INVESTIGATION

Variation in Meiotic Recombination Frequencies Between Allelic Transgenes Inserted at Different Sites in the Drosophila melanogaster Genome

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ABSTRACT Meiotic crossovers are distributed nonrandomly across the genome. Classic studies in Drosophila suggest that the position of a gene along a chromosome arm can affect the outcome of the recombination process, with proximity to the centromere being associated with lower crossing over. To examine this phenomenon molecularly, we developed an assay that measures meiotic crossovers and noncrossover gene conversions between allelic transgenes inserted into different genomic positions. To facilitate collecting a large number of virgin females, we developed a useful genetic system that kills males and undesired classes of females. We found that the recombination frequency at a site in the middle of the X chromosome, where crossovers are normally frequent, was similar to the frequency at the centromere-proximal end of the euchromatin, where crossovers are normally infrequent. In contrast, we recovered no recombinants—crossovers or noncrossovers—at a site on chromosome 4 and at a site toward the distal end of the X chromosome. These results suggest that local sequence or chromatin features have a stronger impact on recombination rates in this transgene assay than position along the chromosome arm.

Keywords: meiotic recombination Drosophila

Meiotic recombination serves several important functions. First, in many organisms the pairing of homologous chromosomes in meiosis is dependent on recombination (reviewed in Bhalla and Dernburg 2008). Second, chiasmata resulting from crossovers promote accurate segregation of homologous chromosomes at the reductive division, allowing the production of gametes or spores with the correct haploid number of chromosomes (reviewed in Martinez-Perez and Colaiácovo 2009). Third, recombination produces new combinations of alleles at different loci, resulting in greater genetic variation upon which natural selection can act and allowing detrimental mutations to be removed from chromosomes bearing favorable alleles at other loci (reviewed in Otto and Lenormand 2002). Not all meiotic recombination events are equally able to achieve all of these benefits. For example, crossovers promote disjunction of homologous chromosomes, but noncrossover recombination events do not. Moreover, some crossovers may be more capable of promoting disjunction than others. Studies of meiotic nondisjunction of human chromosome 21 and the Drosophila X chromosome suggested that a single distal crossover is less able to ensure correct disjunction than a single medial crossover and that extremely proximal crossovers actually disrupt proper segregation (Koehler et al. 1996; Lamb et al. 1996). These observations may explain why crossovers tend to be more frequent in the medial regions of chromosomes in Drosophila and human female meiosis.

Sturtevant (1913) generated the first genetic maps, based on crossover frequencies between pairs of genes, 100 years ago. A decade later, Muller’s (1927) discovery that ionizing radiation induces chromosome rearrangements made it possible to build physical maps of chromosomes. Within a few years several researchers, including Painter and Muller (1929), Dobzhansky (1930), and Beadle (1932), concluded from comparison of physical and genetic maps that the frequency of crossovers per unit distance varies along Drosophila chromosomes, with the most noticeable effect being suppression of crossing over near the centromere. Conclusive evidence that this effect is caused by proximity to the centromere was demonstrated by Sturtevant and Beadle (1936), who found that crossover frequencies in the proximal euchromatin increase when this region is moved away from the centromere by inversion. Green (1955) took advantage of this phenomenon to
study crossovers between different alleles of forked (f). This gene is located about a quarter of the distance from the pericentric heterochromatin of the X to the telomere, a region where crossovers are relatively infrequent. Green recovered a single crossover among 45,000 progeny of f/ f females. He then crossed each allele onto In(1)ac, which inverts most of the chromosome arm, starting a few hundred kilobases from the telomere and ending in the middle of the pericentric heterochromatin. This moved forked much farther from the centromere but left flanking sequences unchanged for at least five megabase pairs in each direction. In this configuration, the frequency of crossovers between f and f was elevated eightfold. This result suggests that meiotic crossover frequency is not an intrinsic property of a given gene or region but rather is determined at least in part by the position of that gene or region along a chromosome.

