ON THE IDENTIFICATION OF THE *ROSY* LOCUS DNA IN *DROSOPHILA MELANOGASTER:* INTRAGENIC RECOMBINATION MAPPING OF MUTATIONS ASSOCIATED WITH INSERTIONS AND DELETIONS

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ABSTRACT

DNA extracts of several *rosy*-mutation-bearing strains were associated with large insertions and deletions in a defined region of the molecular map believed to include the *rosy* locus DNA. Large-scale, intragenic mapping experiments were carried out that localized these mutations within the boundaries of the previously defined *rosy* locus structural element. Molecular characterization of the wild-type recombinants provides conclusive evidence that the *rosy* locus DNA is localized to the DNA segment marked by these lesions.—One of the mutations, ry^{2101}, arose from a *P-M* hybrid dysgenesis experiment and is associated with a *copia* insertion. Experiments are described which suggest that *copia* mobilizes in response to *P-M* hybrid dysgenesis.—Relevance of the data to recombination in higher organisms is considered.

UTILIZING the molecular and cytogenetic methodology of chromosomal walking, BENDER, SPIERER and HOGNESS (1983) isolated DNA segments that together constitute 315 kb of DNA of the 87 DE region of the polytene third chromosome of *Drosophila melanogaster.* This region includes the *rosy* locus (*ry*: 3-52.0) as well as several other genetic units that were defined by genetic complementation tests and were ordered against an array of overlapping deficiencies (HILLIKER *et al.* 1980). The endpoints of the deficiencies then were located on the 315-kb molecular map by *in situ* hybridization and whole genome Southern analyses, thereby further localizing these genetic units (SPIERER *et al.* 1983). In this way, the *rosy* DNA was localized to a segment of approximately 30 kb. Still more precise localization of the *rosy* locus DNA was accomplished by whole genome Southern analyses carried out on a large number of strains carrying various spontaneous and induced *rosy* mutations. This study (COTÉ *et al.* 1986) succeeded in associating several of the *rosy*-mutation-bearing strains with specific DNA lesions.

Figure 1A presents a genetic map of the *rosy* locus indicating the relative map positions of mutations and variant sites ordered in prior fine-structure mapping experiments (reviewed in HILLIKER and CHOVNICK 1981; CLARK *et*

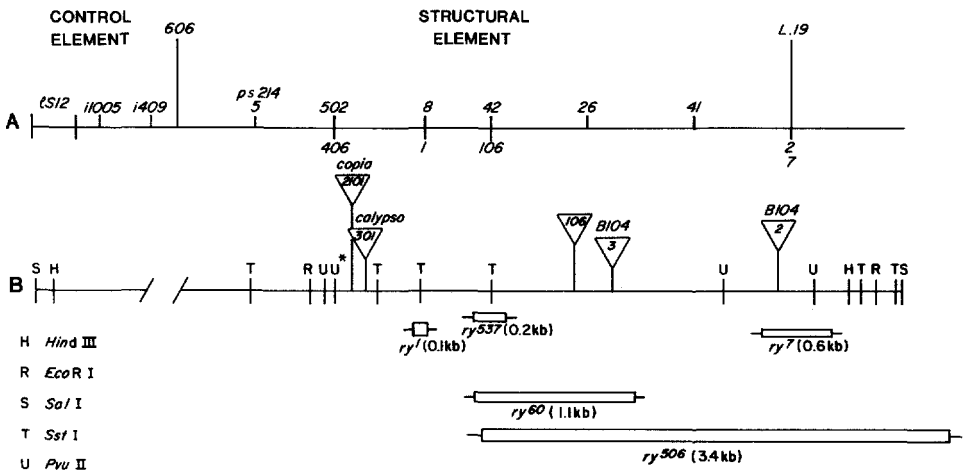

FIGURE 1.—A, Genetic map of the *rosy* locus. The relative map positions of variant sites were ordered in previous studies (reviewed in HILLIKER and CHOVNICK 1981; CLARK *et al.* 1984). Mutants listed below the line have been localized on the DNA restriction map as well. Map not drawn to scale. B, DNA restriction map of the 8.1-kb *Sal*I fragment of *rosy* region DNA. Insertions are indicated by triangles drawn above the restriction map. The number inside each triangle denotes the *rosy* allele associated with the DNA insertion. The names of those identified as mobile elements are noted above the triangles. The ry^{106} allele is associated with an undefined insertion larger than 5 kb. Rosy mutant alleles associated with DNA deletions are illustrated below the restriction map. Rosy allele designation and the approximate size of each deletion is indicated. The open rectangles represent the deleted segment, and the thin lines on the ends of each rectangle define the limits of the deletion's location on the restriction map. The starred *Pvu*II site is polymorphic (see text). The DNA map is drawn only to approximate scale and is taken largely from COTÉ *et al.* (1986).

