noted: (1) The crossover frequency increases, as expected, as a function of

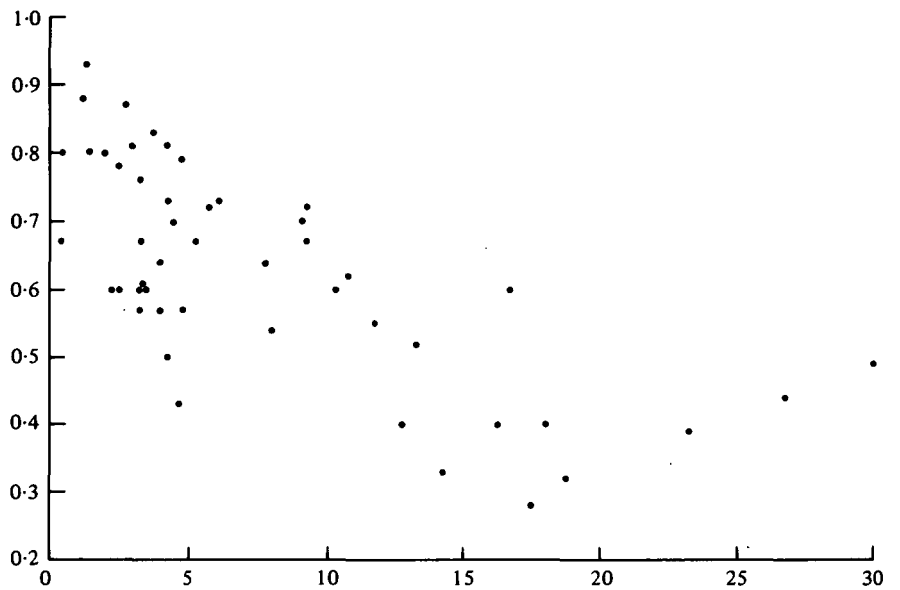

Fig. 5. Proportion of recombinants with parental flanking markers as a function of map distance between mutant heteroalleles under test. Ordinate: Proportion of recombinants with parental flanking markers. Abscissa: Crossover frequency ($\times 10^6$) between mutant heteroalleles under test. (To convert to map distance, mutliply by 200.)

distance; (2) The conversion frequency of a mutant site (*e.g.*, ry^5) appears to be constant (*i.e.*, within Poisson limits) with the exception of tests against very close sites. In these cases, the two mutant sites are commonly included in the same conversion event (*i.e.*, co-conversion), and a wild-type recombinant is not generated. Hence, the apparent reduction of the conversion frequency is seen in Table 1. (3) Conversion frequencies of heteroallelic mutant sites may be quite different.

Since that time, we have accumulated much additional recombination data involving *rosy* locus fine structure tests. Table 2 summarizes the results of 51 fine structure experiments testing for recombination between the specific mutant sites indicated on each row. These represent all the relevant experiments we have undertaken (there are several instances where the same pair of mutant sites is tested with differing distributions of the flanking markers, and these are recorded separately).

For each experiment, we compare the map distance (as estimated by the

frequency of ry^+ crossovers), with the proportion of total recombinants that exhibited parental flanking markers (conversions) in Fig. 5. It is clear from inspection of Fig. 5 that, despite the inherent variation in these experiments (performed over a number of years and involving different genetic backgrounds), the proportion of recombinants exhibiting parental flanking markers is inversley correlated with the length of the recombination interval under test. Correlation analysis of the data, subjected to the angular transformation, yields a very highly significant correlation coefficient ($r = -0{\cdot}728$). For the hypothesis of no correlation, $P < 0{\cdot}001$. Consider next, all crosses in which the crossover frequency was less than 3×10^{-6}, approximately 1/10 of the crossover map. In these experiments, we expect most, if not all recombination events to involve conversion of one or another of the mutant heteroalleles (See Chovnick *et al.* 1971; Smith *et al.* 1970). For this group of experiments, the proportion of recombinants associated with flanking marker exchange is 22% (Fig. 5).