Although different patterns of crossover distribution along chromosomes may be exhibited by different species, or even between the sexes of one species, these patterns are nonetheless highly regulated. The mechanisms used to control crossover distribution are poorly understood (reviewed in Phadnis et al. 2011; Youds and Boulton 2011). Meiotic recombination is initiated through the introduction of double-strand breaks (DSBs), so a simple method to control crossover distribution might be to make DSBs exclusively at sites at which crossovers are desired and then generate a crossover at each DSB site. However, there are more DSBs than crossovers, by an order of magnitude or more in some species (e.g., Moens et al. 2002). Most meiotic DSBs are repaired through pathways that do not produce crossovers; these noncrossover outcomes can be detected when they involve gene conversion, the unidirectional transfer of information from one DNA duplex to another (reviewed in Andersen and Sekelsky 2010). Thus, to understand crossover control, it is necessary to understand the factors that influence initiation as well as the factors that control the crossover/noncrossover decision.

Studies in Drosophila suggest that chromosomal position effects on crossover frequency may be mediated through the crossover/noncrossover decision. Chovnick et al. (1993), Smith et al. (1970), and Finnerty et al. (1970) conducted experiments to dissect the fine-structure of the maroon-like (mal) gene, which is located toward the centromere-proximal end of the euchromatin, approximately 2 Mb from the pericentric heterochromatin. When mal was in its endogenous location, almost all recombination events recovered were noncrossover gene conversions (Smith et al. 1970). These researchers then used the aforementioned method used by Sturtevant and Green, crossing mal mutations into inverted chromosomes. In this configuration, intragenic crossovers were recovered much more frequently, even though sequences surrounding mal were unchanged for several megabases to the right (including a large block of pericentric heterochromatin) and about 20 Mb to the left (almost the entire euchromatic sequence of the X chromosome) (Finnerty et al. 1970). This finding suggests that the longitudinal position of a gene along a chromosome arm affects either the total frequency of recombinant (i.e., the frequency of DSBs), the probability that an initiated event becomes crossover, or both. The data do not distinguish between these possibilities because different alleles of mal were used in the different experiments.

More recently, fine-scale mapping of crossovers in Drosophila have found substantial variation in crossover rates at smaller scales (~5–50 kb) (Cirulli et al. 2007; Singh et al. 2009; Comer et al. 2012; Singh et al. 2013). Several genomic features, including sequence motifs and gene content, were found to be significantly associated with greater crossover rates (Comer et al. 2012; Miller et al. 2012; Singh et al. 2013). In contrast, the frequency of noncrossover gene conversion appears to be similar throughout the euchromatic genome (Comer et al. 2012), which suggests that the distribution of DSBs is much more uniform than the distribution of crossovers. Considered in light

### Table 1 Polymorphisms in pRatt-ry transgenes

| Position | ry+  | ry_ATG | ry_ATGA | Comments |
|----------|------|--------|--------|----------|
| −3149    | GTAC | –      | GTAC   | Destroys KpnI site for flanking marker |
| −2914    | G    | G      | C      | Destroys PstI site |
| −710     | C    | A      | C      | From ry606 chromosome |
| −679     | A    | G      | A      | From ry606 chromosome |
| −527     | G    | G      | T      | From ry606 chromosome |
| −468     | G    | G      | A      | ry606 mutation |
| −332     | T    | T      | G      | From ry606 chromosome |
| −323     | T    | T      | C      | From ry606 chromosome |
| −320     | A    | A      | C      | From ry606 chromosome |
| +73      | G    | T      | G      | From ry606 chromosome |
| +239     | C    | T      | C      | From ry606 chromosome |
| +355     | T    | A      | T      | From ry606 chromosome |
| +449     | T    | C      | C      | From ry606 chromosome, creates AgeI site |
| +467     | C    | T      | C      | From ry606 chromosome |
| +1103    | C    | G      | G      | Destroys PstI site (with +1103) |
| +1106    | C    | T      | T      | Destroys PstI site (with +1106) |
| +2950    | A    | G      | A      | Destroys BamHI site |
| +3358    | C    | C      | G      | Destroys SphI site |
| +3506    | G    | G      | A      | ry609 mutation |
| +3511    | A    | A      | T      | Creates BglII site (with +3506) |
| +3610    | C    | C      | A      | Creates EcoRI site |
| +3755    | A    | A      | C      | Creates BamHI site |
| +4163    | –    | –      | GATATCGAATT | Inserts EcoRV and EcoR site sites |
| +4169    | C    | C      | G      | Destroys NheI site |
| +6998    | C    | C      | G      | Destroys AgeI site for flanking marker |

