The Effect of DNA Sequence Polymorphisms on Intragenic Recombination in the *rosy* Locus of *Drosophila melanogaster*

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ABSTRACT

The effect of simple DNA sequence polymorphisms on intragenic recombination in the *rosy* locus of *Drosophila melanogaster* was assayed. Two crosses were performed involving nearly identical molecular distances between selective *ry* null mutations (3778 nucleotides and 3972 nucleotides). In one heterozygote (ry^{606}/ry^{531}), in addition to the nucleotide substitution ry^- mutations, there were 11 simple nucleotide polymorphisms between the selective markers as well as additional flanking simple nucleotide polymorphisms within the *rosy* locus. In the other heterozygote (ry^{606}/ry^{609}), there were no additional polymorphisms because the two *rosy* nucleotide substitution mutations were induced on the same *rosy* isoallele (ry^{+6}). From ry^{606}/ry^{531} heterozygous females, 27 intragenic crossovers and five marker conversions were seen among 4.53×10^5 progeny. From ry^{606}/ry^{609} heterozygous females, 23 intragenic crossovers and eight marker conversions were seen among 4.18×10^5 progeny. The intragenic crossover frequencies per kilobase of DNA were very similar, 1.6×10^{-5} for ry^{606}/ry^{531} and 1.4×10^{-5} for ry^{606}/ry^{609}. Thus, simple DNA sequence polymorphisms neither inhibit nor promote intragenic recombination in *D. melanogaster*.

GENETIC fine structure analysis of the *rosy* locus of *Drosophila melanogaster* has revealed important features of recombination. Meiotic crossing over or linked exchange in *D. melanogaster* has its origin in gene conversion, the nonreciprocal transfer of genetic information between homologous regions of nonsister chromatids (reviewed in HILLIKER and CHOVNICK 1981; HILLIKER, CLARK and CHOVNICK 1988; CLARK, HILLIKER and CHOVNICK 1988; see also CURTIS *et al.* 1989). A small proportion of gene conversions (less than 20%) results in crossing over; *i.e.*, physical exchange between homologous nonsister chromatids (HILLIKER and CHOVNICK 1981).

The *rosy* locus has been cloned and the molecular lesions of many *rosy* locus mutations have been determined (COTE *et al.* 1986; CLARK *et al.* 1986; LEE *et al.* 1987; KEITH *et al.* 1987; LINDSLEY and ZIMM 1990). The DNA sequences have been determined for several different *rosy* wild-type isoalleles from which mutations have been generated (CURTIS *et al.* 1989; LINDSLEY and ZIMM 1990; D. CURTIS and W. BENDER, personal communication; GRAY *et al.* 1991). In conjunction with fine structure analysis this molecular information allows further analysis of the nature of recombination in *Drosophila*.

The links between recombination and DNA repair are well established in prokaryotes and eukaryotes. In *D. melanogaster*, several mutagen sensitive mutants, likely to be defective in DNA repair, have been shown to drastically effect meiotic crossing over (reviewed in BAKER *et al.* 1976; see also CARPENTER 1982). Since DNA repair operates by recognizing DNA lesions (often base pair mismatches) one could reasonably speculate that DNA sequence polymorphisms are important in crossing over. The localized DNA heteroduplexes formed from complementary strands of DNA (from nonsister chromatids) are probably the substrate for the further single strand nicks and/or other events necessary to cause the heteroduplex to be resolved as a crossover. Base pair mismatches in the heteroduplex may stimulate these further events leading to a crossover to occur, by analogy with DNA repair.

It should be noted, however, that in *Saccharomyces cerevisiae* BORTS and HABER (1987) observed that the introduction of 9 heterologies into a 9-kilobase (kb) interval resulted in a marked *decrease* in meiotic crossing over within this interval. However, the specific interval analysed was unusual in that it had an extremely high level of meiotic crossing over (23.4% of all tetrads!), was a duplication of the MAT locus and included sequences derived from the pBR322 cloning vector.