(ii) *Co-conversion*

Co-conversion of recombinationally separable sites within the *rosy* locus was inferred by Chovnick *et al.* (1971) from data such as those presented in Table 1. However, a clear demonstration of co-conversion emerged only from the first fine structure experiments involving heterozygosity for an unselected electrophoretic site in addition to heterozygosity for selective XDH^- *rosy* mutant sites (McCarron *et al.* 1974). The utility of co-conversion as a tool in gene organization studies in *Drosophila* is discussed elesewhere (Chovnick *et al.* 1974; Chovnick *et al.* 1977). In fungi, the frequency of co-conversion is inversely proportional to the distance between the co-converting sites (Fogel, Hurst & Mortimer, 1971). That such a relationship exists for the *rosy* locus emerges from analysis of the data presented in Table 3. These data are taken from a series of recombination experiments wherein it is possible to determine both the map distance between a selective mutant site and a specific unselected electrophoretic site, and the proportion of conversions of the selective site that were also co-conversions of the electrophoretic site. These data (Table 3), presented as frequency of ry^+ crossovers and co-conversion frequency, were subjected to the arcsin transformation and a correlation analysis performed upon the transformed data. The correlation coefficient obtained ($r = -0{\cdot}809$) is highly significant ($p < 0{\cdot}01$). Clearly, co-conversion frequency is inversely proportional to the map distance between the co-converting sites.

One point that should be noted concerns the distinction between co-conversion and double conversion. By co-conversion, we imply a single event involving information transfer of a segment of the gene extending to cover both of the sites being followed. In contrast, double conversion involves two distinct conversion events. Ballantyne & Chovnick (1971) have shown that double conversions may be recovered in a *rosy* locus fine structure experiment involving a half-tetrad analysis using three XDH^- *rosy* mutant sites spanning much of the *rosy* locus map. While it is unlikely that double conversion events significantly contaminate the

Table 3. *Summary data of recombination experiments providing both map distance and co-conversion frequency between the indicated mutant and electrophoretic sites*

Rosy mutant site	Electrophoretic site	Crossover frequency ($\times 10^6$)	Co-conversion frequency	N ($\times 10^{-6}$)
502	e507	0·3350	0·8478	5·97
5	e217	1·3333	0·5000	1·50
5	e507	2·3810	0·5000	0·84
ps214	e217	4·1420	0·2000	1·69
23	e217	5·5762	0·0000	0·54
219	e217	7·4349	0·1111	0·54
203	e217	11·3333	0·0000	1·50
ps218	e217	13·4003	0·0000	0·60
ps223	e217	13·6585	0·0588	2·05
*41	e217	15·7890	0·1429	0·95

* See discussion.

co-conversion data of Table 3, it is not possible to exclude such events. However, it should be noted that double events, should they occur, would bias the data against the inverse relationship.

(iii) *Conversion frequency and map position*

This section addresses two questions: (1) Are there real differences in *rosy* allele conversion frequencies? (2) If such differences exist, are they a function of map position within the *rosy* locus?

Figure 6 presents *rosy* mutant allele conversion frequencies as a function of map position for a group of unambiguously positioned sites (Figs. 2, 3) that have been subjected to extensive mapping experiments. Exceptions are ry^{t1005L}, ry^{23} and ry^{26} which have been examined in only a limited number of tests, sampling 6·17, 2·9 and $4{\cdot}33 \times 10^6$ progeny, respectively. They are included merely to indicate consistency of the U-shaped pattern of the available conversion data, and its persistence since it was first reported (Chovnick *et al.* 1971) despite tremendous increases in sampling. Certainly, there are differences in allele conversion frequency, and these differences may be a function of map position. Initially, we believed that both of these conclusions may have a common trivial explanation as follows: For any given pair of mutant heteroalleles under test, their apparent conversion frequencies will be strictly limited by the interval between them. If the mean size of a conversion segment is a significant fraction of the *rosy* locus, then in a ry^x/ry^y female where ry^x ry^y are separable, but close to each other, many conversions of ry^x will include the ry^y site, and hence not be recovered as ry^+ exceptions. Thus, even if the real conversion rates were uniform per mutant site, one would expect to see a U-shaped curve when one plots conversion frequency vs. map position for *rosy* alleles that had been tested in a large number of recombination experiments. Those sites at either end of the locus would be more often involved in recombination

tests spanning large intragenic intervals, thereby maximizing the frequency of recovery of ry^+ convertants. In contrast, mutant sites lying closing to the center of the locus would have been involved more often in recombination tests spanning shorter intervals, and thus subject to the reduction in apparent conversion frequency due to co-conversion, as described above.

