DOI: 10.1534/genetics.105.044834

Heterozygous Insertions Alter Crossover Distribution but Allow Crossover Interference in *Caenorhabditis elegans*

Marc Hammarlund,[1] M. Wayne Davis,[1] Hung Nguyen, Dustin Dayton and Erik M. Jorgensen[2]

Department of Biology, University of Utah, Salt Lake City, Utah 84112-0840

Manuscript received April 25, 2005
Accepted for publication July 29, 2005

ABSTRACT

The normal distribution of crossover events on meiotic bivalents depends on homolog recognition, alignment, and interference. We developed a method for precisely locating all crossovers on *Caenorhabditis elegans* chromosomes and demonstrated that wild-type animals have essentially complete interference, with each bivalent receiving one and only one crossover. A physical break in one homolog has previously been shown to disrupt interference, suggesting that some aspect of bivalent structure is required for interference. We measured the distribution of crossovers in animals heterozygous for a large insertion to determine whether a break in sequence homology would have the same effect as a physical break. Insertions disrupt crossing over locally. However, every bivalent still experiences essentially one and only one crossover, suggesting that interference can act across a large gap in homology. Although insertions did not affect crossover number, they did have an effect on crossover distribution. Crossing over was consistently higher on the side of the chromosome bearing the homolog recognition region and lower on the other side of the chromosome. We suggest that nonhomologous sequences cause heterosynapsis, which disrupts crossovers along the distal chromosome, even when those regions contain sequences that could otherwise align. However, because crossovers are not completely eliminated distal to insertions, we propose that alignment can be reestablished after a megabase-scale gap in sequence homology.

MOST eukaryotes require crossover events to properly align and segregate chromosomes during meiosis. The placement of these crossovers on meiotic bivalents is subject to a variety of controls. First, homologs must recognize each other, align homologous sequences, and synapse to recombine and segregate. Although nonhomologous sequences can sometimes synapse, recombination happens only between homologous sequences. Thus, homologous sequences that fail to align will fail to recombine. In *Caenorhabditis elegans*, homolog segregation is strongly influenced by a specialized region at one end on each chromosome, the homolog recognition region (McKim *et al.* 1988). In chromosomal rearrangements such as translocations and insertions, chromosome segments that are separated from their homolog recognition region usually fail to recombine (Rosenbluth and Baillie 1981; McKim *et al.* 1993). Despite the importance of the homolog recognition region for the normal completion of meiosis, its exact function is unclear. One possibility is that this region is required only for an initial recognition step and that alignment itself is accomplished by specialized sites (Rosenbluth *et al.* 1990; McKim *et al.* 1993). Alternatively, the homolog recognition region could be required for both recognition and the initiation of synapsis, which would then propagate unidirectionally along the bivalent (Villeneuve 1994).

Second, homologs must undergo crossing over. In most organisms, the distribution of crossovers is regulated. Although recombination is initiated at multiple sites along each chromosome, not all of these recombination intermediates mature into crossovers. In many organisms, the positions of multiple crossovers are not random relative to one another. Rather, the likelihood of crossovers occurring close together is less than expected. This process is called interference, and it results in more widely spaced crossovers than expected by a random distribution. Although interference was first observed by Sturtevant in 1913 (Sturtevant 1913), its molecular mechanism remains unknown. Most current models for interference involve the transmission of information along meiotic chromosomes, so that commitment to crossing over at one site inhibits its progression at other local sites. Any model for the transmission of interference must account for the fact that information must be transmitted over multiple megabases of DNA. An extreme example of interference is found in *C. elegans*, where each chromosome is at least 13 Mb long, and each experiences almost exactly one crossover per bivalent (Brenner 1974; Hodgkin *et al.* 1979; Barnes *et al.* 1995; Meneely *et al.* 2002; Nabeshima *et al.* 2004).

[1]These authors contributed equally to this work.

[2]*Corresponding author:* Department of Biology, University of Utah, 257 S. 1400 E., Salt Lake City, UT 84112-0840.
E-mail: jorgensen@biology.utah.edu

To determine in detail how recombination is controlled by homolog alignment and interference, one would ideally like to know the location of every crossover event from a given meiosis. By contrast, previous studies of recombination in *C. elegans* have typically compared the frequency of recombination between specific pairs of markers under various conditions. Since only a specific interval is studied, recombination activity outside the marked region cannot be examined. Thus, information such as the total number of crossovers per bivalent cannot be directly measured. Further, experiments that affect the distribution of crossovers along a chromosome must rely on the successive measuring of multiple intervals. However, by using single-nucleotide polymorphisms between the wild type and a closely related strain of *C. elegans*, entire chromosomes can be surveyed for recombination (Wicks *et al.* 2001). In this article, we describe whole-chromosome measurements of recombination.