* Positions are according to Cote et al. (1986); +1 is 3R:8,859,890 on genome assembly release 5.44. Coordinates from −3131 to +4158 are genomic DNA from the ry region. Sequences outside of this region are from the vector.
of the aforementioned genetic studies, these findings suggest that chromosomal position has a major influence on the crossover/noncrossover decision.

We sought to develop a system that would allow us to use genetic and molecular tools to understand the effects of chromosomal position on the crossover/noncrossover decision. We chose rosy (ry) because it is possible to select for rare wild-type recombination products from females heteroallelic for ry mutations, allowing detailed studies of intragenic recombination (Chovnick et al. 1993). The endogenous ry gene is in the middle of chromosome arm 3R. We made use of technology that allows integration of different transgenes into the same genomic location to assay recombination within ry at four different genomic locations: proximal, medial, and distal sites on the X chromosome, and a site on chromosome 4. Surprisingly, recombination rates were identical at the proximal and medial chromosome, and a site on chromosome 4. Surprisingly, recombination rates were identical at the proximal and medial chromosome locations, and recombination was completely absent at the distal X and chromosome 4 sites. These findings suggest that short-range effects on recombination are stronger than effects of position along the chromosome in this assay.

MATERIALS AND METHODS

Generation of transgenes

Descriptions of ry follow the coordinate system of Coté et al. (1986), in which +1 is within an EcoRI site in the second exon. This corresponds to 3R:8,859,890 on the genome assembly (release 5.44). Sequences toward the 3’ end of ry are positive coordinates and are also increasing coordinates on the genome assembly. To generate the ry+ transgene, the following fragments were cloned into the pMTL23P cloning vector (Chambers et al. 1988): (1) a 285-bp phiC31 attB sequence; (2) the 3xP3::dsRed marker gene from pM[3xP3-RFPattP] (Bischof et al. 2007), including the downstream loxP site; and (3) the 7289 bp ry genomic HindIII rescue fragment (ry1972) from pP[Car20] (Rubin and Spradling 1982), spanning −3131 to +4158. The product, pRatt-ry, is 12,287 bp. To generate pRatt-ry606N, the segment from a ry+5 fly and the ry609 mutation (G to A at 3506) was introduced by site-directed mutation. At the same time, A at 3511 was changed to T, a silent mutation that generates a BgIII site overlapping ry609. Additional polymorphisms were generated by site-directed mutagenesis (Table 1).

To generate pWhiteReaper, a 235-bp sequence containing the rpr coding region was amplified from genomic DNA and cloned into pUAST (Rorth et al. 1998), generating pUAST-rpr. The following fragments were then cloned into the pMTL23P cloning vector (Chambers et al. 1988): (1) a fragment from pStinger (Barolo et al. 2000) containing eGFP-nls flanked by gypsy insulator sequences; (2) a 985-bp fragment containing the tub84B promoter, first exon, and first intron, inserted upstream of eGFP-nls; (3) a phiC31 attB site, inserted outside of the upstream insulator; (4) a 1593-bp fragment from pUAST-rpr, containing the UAS enhancers, hsp70 promoter, rpr coding sequence, and SV40 3′ UTR, between the upstream insulator and the tub::GFP gene. The resulting plasmid, pGreenReaper, is 7624 bp. We were unsuccessful in obtaining integrations of this plasmid into the Y chromosome P[Cary.attP] landing site after screening for green fluorescence. We then generated pWhiteReaper by replacing most of the tub::GFP gene with a fragment in which the hsp70 basal promoter was fused to a w+ cDNA. Euchromatic transformants with this w+78c chimera gene have a nearly wild-type eye color regardless of genomic insertion location (S. McMahan and J. Sekelsky, unpublished data).