al. 1984). Figure 1B presents a DNA restriction map of an 8.1-kb *Sal*I fragment of the *rosy* region indicating the DNA lesions associated with the indicated *rosy*-mutation-bearing strains. That the DNA lesions are the *rosy* mutations themselves was inferred from the following: (1) All of the DNA lesions fall within a segment of less than 5 kb, consistent with estimates of the minimal length of DNA required to code for a peptide of 150,000 daltons (CHOVNICK *et al.* 1977). (2) The poly(A)$^+$ RNA transcribed from this region is approximately 4.7 kb (COVINGTON, FLEENOR and DEVLIN 1984; RUSHLOW, BENDER and CHOVNICK 1984; CLARK *et al.* 1984; COTÉ *et al.* 1986). (3) Comparison of the molecular map order of the DNA lesions with the genetic map order indicates a striking colinearity. In particular, the relative map positions of the *rosy* mutations listed below the line of Figure 1A (reviewed in HILLIKER and CHOVNICK 1981) are associated with deletions (ry^1 and ry^7) and insertions (ry^{106} and ry^2) indicated on the molecular map (Figure 1B). (4) Localization of the allele complementing site mutant, ry^{406}, on the molecular map was accomplished in a half-tetrad recombination experiment. Conversions of ry^{406} exhibited 100% co-conversion (eight independent conversion events) with a polymorphism for the starred *Pvu*II site (Figure 1B), which was an unselected heterozygous marker in the cross (S. H. CLARK and A. CHOVNICK, unpub-

lished), indicating the close proximity of these sites (see discussion in HILLIKER and CHOVNICK 1981).

The present investigation, carried out in concert with the work of COTÉ *et al.* (1986), consisted of recombination experiments designed to genetically map the locations of mutations associated with large DNA insertions and deletions. Molecular characterization of the ry^+ recombinants of the insertion and deletion experiments provided further and conclusive evidence that the *rosy* DNA is indeed localized to the DNA segment described in Figure 1B. Subsequent elegant confirmation of this point came from transformation experiments that demonstrated the association of this DNA fragment with *rosy* locus expression (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1983). Finally, the present data are considered with respect to recombination *per se*. Of particular interest is the fact that intragenic rearrangement heterozygosity has little effect on recombination, except in the immediate region of the rearrangement itself. Thus, frequencies of conversion of the mutant allele associated with the rearrangement to wild type are diminished significantly in the case of smaller rearrangements and are totally absent for the large rearrangements. With respect to the mutations that are intragenic deletions, conversion to wild type permits a direct assessment of the size of heteroduplex corrections.

MATERIALS AND METHODS

The genetic system: A genetic map of the *rosy* locus (*3*-52.0) is presented in Figure 1A and indicates the relative map positions of mutations and variant sites ordered in prior intragenic mapping experiments and utilized in the present report (reviewed in HILLIKER and CHOVNICK 1981). The mutation, ry^{1108}, was recovered from an EMS mutagenesis by A. J. HILLIKER and S. H. CLARK. The spontaneous mutant, ry^3, is described in LINDSLEY and GRELL (1968). The origin of other *rosy* locus mutations is described herein. Balancer chromosomes, *rosy* region deletions and other markers are described either in HILLIKER *et al.* (1980) or in LINDSLEY and GRELL (1968).

Selective system matings: Large-scale fine-structure experiments were carried out utilizing a purine selection protocol satisfactory for discriminating between XDH^- (or almost XDH^-) and wild-type levels of activity (CHOVNICK 1973).

DNA extraction and restriction analysis: Procedures are described in CLARK *et al.* (1984), and probes used are described in COTÉ *et al.* (1986).

RESULTS AND DISCUSSION

Confirmation of the insertional nature of ry^3 and ry^{301}: While it seemed most likely that the colinearity data of COTÉ *et al.* (1986) are correct in identification of the *rosy* locus DNA (Figure 1B), fine-structure recombination experiments were carried out that were designed to specifically map the locations of ry^3 and ry^{301} on the genetic map and to examine a sample of the resulting intragenic recombinants for the presence of the pertinent DNA lesions.