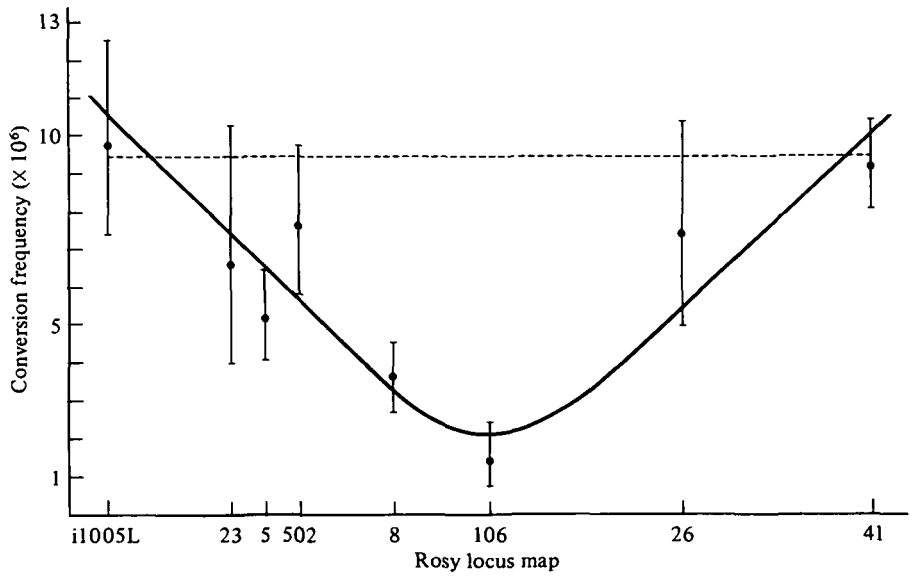

Fig. 6. Summary of *rosy* mutant allele conversion frequency as a function of relative map position.

That such a trivial explanation of the U-shaped pattern is not the case emerges from a consideration of Table 4, summarizing conversion frequency data for the five *rosy* alleles of Fig. 6 that have been subjects of extensive recombination tests. The extensively studied *rosy* alleles included in Table 4 have been examined in tests ranging from a minimum of five different heteroallele tests to a maximum of twenty-two different tests, and involve a total of $77{\cdot}76 \times 10^6$ screened progeny.

If the conversion frequency of an allele, ry^x, is a function of its map position for the trivial reason outlined above, then the ry^y conversion frequency (the average conversion frequency of all *rosy* alleles tested against a given ry^x) should be elevated or depressed from the overall mean ry^y conversion frequency in the same direction and to a similar extent that the ry^x frequency departs from its overall mean. Mere inspection of the data indicates that this is not the case, and correlation analysis (employing the arcsin transformation) yields a correlation coefficient not significantly different from zero ($r = -0{\cdot}282$). Thus, the trivial explanation is invalid, and another basis for the U-shaped polarity of conversion frequencies must be sought.

For the most part, conversion frequencies of *rosy* alleles that have not been

extensively tested appear not to deviate significantly from expectation on the basis of map position. For example, we have already noted the positions of ry^{i1005L}, ry^{23} and ry^{26} on the U-shaped curve (Fig. 6). Additionally, ry^{203}, which is located at the right end of the map, inseparable from ry^{41} (Fig. 2), has a high conversion frequency ($9{\cdot}11 \times 10^{-6}$ in a total of $4{\cdot}61 \times 10^{6}$ screened progeny), while ry^{42} centrally located somewhere to the left of ry^{26} has a moderate value ($4{\cdot}69 \times 10^{-6}$ in a total of $6{\cdot}84 \times 10^{6}$ screened progeny). One striking exception to the pattern is the ry^{2} allele. It exhibits a conversion frequency of $0{\cdot}36 \times 10^{-6}$ in a total of $5{\cdot}62 \times 10^{6}$ screened progeny, an order of magnitude smaller than the overall mean *rosy* allele conversion frequency of 5×10^{-6}, and yet maps very close to ry^{41} and ry^{203}, at the high point of the U-shaped curve. Such a deviation from the pattern of observed conversion frequencies has been termed a 'marker effect' (See review, Hastings, 1975).