Tests of purine treatment for transgenes

To determine whether flies carrying a ry+ transgene are able to survive purine treatment, we set up bottles with 30 y; kar ry+; cv-c virgin females crossed to 10 males whose genotype was wild-type (endogenous ry), hemizygous for M[ry](2A, 6E, or 20C), and homozygous for ry006 or homozygous for M[ry](102D) and for ry506. For each genotype, three bottles were treated with purine as described in the section Infragenic recombination assays and three were untreated. Adult males and females that emerged were counted. Two bottles (one treated, one untreated) for 102D were discarded because of contamination with an unknown pathogen that causes larval lethality. Treated and untreated counts were compared in an unpaired t-test with the use of Prism 6.02 (Graphpad), with each bottle considered a biological replicate. For wild-type and 102D, male and female counts were summed before doing the t-test, but for the X insertions males and females were compared separately because males did not inherit a transgene and therefore did not survive the treatment with purine.

Figure 1 Structure of ry transgenes used in this study. (A) Transgenes carried a 7.3-kb genomic fragment spanning the ry gene (black line) and 3xP3::dsRed marker for transgenesis. The structure of the ry transcript is shown below. Transgenes were integrated into landing sites with Mariner (M) ends. Integration includes the pUC plasmid backbone. The locations of the ry606N and ry609N mutations are indicated, as are the KpnI and Agel sites used as flanking markers and polymorphisms introduced for conversion tract mapping (black lollipops). Regions in dotted boxes are magnified in (B) and (C) to show polymorphism distribution (see Table 1 for exact sequence changes).
Intragenic recombination assays

Each transgene was inserted into each of four landing sites: $M\{3xP3-RFP.attP\}ZH-6E$, $M\{3xP3-RFP.attP\}ZH-20C$, $M\{3xP3-RFP.attP\}ZH-102D$, and $M\{3xP3-RFP.attP\}ZH-2A$. Before integration, we removed the $3xP3::RFP$ gene from the landing site by Cre-mediated recombination between flanking loxP sites. This allowed us to use the $3xP3::RFP$ gene on our construct as a marker for transformation. For sites on the $X$ chromosome, we built stocks homozygous for the landing site construct and the $M\{vas-int.Dm\}ZH-102D$ integrase construct on chromosome 4. For the site on chromosome 4, the stock was homozygous for this site and for the $M\{vas-int.Dm\}ZH-2A$ integrase construct. After injection, the integrase was crossed out and stocks were made homozygous for ry506 on chromosome 3. In stocks with the $ry606N$ transgene, this chromosome also carried $P\{GawB\}^{h12}$. In stocks with the $ry609N$ transgene, the Y chromosome carried $P\{UAS-rpr\}\cdot$. Virgin females heteroallelic for $ry606N$ and $ry609N$ constructs were crossed to $y/y+Y$; $kar ry506$ ev-c males. Crosses were set up in bottles containing 25 mL of food medium using 30 females and 10 males. After 3 d, the adults were transferred to new bottles to generate the second brood, and 0.75 mL of 0.2% purine was added to the first brood bottles. Three days later adults were removed from second brood bottles and discarded, and purine was added to these bottles. In each tray of 25 bottles, one bottle, selected at random, was left untreated. Progeny from this bottle were counted to estimate the total number of progeny screened.