The topic that this report investigates is the relationship between DNA sequence polymorphisms and recombination. If DNA sequence polymorphisms promote physical exchanges, then the selection for crossing over in the female germline may facilitate the maintenance of polymorphisms. In the absence of meiotic crossing over, chromosome segregation is pro-

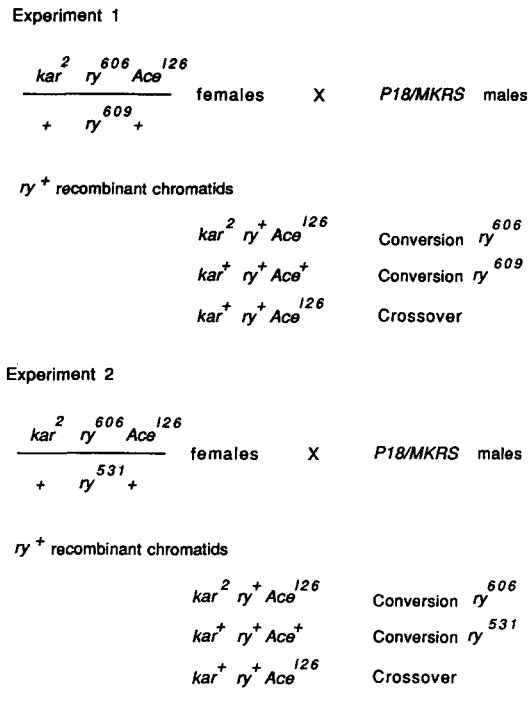

FIGURE 1.—Mating schemes employed in the recombination experiments and classes of recombinant events recovered. In a rosy heteroallelic female three classes of ry^+ recombinants are typically recovered, a flanking marker exchange ry^+ recombinant class, which represents an intragenic crossover, and two parental classes, nonexchange for flanking markers, which represent conversions of a marker allele (reviewed in HILLIKER and CHOVNICK, 1981).

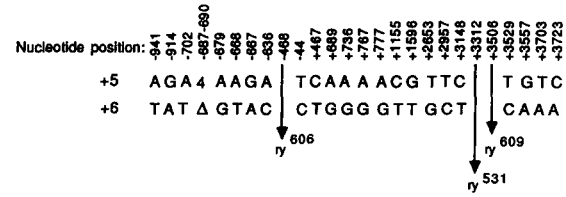

FIGURE 2.—Relevant rosy locus DNA polymorphisms in ry^{606}/ry^{531} heterozygotes. DNA polymorphisms in the interval defined by ry^{606} and ry^{531} and 500 bp to the left and right are presented. All 11 polymorphisms between ry^{606} and ry^{531} are transition mutations. In the 500-bp subinterval to the left of ry^{606} there are four transitions, three transversions and a 4-bp deletion. In the 500-bp subinterval to the right of ry^{531} there are three transitions and one transversion.

foundly disrupted in female meiosis, leading to a very high segregation load (reviewed in BAKER *et al.* 1976).

MATERIALS AND METHODS

Chromosomes, balancers and mutations: Several abbreviated designations are employed. These include *MKRS* = *Tp(3)MKRS, M(3)S34 kar ry*^{2} Sb; *P18* = *In(3L)P* + *In(3R) P18, Ubx ry*^{41} e^4 of the *rosy* alleles employed ry^{606} is a G → A null mutation at *ry* locus nucleotide position −468; ry^{609} is a G → A null mutation at *rosy* locus nucleotide position +3506 and ry^{531} is a G → A null mutation at *rosy* locus nucleotide position +3312 (GRAY *et al.* 1991). Further information on these mutations and rearrangements may be found in LINDSLEY and ZIMM (1987, 1990).

Selective system mating system: Selective system matings were carried out following a purine selection protocol (CHOVNICK 1973) suitable for (1) discriminating between XDH$^-$ and XDH$^+$ individual progeny, (2) permitting detection of clusters of exceptional progeny, and (3) estimating the total number of progeny sampled in each experiment.