Table 4. *Conversion frequencies of extensively tested* rosy *alleles* (ry^{x})

ry^{x}	Conversion frequency* of ry^{x} ($\times 10^{6}$)	Arcsin $\sqrt{f}$	Conversion frequency† of ry^{y} ($\times 10^{6}$)	Arcsin $\sqrt{f}$	N ($\times 10^{-6}$)
5	5·13	0·1298	6·56	0·1467	14·63
502	7·59	0·1578	4·28	0·1185	8·17
8	3·57	0·1083	4·07	0·1156	19·90
106	1·38	0·0673	5·76	0·1375	8·68
41	9·14	0·1732	4·93	0·1272	26·38

* Average conversion frequency for each of the indicated alleles, ry^{x} over a large number of tests against many heteroalleles, designated ry^{y}.

† Average conversion frequency of many alleles (designated ry^{y}), as seen in tests against a given ry^{x} allele.

(iv) *Gene conversion and chromosomal interference*

As was first noted by Muller (1916) and discussed in detail by Stevens (1936), crossing-over within a genetic interval interferes with the occurrence of coincident crossovers within closely linked intervals in the same chromosomal arm. This phenomenon is termed chromosomal or chiasma interference. We are able to examine the question of chromosomal interference in association with *rosy* locus recombination classes by examining coincident exchange within the adjacent *Ace*–*Sb* interval, which has a standard distance of 6·0 map units (Fig. 1).

The pertinent data are presented in summary form in Table 5. This summary was prepared by pooling the data from 41 different recombination experiments involving females of the genetic constitution *kar* ry^{x} *Ace Sb*/+ry^{y}++ (Fig. 4A), where ry^{x} and ry^{y} are mutant heteroalleles. Crossovers are ry^{+} exceptional progeny associated with exchange for the flanking markers *kar* and *Ace*. Conversions are ry^{+} exceptionals not associated with flanking marker exchange. All of the exceptional progeny were assayed for coincident recombination in the *Ace* to *Sb* interval. The 41 recombination experiments screened a total of $4{\cdot}5 \times 10^{7}$ progeny.

Table 5. *Coincident exchange within the* Ace-Sb *interval among* rosy *locus crossovers and conversions*

ry^+ recombinant class	Observed number	Coincident crossovers in *Ace-Sb* interval	Crossover frequency in *Ace-Sb* interval
Crossovers	281	2	0·00712
Conversions	420	22	0·0524

Examination of Table 5 reveals that crossing-over within the *rosy* locus strongly interferes with crossing-over in the nearby *Ace–Sb* interval, while conversion events within the *rosy* locus do not.

4. DISCUSSION

In all respects intragenic recombination in *Drosophila* appears analogous to that observed in fungal systems. All recombination would appear to involve gene conversion (i.e., non-reciprocal information transfer) in the immediate region of the exchange event in both fungi (Fogel *et al.* 1978) and *Drosophila* (Chovnick *et al.* 1971). As in fungal systems (Fogel *et al.* 1971), we observe that conversion events may include adjacent markers within a single locus (McCarron *et al.* 1974) or even extend to an adjacent locus (McCarron *et al.* 1979) and further, that the frequency of co-conversion of two heteroallelic sites is inversely proportional to the map distance between them (Table 3). Additionally, gene conversion in *Drosophila*, as in fungi, is associated with molecular fidelity in that the recombinants are truly derivative of the parental genetic information (McCarron *et al.* 1974).

As in *Neurospora* (Stadler, 1959) and *Saccharomyces* (Mortimer & Fogel, 1974), with *Sordaria* a possible exception (Kitani, 1978) we observe in *Drosophila* that intragenic recombinants associated with flanking marker exchange interfere with crossing over in adjacent regions, whereas parental flanking marker recombinants do not (Table 5).

As in fungal systems (Stadler, 1973) we observe allele conversion frequency differences (Table 4, Fig. 6) within the *rosy* locus. However, we do not see a simple polarity in allele conversion frequencies as a function of map position as in a number of fungal gene loci (Stadler, 1973), although the data do suggest that we may be observing a bipolar pattern (Fig. 6) reminiscent of the earlier results of Pees (1967) obtained from fine structure analysis of the *lys-51(FL)* locus of *Aspergillus nidulans*. Of some interest in this connection, is the exceptionally low conversion frequency associated with the spontaneous mutant, ry^2 (Hadorn & Schwink, 1956), which may be related to the observation of Bender (personal communication) that ry^2 is associated with a sizeable insertion at the right end of the *rosy* locus DNA. This insertion is seen consistently in whole genome Southern analyses of ry^2 mutant stocks as compared to other mutant and ry^+ isoallele stocks.