To distinguish crossovers from noncrossovers the flanking KpnI and AgeI sites were checked by polymerase chain reaction (PCR)-amplifying fragments spanning these sites and digesting with the appropriate enzyme. To map gene conversion tracts in noncrossover recombinants we sequenced PCR fragments spanning the mutant sites ($ry606N$ or $ry609N$).

An online calculator at http://www.graphpad.com/quickcalcs/ was used to determine two-sided $P$ values using the Fisher exact test, based on number of recombination events per 1000 progeny (e.g., 112 crossovers from 3710 thousand progeny at the endogenous $ry$ location).
RESULTS AND DISCUSSION

A transgenic system to measure recombination rates between the same allelic pair at different genomic locations

To assess the effects of genomic position on recombination rates within identical sequences, we used the pC31 site-specific recombination system (Bischof et al. 2007). This allowed us to insert similar transgenes into allelic positions on homologous chromosomes. We first built a transgene that carries a 7.3-kb wild-type ry genomic fragment, a 3xp3:RFP marker gene, and a pC31 attP site (Figure 1). We used site-directed mutagenesis to generate derivatives with the mutations in ry606 and ry609. Although ry606 and ry609 are considered to be genetically null when homoyoxous flies are compared with hemizygous flies, these alleles complement one another for eye color (Gelbart et al. 1976). The ry gene encodes xanthine dehydrogenase (XDH). Gelbart et al. (1976) found XDH activity in ry606/ry606 mutants to be 2.5% of wild-type levels, likely the result of weak activity of hybrid multimers, since XDH functions as a homodimer and these mutations alter residues in different domains of the polypeptide (Gelbart et al. 1976; Hille 1996). Additional sites were modified to provide polymorphisms for mapping gene conversion tracts (Table 1); we refer to these derivative alleles as ry606N and ry609N.

To generate flanking markers for distinguishing crossovers from noncrossover gene conversions, we destroyed a KpnI restriction site 2.7 kb upstream of the ry606N mutation and an AgeI site 3.5 kb downstream of the ry609N mutation. In our previous studies of gene conversion at the endogenous ry locus, the average span of co-converted markers was 551 ± 98 bp (n = 29), with the longest tract being 2298 bp (Blanton et al. 2005). If gene conversion tracts are similar in length in different locations of the genome, the sites we modified will rarely be co-converted with mutant sites, allowing us to use the modified sites as flanking markers to distinguish crossovers from noncrossovers.

Each of the three constructs (ry+, ry606N, and ry609N) was introduced first into a site in cytological region 6E on the polytene chromosome map. Crossovers are relatively frequent in this region of the chromosome, at least on a broad scale. We refer to these insertions as M[ry⁺][6E], M[ry606N][6E], and M[ry609N][6E]. We generated stocks homozygous for each of these transgenes on the X chromosome and for ry206, a deletion that removes most of the endogenous ry locus, on chromosome 3.

A genetic system to generate virgin females

Because intragenic recombination between ry alleles is rare, we screen approximately 10⁶ progeny of ry mutant females in a typical experiment (Blanton et al. 2005; Radford et al. 2007a,b). Collection of the large number of virgin females required to generate these progeny can be labor-intensive. To accelerate this process we developed a system that automatically kills male progeny. An existing method makes use of a Y chromosome transgene that expresses the cell death gene hid under the control of the hsp70 (cited in Starz-Gaiano et al. 2001). This method is not suitable for our purposes because heat shock affects recombination frequencies (Plough 1917) and because effectiveness (percentage of males killed) variable in our laboratory. As an alternative, we generated a Y chromosome carrying a rpr cell death transgene under the control of a Gal4-inducible promoter, similar to the P[w+mc=UAS-rpr.C] transgene of Aplin and Kaufman (1997). It can be difficult to recover transpositions of P elements onto the Y chromosome, so we rebuilt a UAS:rpr fusion gene and cloned it into vectors for pC31-mediated transgenesis (see Materials and Methods). This was then integrated into a pre-existing landing site on the Y chromosome (S. Russell, personal communication), producing Y, P[w+mc=UAS-rpr.Y].