The first four rows of Table 1 present the results of recombination experiments that locate both ry^3 and ry^{301} within the *rosy* locus structural element defined by ry^{606} as the left boundary (GELBAR, MCCARRON and CHOVNICK 1976) and $ry^{L\text{-}19}$ as the right boundary (MCCARRON *et al.* 1979).

Next, we consider the likelihood that the large DNA insertions found in the ry^3 and ry^{301} strains are the *rosy* DNA lesions themselves, and not insertion

TABLE 1

Number and classes of ry^+ chromosomes recovered from progeny of crosses of the indicated females to tester males of the genotype *Tp(3)MKRS, M(3)S34 kar ry² Sb/In(3R)P18, kar ry⁴¹ Ubx e⁴*

Female parent ry^x/ry^y	Crossovers $kar^+\ ry^+\ Ace^{i26}$	Crossovers $kar\ ry^+\ Ace^+$	Conversions ry^x $kar^+\ ry^+\ Ace^+$	Conversions ry^y $kar\ ry^+\ Ace^{i26}$	Progeny sampled ($\times 10^6$)
$\frac{h\ st\ +\ ry^3\ +\ ss^a}{+\ +\ kar^2\ ry^{606}\ Ace^{i26}\ +}$	46	0	1[b]	25	2.53[a]
$\frac{h\ st\ +\ ry^3\ +\ ss^a}{+\ +\ kar^2\ ry^{L.19}\ Ace^{i26}\ +}$	0	11	0	11	2.18
$\frac{+\ ry^{301}\ +}{kar^2\ ry^{606}\ Ace^{i26}}$	1	0	0	2	1.01
$\frac{+\ ry^{301}\ +}{kar^2\ ry^{L.19}\ Ace^{i26}}$	0	13	0	5	1.08
$\frac{+\ +\ ry^3\ +\ +\ +}{cu\ kar\ ry^8\ Ace^{i26}\ Sb\ Ubx}$	1	0	0	7	0.83[c]
$\frac{+\ +\ ry^{301}\ +\ +\ +}{cu\ kar\ ry^{41}\ Ace^{i26}\ Sb\ Ubx}$	0	36	0	18	2.53[c,d]
$\frac{+\ ry^{301}\ +}{kar^2\ ry^5\ Ace^{i26}}$	1	0	0	1	0.54

Progeny were reared on purine-supplemented medium, permitting only rare ry^+ offspring to survive.

[a] This cross involved tester males of the genotype *Tp(3)MKRS, M(3)S34 kar² ry² Sb/Df(3R)* ry^{36}.

[b] This exceptional chromosome was confirmed to be *h st* $kar^+\ ry^+\ Ace^+\ ss^a$.

[c] This cross involved tester males of the genotype *Dfd, Df(3) kar³¹* ry^{60}/kar^2, *Df(3)* ry^{75}.

[d] Data taken from GELBART *et al.* (1974).

polymorphisms in nearby, non-*rosy* locus DNA. Essential to the argument is that all conversions of ry^3 and ry^{301} to ry^+ should be free of the insertion. Indeed, all ry^+ recombinants should be free of the insertions. Thus, if the insertion associated with the ry^3 or ry^{301} chromosome were located outside and to the left of the *rosy* structural element (Figure 2A), the ry^+ crossovers between ry^{606} and the ry^3 or ry^{301} site would still carry the insertion, whereas crossovers between ry^3 or ry^{301} and $ry^{L.19}$ would be free of the insertion. If the insertion were to the right of $ry^{L.19}$ and the *rosy* structural element, then the reverse would obtain (Figure 2B).

Figure 3 presents a Southern blot that illustrates the results of a restriction analysis of the DNAs from a sampling of all parental and recombinant classes of the first four rows of Table 1. Utilizing a 4.6-kb *Eco*RI fragment from this region as a probe to identify fragments generated by *Pvu*II digestion (Figure 1B), distinction is possible between the DNAs associated with the parental allele complementing site mutations, ry^{606} (lane 2, Figure 3) and $ry^{L.19}$ (lane 3, Figure 3), on the one hand, and the insertion-bearing parental strains, ry^3 (lane 1, Figure 3) and ry^{301} (lane 10, Figure 3), on the other hand. Both ry^3 and ry^{301}