The probability that a given gene conversion involves flanking marker exchange is markedly less than 50% in *Drosophila melanogaster*, an observation that is

consistent with most of the fungal data (Stadler, 1973; DiCaprio & Hastings, 1976; Fogel *et al.* 1978; Perkins, 1979). However, an apparent difference is that in *Drosophila* only one class of flanking marker exchange is recovered from each cross (See Table 2), in contrast to most fungal data which show both major and minor crossover classes. The major crossover class of fungal data parallels the single crossover class of *Drosophila* in providing for unambiguous mapping. Perhaps the minor crossover class reflects the extreme rarity, in *Drosophila* as compared to fungi, of multiple recombination events in short intervals.

A key feature of molecular models of recombination is the production of a region of 'hybrid' (*i.e.*, heteroduplex) DNA. The existence of such an intermediate in recombination was first inferred from observations of postmeiotic segregation in analyses of *fungal* tetrad data, which are interpreted as the segregational products of uncorrected heteroduplexes. Chovnick *et al.* (1971) concluded that such events were infrequent in *Drosophila*. They argued that failure to correct a heteroduplex would lead to somatic segregation at the first postmeiotic mitosis, and the production of a somatic mosaic individual. In view of the nonautonomous nature of the *rosy* mutant eye colour phenotype and the fact that flies with less than normal levels of XDH can survive growth on the selective medium, a significant portion of the mosaics would be expected to survive and be scored among the putative recombinants. If all or most of its gonadal tissue derived from the ry^+ bearing nucleus, then such a mosaic individual would not be distinguished from other recombinants by the present system of analysis. However, one would expect that some mosaic individuals would possess gonadal tissue derived from the mutant nucleus, and be identified as ry^+ exceptionals that reproduced as mutants. At that time, only two such individuals were scored in 498 ry^+ exceptionals analysed.

In an analysis of sex-linked meiotic mutants, Baker & Carpenter (1972) identified a gene (*mei-9*) that was inferred to specify some component of the exchange process in meiosis in females. Chovnick (unpublished) examined the effect of homozygosis for the mutation, $mei\text{-}9^b$, upon recombination in the ry^5/ry^{41} heterozygote, a genotype that previously had been the subject of intensive analysis (Table 2). Due to the extremely poor fertility of $mei\text{-}9^b$ homozygous females, only a small sample was possible, and the experiment was terminated with 4 recovered recombinants. Three were simple conversions, and one was a clear-cut gonadal mosaic. The mosaic individual's progeny are most simply interpreted as reflexions of an uncorrected heteroduplex with one ry^+ convertant strand and the other carrying a maternally derived rosy mutant chromosome. With the realization that *mei-9* functions in repair replication (Nguyen & Boyd, 1977) and excision repair (Boyd, Golino & Setlow, 1976), this investigation was reopened (Romans, 1980*a*, *b*), and succeeded in demonstrating the following: (1) Recombinants in *rosy* heteroallele crosses do arise in $mei\text{-}9^a$ mutant homozygotes as frequently as in $mei\text{-}9^+$ control experiments. (2) Crossover events are suppressed in the $mei\text{-}9^a$ mutant crosses, with a concomitant increase in the conversion frequency. (3) Of twenty recombinants recovered from the $mei\text{-}9^a$ mutant crosses, five are most simply interpreted as postmeiotic segregants. Three exhibited ry^+ phenotypes, but transmitted as mutants, while two of the five were gonadal mosaics, reflecting the

maternal transmission of both strands of an uncorrected heteroduplex. Such exceptions were not seen among the conversions recovered from the *mei-9*$^+$ control crosses. These observations should be considered as minimal reflections of the extent of uncorrected heteroduplexes resulting from the *mei-9* defect. Clearly, these observations parallel fungal post-meiotic segregants and imply the production of a heteroduplex intermediate in recombination in *Drosophila*. Moreover, these data further support the notion that the *mei-9* gene product, presumably an enzyme required for heteroduplex mis-match correction, also plays an essential role in completing the process leading to the production of a flanking marking crossover.

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