Males of the genotype w¹¹¹⁸/Y, P[w+mc=UAS-rpr.Y] have variegated eyes, indicating that the transgene is subject to position-effect variegation, despite the presence of gypsy chromatin insulators on the construct. However, when these males are crossed to P[GawB]h¹³³/TM3, Sb females, no males inheriting the P[GawB]h¹³³ chromosome survive to adulthood. The combination of P[GawB]h¹³³, which expresses Gal4 in the pattern of the hairy (h) gene, and Y, P[w+mc=UAS-rpr.Y] is completely lethal. We used the crossing scheme shown in Figure 2A to take advantage of this lethality and generate virgin females for our recombination experiments.

To extend the functionality of this system, we jumped an existing X chromosome insertion of P[w+mc=UAS-rpr.C] onto chromosome 2 and 3 balancers. Both insertions cause complete lethality when combined with P[GawB]h¹³³. The UAS:rpr transgenes are also lethal when combined with TM3, P[w+mc=GA14-tw].G2.3, P[UAS-2xEGFP]AH2.3, Sb Ser. This facilitates more complex crosses like the one shown in Figure 2B. We used this scheme in another recombination assay and were able to generate 145,000 virgin females with relative ease (K. P. Kohl and J. Sekelsky, unpublished data). In some
crosses there are surviving males carrying Y, \( P[w^{+}c = UAS-rpr; Y] \) and a Ga4 driver, but these are still infrequent. Stocks with the chromosomes described above have been deposited into the Bloomington Drosophila Stock Center.

### Recombination within ry transgenes at ectopic locations

Before assaying recombination, we asked whether local position effects alter functionality of the \( ry \) transgenes. Because selection demands expression of a functional \( ry \) gene, we confirmed that \( M[ry^{+}16E; ry506] \) larvae survive purine selection. Flies that are heterozygous for the \( ry506 \) deletion are completely viable after purine treatment (Figure 3). Similarly, flies homozygous for \( ry506 \) but carrying one copy of a \( ry^{+} \) transgene, which is the genotype that is selected for in our experiments, are completely viable at 6E and at each of the other insertion sites tested. Additional support for complete function of the transgenes comes from observations of heteroallelic genotypes. Flies heteroallelic for \( ry506 \) and \( ry609 \) have 2.5% of the wild-type level of XDH activity, which is sufficient for wild-type eye color but insufficient for survival on purine selection (Gelbart et al. 1976). The alleles we constructed \( in \) \( vitro \) recapitulate this behavior when inserted into the 6E site as well as other sites used in this study. Thus, the different genomic locations we used do not cause any observable changes in \( ry \) function compared to the endogenous location.

We used a purine selection experiment to measure the frequency of intragenic meiotic recombination. In previous studies of the endogenous \( ry \) locus in this laboratory, 112 crossovers and 53 noncrossover gene conversions were recovered after screening \( 3.7 \times 10^{6} \) progeny (Blanton et al. 2005; Radford et al. 2007a,b). At the 6E site, we recovered six crossovers and ten gene conversions in a screen of 943,000 progeny (Table 2). Although the frequency of noncrossover gene conversion was not significantly different between these experiments (\( P = 0.53 \)), crossovers were about fivefold more frequent at the endogenous locus than at 6E (\( P < 0.0001 \)), despite the fact that both locations are in medial positions along the chromosome arm (Table 2).