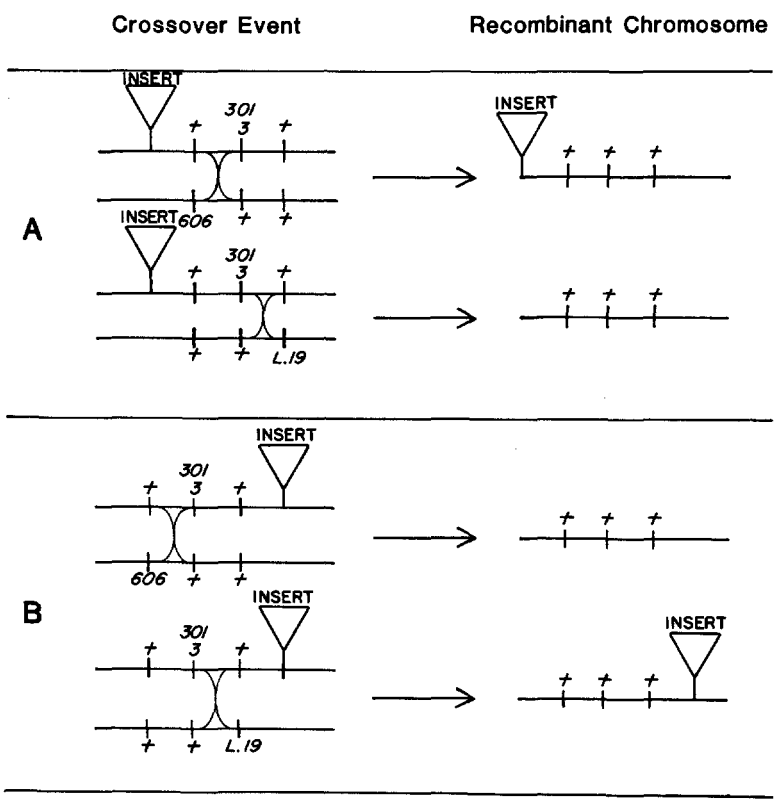

FIGURE 2.—A, Possible crossover events and resulting recombinant chromosomes if the DNA insertion associated with ry^{301} or ry^{3} marks a segment of DNA located to the left of ry^{606}. B, Possible crossover events and resulting recombinant chromosomes if the DNA insertion associated with ry^{301} or ry^{3} marks a segment of DNA located to the right of $ry^{L.19}$.

are associated with large transposable element insertions within the same *Pvu*II fragment (2.2 kb). That the ry^{3} and ry^{301} insertions differ from each other is evident from differences exhibited by their specific *Pvu*II restriction patterns (Figure 3). The fact that all ry^{+} recombinants, including the one conversion of ry^{3} to ry^{+}, are free of the insertions demonstrates that the insertions are located within the genetically defined structural element of the *rosy* locus (Figure 1).

The remaining data of Table 1 were collected some years ago, and they are presented to refine the genetic map positions of ry^{301} and ry^{3} to augment the total available intragenic recombination data involving mutations associated with these insertions. In particular, we call attention to the exceedingly low frequency of conversion of ry^{3} to ry^{+} ($1/5.54 \times 10^{6}$) and ry^{301} to ry^{+} ($0/5.16 \times 10^{6}$), in contrast to the much greater frequency of conversion events involving the various site mutant heteroalleles used in these tests (Table 1), and other *rosy* alleles in general (HILLIKER and CHOVNICK 1981). Similarly, low conversion frequency was seen earlier for ry^{2} to ry^{+} ($2/6.25 \times 10^{6}$) and for ry^{106} to ry^{+} ($20/10.73 \times 10^{6}$) in fine-structure mapping experiments reviewed