Experimental system: Mutant heteroallele females, kar^2 ry^{606} $Ace^{126}/+$ $ry^{609}+$ in Experiment 1 and kar^2 ry^{606} $Ace^{126}/+$ $ry^{531}+$ in Experiment 2, were crossed to P18/MKRS tester males and their progeny were reared on purine supplemented selective medium at approximately 25°.

Both experiments were performed at the same time and under the same experimental and environmental conditions. Figure 1 illustrates these matings and the types of intragenic recombinants recoverable with respect to the flanking markers kar^2/kar^+ and Ace^{126}/Ace^+. Surviving ry^+ exceptions, occurring as rare single individuals among the matings, were crossed to the tester stock to establish lines for which the flanking markers could be confirmed (for kar/kar^+) or determined (for Ace^{126}/Ace^+).

RESULTS AND DISCUSSION

Although the molecular distance between ry^{606} and ry^{609} is nearly identical to that between ry^{606} and ry^{531} (3.97 kb and 3.78 kb, respectively) (GRAY *et al.* 1991) there are 11 simple nucleotide sequence polymorphisms between ry^{606} and ry^{531} (Figure 2). (There are no simple nucleotide sequence polymorphisms between ry^{606} and ry^{609} as they were induced on the same wildtype *rosy* isoallele, ry^{+6}.) In addition to the 11 polymorphisms between the ry^{606} and ry^{531} mutations in ry^{606}/ry^{531} heterozygotes, there are also seven simple nucleotide sequence polymorphisms and one, 4-base pair (bp) deletion polymorphism in the 500-bp region immediately to the left of ry^{606} and four simple nu-

TABLE 1

Number and classes of ry^+ chromosomes recovered from progeny of the indicated females crossed to tester males

Expt. No.	Females ry^x/ry^y	DNA heterozygosities	Crossovers		Conv ry^x kar^2 ry^+Ace^{126}	Conv ry^y $kar^+ry^+Ace^+$	Zygotes sampled (×10^6)
			$kar^2ry^+Ace^+$	kar^+ ry^+Ace^{126}			
1	$\frac{kar^2ry^{606}Ace^{126}}{+\ ry^{609}\ +}$	0	0	23	5	3	0.418
2	$\frac{kar^2ry^{606}Ace^{126}}{+\ ry^{531}\ +}$	11	0	27	1	4	0.453

cleotide sequence polymorphisms in the 500-bp region immediately to the right of ry^{531} (Figure 2).

Thus, if nucleotide sequence polymorphisms influence meiotic recombination, we would expect a marked difference in recovery of ry^+ intragenic crossovers from ry^{606}/ry^{609} females relative to ry^{606}/ry^{531} females (see Introduction). However, the two experiments yielded similar results (Table 1). From ry^{606}/ry^{609} females, 23 crossovers were recovered from 4.18×10^5 progeny and from ry^{606}/ry^{531} females 27 crossovers were recovered from 4.53×10^5 progeny. The ry^+ crossover frequencies per kilobase of DNA were 1.4×10^{-5} for ry^{606}/ry^{609} and 1.6×10^{-5} for ry^{606}/ry^{531}. This crossover frequency is an underestimate by a factor of 2 as only ry^+ crossovers are recovered by the selection system. (The double mutant reciprocal crossover class, $ry^x\ ry^y$, is not recoverable.) The corrected map distances are 0.011 cM for $ry^{606} - ry^{609}$ (3.97 kb) and 0.012 cM for $ry^{606} - ry^{531}$ (3.78 kb).

Thus, DNA simple sequence polymorphisms do not significantly effect crossing over in *D. melanogaster*. They are clearly not required for recombination to occur and their absence would not result in an elevation in segregational load due to meiotic chromosomal nondisjunction (which accompanies a reduction or elimination of crossing over in the female). There is no relationship between the mechanism of meiotic crossing over and the maintenance of DNA simple sequence polymorphisms.

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