Several factors may contribute to the differences between crossover rates at the endogenous \( ry \) locus and between our transgenes at 6E. These include chromosomal location, chromatin environment, strain differences (background effects), and local sequence context, including the composition of the transgene or the \( Mariner \) landing site. To determine whether chromosome position affects recombination rates, we repeated the experiment with transgenes located in 20C, only 200 kb from the end of the assembled euchromatic sequence. Because crossovers are less frequent in proximal regions of the \( X \) than in medial regions, we hypothesized that the \( ry \) transgenes integrated into 20C would have a lower crossover rate than at 6E. We screened a similar number of progeny of \( M[ry606N]/M[ry606N] \) females at the 20C site and, surprisingly, recovered a similar number of recombinants (Table 2). At 20C, as at 6E, crossovers were reduced relative to the endogenous \( ry \) locus (\( P = 0.0010 \), but noncrossover gene conversions were not (\( P = 0.14 \)). There was no significant difference between the frequencies of recombinants at 6E and 20C.

These results suggest that if there is an effect of chromosomal position (e.g., medial vs. proximal) it is small compared with other effects experienced by our transgenes. One such effect might arise from sequences on the transgenes themselves: The transgenic \( ry \) constructs may have properties that are sufficient to dictate recombination frequency regardless of insertion position. To test this hypothesis we measured recombination frequencies at two additional regions in which crossovers are normally low (region 2A toward the distal end of the \( X \) chromosome) or absent (region 102D on chromosome 4). We screened 960,000 progeny for the 2A site and 1,285,000 progeny for the 102D site. To our surprise, we recovered no \( ry^{+} \) recombinants – neither crossovers nor noncrossover gene conversions – in either experiment. As at the 6E and 20C sites, \( ry \) function appeared to be normal at these locations: The \( ry^{+} \) transgene conferred survival through purine selection (Figure 3) and \( M[ry606N] \) and \( M[ry606N] \) complemented for eye color but not for survival after purine treatment. Insertion locations were verified by PCR with primers in the flanking genomic sequence and in the transgene. For the 2A site, we also crossed the \( y \) mutation off of the \( M[ry606N] \) and crossed \( ec \) onto the \( M[ry606N] \) chromosome. The ability to obtain the desired recombinants suggests that there are no large chromosome rearrangements associated with these transgenes.

To better understand our results, we compared the crossover rate we measured to the average rate in the genomic neighborhood of each transgene (Figure 3). According to the standard map (Lindsley and Zimm 1992), the 1.75-Mb interval from \( k r \) to \( cv-c \), which spans the endogenous \( ry \) location, is 2.4 map units, for an average of 1.37 map units per Mb. This is not greatly different from the value of 1.60 map units per Mb between \( ry531 \) and \( ry606 \) (112 crossovers from \( 3.7 \times 10^{6} \) progeny; only half of the crossovers – those that are \( ry+c \) – are recovered by purine selection). There is also good agreement at the 20C site. We measured a rate of 0.56 map units per Mb in our \( ry \) transgene experiments at this site. The 2.2-Mb interval from \( m a l \) to \( su(f) \) is 1.1 map units, which is 0.50 map units per Mb. This interval also includes an unknown length of heterochromatin that has not been assembled, so the actual rate would be lower if we included heterochromatic sequences. It should also be noted that the fine-structure mapping of Comer et al. (2012) shows substantial fine-structure variation in crossover rates in this region. Because regions of high and low crossing over varied between different strains, it is not possible to predict local recombination rates in our experiments. It was also not possible to measure these rates directly because of the absence
of polymorphisms between the chromosomes we used due to both being inserted into the same parental landing site chromosome.

In contrast to the endogenous and 20C sites, the 6E site showed a large disparity between the average crossover rate and the rate between our ry transgenes. A 1.6-Mb region that spans this insertion site (rux to cm) is five map units, for an average of 3.13 map units per Mb. In our experiments we measured 0.32 map units per Mb, about a tenth of the average rate in the surrounding neighborhood. At the 2A site, we recovered no crossovers, even though the average rate in this region is about the same as in 20C.