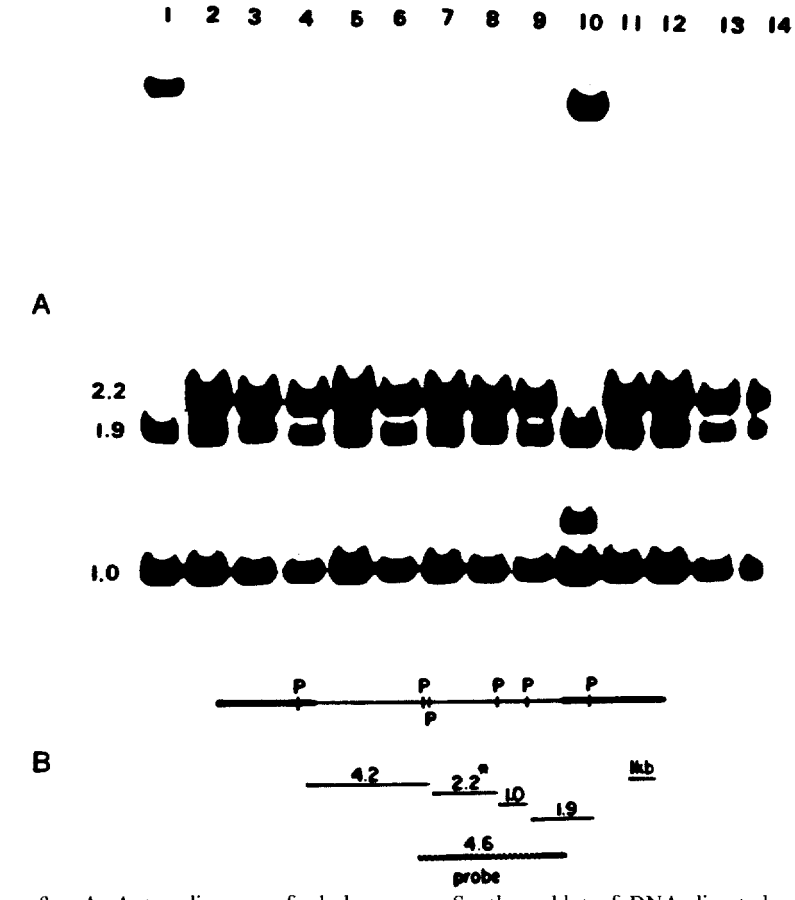

FIGURE 3.—A, Autoradiogram of whole-genome Southern blot of DNA digested with *Pvu*II. The blot was probed with a ^{32}P-labeled, 4.6-kb *Eco*RI fragment from this region (Figure 1B). Each lane contains DNA extracted from adults carrying parental or recombinant *rosy* alleles (Table 1, rows 1 through 4) as indicated below. Unless otherwise noted, all genotypes are hemizygotes with *Df(3R)ry*^{614} (HILLIKER *et al.* 1980). (1) *ry*^{3}; (2) *ry*^{606}; (3) *ry*$^{L.19}$; (4) *ry*$^{+}$ crossover from *ry*^{3}/*ry*^{606}; (5) *ry*$^{+}$ conversion of *ry*^{606} from *ry*^{3}/*ry*^{606}; (6) *ry*$^{+}$ conversion of *ry*^{3} from *ry*^{3}/*ry*^{606}; (7) *ry*$^{+}$ crossover from *ry*^{3}/*ry*$^{L.19}$; (8) *ry*$^{+}$ conversion of *ry*$^{L.19}$ from *ry*^{3}/*ry*$^{L.19}$; (9) *ry*$^{+3}$, the parental wild-type allele of *ry*^{301}; (10) *ry*^{301}/*ry*^{301}; (11) *ry*$^{+}$ crossover from *ry*^{301}/*ry*^{606}; (12) *ry*$^{+}$ conversion of *ry*^{606} from *ry*^{301}/*ry*^{606}; (13) *ry*$^{+}$ crossover from *ry*^{301}/*ry*$^{L.19}$; (14) *ry*$^{+}$ conversion of *ry*$^{L.19}$ from *ry*^{301}/*ry*$^{L.19}$. DNA fragment sizes (kb) are indicated. B, Illustration of the *Pvu*II restriction map of wild-type *ry*$^{+}$ DNA, including all pertinent sites. The narrow line represents the limits of the 8.1-kb *Sal*I fragment, and the flanking genomic DNA is illustrated by a broad line. Below the line are fragments and sizes generated by *Pvu*II restriction. The *ry*^{3} and *ry*^{301} insertions (Figure 1B) are located in the region of the starred *Pvu*II fragment. The 4.6-kb *Eco*RI fragment (Figure 1B) utilized as probe is indicated.

in HILLIKER and CHOVNICK (1981), and both are associated with large insertions (Figure 1B, COTÉ *et al.* 1986).

Studies with *ry*^{2101}: DNA from flies of the *ry*^{2101}-bearing strain possess an insertion of the transposable element, *copia*, located as indicated in Figure 1B, just proximal to the *ry*^{301} insertion, (COTÉ *et al.* 1986). This mutation was one

TABLE 2

Number and classes of ry^+ chromosomes recovered from progeny of the indicated females to tester males of the genotype *Tp(3)MKRS, M(3)S34 kar ry² Sb/In(3R) P18, kar ry⁴¹ Ubx e⁴*

Female parent ry^+/ry^y	Crossovers		Conversions ry^+	Conversions ry^y	Progeny sampled ($\times 10^6$)
	$kar^+\ ry^+\ Ace^{l26}$	$kar\ ry^+\ Ace^+$	$kar^+\ ry^+\ Ace^+$	$kar\ ry^+\ Ace^{l26}$	
$\frac{+\ ry^{2101}\ +}{kar^2\ ry^{606}\ Ace^{l26}}$	7 (3)	0	2 (1)	9 (1)	2.04
$\frac{+\ ry^{2101}\ +}{kar^2\ ry^{L.19}\ Ace^{l26}}$	0	4 (4)	0	1 (1)	0.58
$\frac{+\ ry^{2101}\ +}{kar^2\ ry^{606}\ Ace^{l26}}$ (D)	0	0	1 (1)	2	0.57
$\frac{+\ ry^{2101}\ +}{kar^2\ ry^{L.19}\ Ace^{l26}}$ (D)	0	8 (4)	2 (2)	1	0.83

Progeny were reared on purine-supplemented medium, permitting only rare ry^+ offspring to survive. Southern analysis was carried out on DNAs from 17 recombinant lines, indicated in parentheses.

of several that arose as a result of a *P-M* hybrid dysgenesis mutagenesis experiment conducted several years ago in this laboratory. Harwich (*P* strain) males were crossed to homozygous *cu kar* ry^{+0} (*M* strain) females. The clearly dysgenic F_1 males were mated to *Tp(3)MKRS, M(3)S34 kar* ry^2 *Sb/In(3R)P18, kar* ry^{41} *Ubx* e^4 females, and the ry^{2101} mutation was recovered among the progeny as a ry^- mutation on a *cu* kar^+ chromosome. Since (1) the Harwich strain is not a ry^+ isoallelic line and (2) the ry^{2101} mutation-bearing chromosomes arose with an apparent crossover between *cu* and *kar*, we are uncertain of the parental ry^+ allele of ry^{2101}. The electrophoretic mobility data of ry^+ recombinants involving ry^{2101} is consistent with a ry^+ parent allele that is associated with an XDH peptide of the same electrophoretic mobility as that of ry^{+0}.

Fine-structure recombination experiments were carried out that were designed to locate ry^{2101} on the genetic map (Table 2). It was evident that the ry^{2101} stock was behaving as a *P* strain. In crosses to standard laboratory strains, the F_1 progeny were associated with the syndrome of germline abnormalities characteristic of *P-M* hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977; BREGLIANO and KIDWELL 1983; ENGELS 1983). Consequently, the mapping study was carried out utilizing heterozygous female parents produced from reciprocal crosses. Those females that were not dysgenic (Table 2, rows 1 and 2) were used as parents in crosses distinct from those utilizing dysgenic females (Table 2, rows 3 and 4). This diagnosis of the character of the crosses of Table 2 was confirmed by our examination of the control crosses. Our standard method for estimation of total progeny has been to rear a portion of the crosses on nonselective medium, thereby permitting estimation of total progeny by direct count. The crosses of Table 2, rows 3 and 4 were relatively infertile, and a number of visible mutations were recovered among the progeny in the control bottles (data not shown).

The crossover data of Table 2 serve to locate ry^{2101} within the *rosy* locus structural element on the genetic map (Figure 1A). Southern analysis was carried out on DNAs from 17 recombinant lines, indicated in parentheses in Table 2. The experimental protocol is modified from that of Figure 3, but the results are identical. Utilizing an 8.1-kb *Sal*I fragment that covers the entire *rosy* locus (Figure 1B) as a probe to identify fragments generated by *Sst*I digestion, the DNAs from the parental allele complementing mutants, ry^{606} and $ry^{L.19}$ are indistinguishable from each other, and both are distinguishable from ry^{2101}. In particular, the 1.5-kb *Sst*I fragment present in ry^{606} and $ry^{L.19}$ is missing in ry^{2101} and is replaced by a 6.5-kb fragment representing the *copia* insertion. All recombinants, including the conversions of ry^{2101}, exhibited a normal *Sst*I restriction pattern free of the *copia* insertion. Thus, we conclude that the *copia* insertion is, indeed, the lesion of ry^{2101} and, further, that this insertion serves as a DNA marker located within the genetically defined *rosy* locus structural element.

We have separated the presentation of the ry^{2101} data from the ry^{3} and ry^{301} analysis because of our concern over the nature of the ry^{2101} conversions. Although there is no doubt about the crossover map position and molecular map position of ry^{2101}, we suggest that some or all of the ry^{2101} conversions may, in fact, be precise excisions of the *copia* element leading to restoration of a ry^{+} DNA sequence and phenotype. Certainly the greater frequency of apparent conversion of ry^{2101} observed in the dysgenic crosses, compared to that seen with the other transposable element insertions (Table 5B), suggests caution in simply classifying them as conversions.