Factors that influence recombination rates and outcomes

Recombination rates in our assay area appear to be determined primarily by local effects of insertion location, perhaps in combination with properties inherent to the transgenes. There were no significant differences between the 6E and 20C sites for crossover frequency, noncrossover frequency, or conversion tract length (Table 2 and Figure 5). Compared with the endogenous ry gene, however, the crossover frequencies at these sites were reduced by approximately 80%, whereas noncrossover frequencies and conversion tract lengths were

**Figure 4** Recombination rates at various locations in the genome. Red bars are map units per megabase measured within ry at the endogenous location (86E) and at four different ectopic locations. Blue bars are the measured frequency of noncrossover gene conversion events at the same locations. Yellow segments are map units per megabase across a region flanking each site, according to Lindsley and Zimm (1992). The width of these bars is proportional to the size of the region that was used to calculate recombination rates. For 86E, this is 1.75 Mb (kar to cv-c); for 2A, it is 1.16 Mb (su(w) to pn); for 6E, it is 1.6 Mb (rux to cm); for 20C, it is >2.2 Mb (mal to su(f), which includes an unknown amount of unsequenced heterochromatin); and for 102D, it is 1.35 Mb (the entire assembled euchromatin on 4). The drawings below represent 3R, X, and 4R. Stippled regions are pericentric heterochromatin. Green lines show approximate positions where recombination measurements were made.

**Figure 5** Gene conversion tracts at ectopic locations. (A) Schematic of the rosy locus. Intron/exon structure is shown, with coding sequences filled. The positions of the selected sites corresponding to the ry606N and ry609N mutations are shown. Heterologies between the ry606N and ry609N transgenes are indicated as lollipops on the scale bar. These are all single nucleotide polymorphisms except for the KpnI site at –3149 and an insertion in ry609N at +4163 (Table 1). The scale is in bp, using the coordinate system of Coté et al. (Coté et al.). (B and C) Tract lengths observed in NCOs recovered at 6E (B) and 20C (C). Each bar represents an independent event, with the open circle denoting the selected marker (ry606N and ry609N mutant sites). Black bars represent the minimum tract length for each event, with co-converted sites marked by white lines. Dotted lines indicate the maximum tract length possible based on the next unconverted polymorphism.
ry

are not in possible that the rates are determined entirely by local features and sites suppress all recombination between the transgenes. It is also to achieve this rate, whereas restrictive properties of the 2A and 102D sive properties of the 6E and 20C insertion sites allow the transgenes at any of the interesting to incorporate motifs associated with elevated crossover rates (Comeron et al. 2012; Singh et al. 2013) have an effect on this assay, since our insertion method resulted in more than four kb to the left of ry being identical between insertion locations. Nonetheless, it would interesting to incorporate motifs associated with elevated crossover rates into our transgenes to see whether an effect can be observed at any of the X chromosome sites we assayed.

Histone modifications are known to be important in specifying meiotic recombination initiation hotspots in yeast and mammals (Wu and Lichten 1994; Buard et al. 2009). It seems likely that chromatin modifications are likewise involved in the regulation of recombination in Drosophila, but initiation hotspots have not been identified and there have been no analyses of chromatin modifications in cells undergong meiotic recombination.

In conclusion, we set out to develop an assay that could quantify the effects of gross position along a chromosome on the crossover/noncrossover decision. However, we were unable to detect any such effects, probably due to more localized effects on the ability to engage in recombination. A different transgene design, or possibly a gene other than ry, might mitigate these problems. One possibility is to surround the fragment with chromatin insulators (reviewed in Maeda and Karch 2007); however, it is unknown whether insulators function during meiosis or what aspects of recombination they might affect. An alternative is to increase the size of the transgenes. The genomic fragment we used includes parts of the genes immediately flanking ry, but it is possible that a larger genomic fragment will better recapitulate the endogenous properties of the region and resist any local effects of the insertion site. Current technologies allow integration of fragments exceeding 100 kb (e.g., Venken et al. 2009), so it should be possible to develop a system that can assay the effects of chromosomal position on recombination frequency and outcome. Finally, it is possible that the insertion sites we selected are unusual in some way that masked the ability to detect effects of chromosomal position.

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