Finally, we note that the origin of ry^{2101} and the data presented in this section serve to direct attention to the possibility that *copia* mobilizes in response to *P-M* dysgenesis. Observations consistent with this theory have also been reported by RUBIN, KIDWELL and BINGHAM (1982).

Recombination studies involving intragenic deletions: Several of the *rosy* mutations have been found to be associated with intragenic deletions. Our earliest intragenic mapping studies, utilizing a flanking lethal crossover selective system, provided data indicating that both ry^{1} and ry^{7} failed in recombination test against closely linked site mutants which were, themselves, separable. A considerable argument was presented associating ry^{1} with an intragenic rearrangement (CHOVNICK *et al.* 1964).

Table 3 summarizes the results of earlier recombination experiments involving *rosy* alleles that are now known to be associated with deletions and that were presented in prior reports (as indicated). These experiments utilized the purine selective system, thereby permitting the recovery of conversions as well as crossovers. Of interest are the observations that ry^{1} exhibits a low normal conversion frequency (see HILLIKER and CHOVNICK 1981), in contrast to the complete failure to recover conversions of ry^{506} in experiments of approximately the same sample size.

In order to examine this issue further, and to gather additional data bearing on the localization of the *rosy* locus DNA, additional recombination experiments were carried out involving the intragenic deletion mutations ry^{60} and

TABLE 3

Summary of experiments (reported earlier as indicated) involving mutations that are associated with intragenic deletions ry^1 (0.1 kb) and ry^{506} (3.4 kb)

Heteroallelic pair ry^x/ry^y	Crossovers	Conversions ry^x	Conversions ry^y	N ($\times 10^6$)
ry^1/ry^5	3	2	5	0.68[a]
ry^1/ry^{41}	11	2	10	0.82[a]
ry^1/ry^2	20	6	0	0.91[a]
ry^{506}/ry^5	3	0	4	0.90[b]
ry^{506}/ry^{ts214}	4	0	4	1.59[c]

[a] From CHOVNICK, BALLANTYNE and HOLM (1971).
[b] From GELBART *et al.* (1974).
[c] From GELBART, MCCARRON and CHOVNICK (1976).

TABLE 4

Number and classes of ry^+ chromosomes recovered from progeny of crosses of the indicated females to tester males of the genotype *Tp(3)MKRS, M(3)S34 kar* ry^2 *Sb/Df(3R)* ry^{36}

Female parent ry^x/ry^y	Crossovers		Conversions ry^x	Conversions ry^y	Progeny sampled ($\times 10^6$)
	kar^+ ry^+ l	kar^+ ry^+ l^+	kar^+ ry^+ l^+	kar ry^+ l	
$\frac{+\ ry^{60}\ +}{kar^2\ ry^{606}\ Ace^{l26}}$[a]	11	0	1[b]	6	0.68
$\frac{+\ ry^{60}\ +}{kar^2\ ry^{406}\ pic^{lG23}}$[a]	0	0	1[b]	1	0.50
$\frac{+\ ry^{60}\ +}{kar^2\ ry^{L.19}\ Ace^{l26}}$[a]	0	4	1[b]	4	0.52
$\frac{+\ ry^{537}\ +}{kar^2\ ry^{606}\ Ace^{l26}}$	3	0	1	1	0.25[c]

Mutations that are associated with intragenic deletions ry^{60} (1.1 kb) and ry^{537} (0.2 kb) are summarized. Progeny were reared on purine-supplemented medium, permitting only rare ry^+ offspring to survive.

[a] The starred *Pvu*II site (Figure 1) is a nonselective, heterozygous marker in this cross. This site is absent in ry^{60} and is present in the other mutant allele.

[b] The starred *Pvu*II site is not present.

[c] This cross involved tester males of the genotype kar^2 ry^{1108}/kar^2 ry^{1108}.

ry^{537} (Table 4). The ry^{60} mutation was recovered from an X-ray mutagenesis and was found to be associated with a 1.1-kb deletion (Figure 1B) by COTÉ *et al.* (1986). The ry^{537} mutation was recovered from an ethylnitrosourea (ENU) mutagenesis (MCCARRON and CHOVNICK 1981) and was found to be associated with a 0.2-kb deletion (Figure 1B) by M. HUFF and F. L. DUTTON (personal communication). Southern analysis was carried out on DNAs from the parent lines and on all of the conversions of the deletion-associated alleles (Table 4). All conversions of ry^{60} to ry^+ and the single conversion of ry^{537} to ry^+ exhibit a *Pvu*II restriction pattern, indicating that the observed DNA deletion, in each case, is no longer present. With respect to the ry^{60} conversions, none extended

TABLE 5

Conversion frequencies ($ry^x \rightarrow ry^+$) for mutations associated with (A) insertions and (B) deletions

Class	Mutation	Rearrangement size (kb)	No. of conversions	N ($\times 10^6$)	Frequency ($\times 10^{-6}$)
A	ry^2 (*B104*)	8.5	2	6.25	0.320
	ry^3 (*B104*)	8.5	1	5.54	0.181
	ry^{301} (*calypso*)	8.0	0	5.16	0.000
	ry^{106}	5.0	20	10.73	1.86
	ry^{2101} (*copia*)[a]	5.0	2	2.62	0.763
	ry^{2101} (*copia*)[b]	5.0	3	1.40	2.14
B	ry^1	0.1	10	2.41	4.15
	ry^{537}	0.2	1	0.25	4.00
	ry^{60}	1.1	3	1.70	1.76
	ry^{506}	3.4	0	2.49	0.00

Identified transposable elements among the insertions are indicated.
[a] Nondysgenic.
[b] Dysgenic.

to co-convert the heterozygous *Pvu*II site (Table 4). These data (not shown) provide still further support for the identification of the *rosy* locus DNA as described in prior sections.

Intragenic recombination in Drosophila: Studies of the effect of gross rearrangement heterozygosity on intergenic recombination in Drosophila are characterized by suppression of exchange that extends for some distance along the length of the involved chromosome(s), beyond that of the rearrangement itself. This effect is believed to relate to chromosomal pairing problems in regions beyond the rearrangement (*e.g.*, STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954; ROBERTS 1970; HAWLEY 1980). In contrast, the present data, dealing with intragenic rearrangement heterozygosity, shows little evidence of suppression extending beyond the rearrangement itself. We have experienced no serious difficulty in locating these rearrangements on our genetic map (Figure 1A), and there is excellent correspondence between that map and the molecular map (Figure 1B). There is some evidence for slight diminution of exchange in the immediate vicinity of the heterozygosity, but this effect disappears in heteroallelic recombination tests involving more distant sites.

Of particular interest are the conversion data involving the deletion and insertion alleles summarized in Table 5. The conversion data involving the insertions (Table 5A) appear to fall into two very distinct classes when one considers their cumulative conversion frequencies (0.176×10^{-6} for the 8-kb insertions and 1.69×10^{-6} for the 5-kb insertions). This one order-of-magnitude difference remains even if one removes the dysgenic cross from consideration. With the exception of the ry^{106} insertion (X-ray induced), which has not been cloned and examined, all of the insertions are identified transposable elements. It is possible that the infrequent apparent conversions may represent mobilizations in the form of precise excision events.

With respect to the deletion mutations (Table 5B), we note an inverse relationship between deletion size and conversion frequency. Thus, conversion of ry^{60} or ry^{+} occurs within a very much below-normal frequency (see HILLIKER and CHOVNICK 1981), but intermediate to that seen with ry^{1} and ry^{506}, the much smaller and much larger deletion, respectively. Unlike the insertion mutations, we believe that these events must be true conversions.

That gene conversion in Drosophila, as in fungi, results from the correction of a biparental heteroduplex recombination intermediate is implied from the results of recombination studies involving meiotic repair defective mutants which exhibit postmeiotic segregation [see discussion in HILLIKER and CHOVNICK (1981) and CARPENTER (1982)]. Since the Drosophila data is restricted to observations of correction in one direction, we are unable to comment on the issue of parity in the case of deletion mutants, as well as in site mutants (FINK 1974; FINK and STYLES 1974; LAWRENCE *et al.* 1975).

Efforts to estimate the size range of segments that may be corrected in a conversion event have been based on co-conversion mapping data (see HILLIKER and CHOVNICK 1981; CARPENTER 1984). The present data, based on DNA deletions of known extent, permit direct determination of correction lengths. Thus, we note that DNA segments as large as 0.1–0.2 kb are frequently corrected, whereas it is indeed possible in recombinationally normal genotypes to correct as much as 1.1 kb. We recognize that current molecular models consider that correction of large insertions and deletions are special cases (RADDING 1978; SZOSTAK *et al.* 1983).

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