We then apply these methods to investigate models for interference. Interference in *C. elegans* has been shown to be essentially complete not only on wild-type chromosomes (Meneely *et al.* 2002) but also on chromosomes whose physical length available for recombination has been substantially reduced (Zetka and Rose 1992) or increased (Hillers and Villeneuve 2003). The mechanism for interference is not known. Recent studies in yeast have suggested that interference information in that organism may be transmitted along axial elements, rather than the synaptonemal complex as originally proposed (Hunter and Kleckner 2001; Borner *et al.* 2004). In *C. elegans*, a chromosomal discontinuity disrupts normal interference, confirming that some structural aspect of the meiotic bivalent is required for interference (Hillers and Villeneuve 2003). However, these experiments could not distinguish between a requirement for continuous sequence identity and a requirement for chromosomes that are physically intact. To distinguish between these models, we specifically disrupted sequence identity using heterologous insertions, while leaving axial continuity intact. We found that crossovers still displayed essentially complete interference, suggesting that axial continuity is necessary to transmit interference information. Alternatively, for any particular bivalent in the insertion heterozygote, alignment on either side occludes alignment on the other side.

We also used these methods to investigate possible effects of insertions on local recombination frequency. As in previous studies, we found that insertions suppress local recombination, even though all wild-type sequences are present (McKim *et al.* 1993). In addition, because we could locate every recombination event, we were able to determine how local suppression affects the chromosomewide distribution of recombination. All three insertions tested caused a similar redistribution of crossovers, with significant increases observed only on the side of the chromosome containing the homolog recognition region (McKim *et al.* 1993). These data support a model in which synapsis propagates unidirectionally from the homolog recognition region. When a break in identity is encountered some chromosomes continue to synapse, resulting in heterosynapsis. Other chromosomes, however, can align distal to the break in homology. This mixed population of bivalents would account for the altered crossover distribution that we observed in insertion heterozygotes.

MATERIALS AND METHODS

Strains and genetics: The following stains were used in this study: EG3134 *oxIs107 V*, EG3191 *oxIs131 V*, EG1285 *lin-15(n765) oxIs12 X*, EG3300 *oxIs12 X* (introgressed into CB4856), MT406 *unc-60(e677) dpy-11(e224) V*, EG2651 *unc-2(ox198) lon-2(e678) X*, CB4856, and N2 Bristol. The construction of EG3134, EG3191, and EG3300 is described below; EG1285 is described in McIntire *et al.* (1997); all other strains were obtained from the *C. elegans* Genetics Center. Animals were maintained on agar plates seeded with HB101. For all crosses, animals were allowed to mate for 12–24 hr, mated hermaphrodites were cloned onto individual plates, and cross progeny were harvested from plates that segregated 50% males.

Comparing recombination over N2 Bristol and CB4856 chromosomes: N2 or CB4856 males were crossed to strains homozygous for two visible markers. Animals were scored for phenotype in the F_2 generation. For recombination between *dpy-11* and *unc-60* over N2 chromosomes, we obtained 1848 wild-type, 469 Dpy Unc, 181 Dpy, and 177 Unc animals. Over CB4856 chromosomes we obtained 1114 wild-type, 268 Dpy Unc, 139 Dpy, and 119 Unc animals. For recombination between *lon-2* and *unc-2* over N2 chromosomes, we obtained 1168 wild-type, 411 Lon Unc, 61 Lon, and 36 Unc animals. Over CB4856 chromosomes we obtained 1312 wild-type, 371 Lon Unc, 63 Lon, and 53 Unc animals. For each experiment, recombination was calculated as the number of animals with nonparental phenotypes (Dpy, Lon, and Unc) divided by the total number of animals. Ninety-five percent confidence intervals were calculated using VassarStats (http://faculty.vassar.edu/lowry/prop1.html). The chi-square test and two-tailed *P*-values were calculated with GraphPad QuickCalcs (http://www.graphpad.com/quickcalcs/contingency1.cfm).

Single-nucleotide polymorphism mapping: Insertion homozygotes were crossed to CB4856 (Hawaiian) males to generate heterozygotes. Heterozygotes were allowed to self and F_2 recombinant animals were singled onto individual plates. The progeny of the recombinant animals were washed off of the plates into individual wells of a 96-well plate using ddH_2O. Worms were sedimented and liquid was removed to leave 30 μl per well. Ten microliters of 4× single-worm lysis buffer [200 mm KCl, 40 mm Tris (pH 8.3), 10 mm $MgCl_2$, 1.8% NP-40, 1.8% Tween 20, 0.04% (w/v) gelatin, 240 μg/ml proteinase K] was added to each well and mixed. The worms were lysed by freezing at −80° and then incubating the plates at 65° for 1 hr and at 95° for 15 min. The plates were then frozen at −80° until needed. PCR master mix for each 96-well reaction {886.2 μl H_2O, 105 μl 10× buffer [22.5 mm $MgCl_2$, 140 mm $(NH_4)_2SO_4$, 500 mm Tris (pH 9.2)], 21 μl 10 μm dNTP, 6.3 μl 5 units/μl Taq polymerase, 2.5 μl 100 μm forward primer, 2.5 μl 100 μm reverse primer; see Table S1 at http://www.genetics.org/supplemental/} was mixed and 9.8 μl was aliquoted into each well of a 96-well plate. Genomic template was then added to each reaction using a pin replicator. PCR conditions were 94° for

2 min, followed by 35 cycles of 94° for 15 sec, 58° for 1 min, and 72° for 45 sec. PCR reactions were digested by adding 4.15 μl H_2O, 1.6 μl 10× restriction buffer (from the enzyme supplier), and 0.25 μl restriction enzyme (10 units/μl). All PCR products were digested with *Dra*I, except Y73B3A (*Apo*I) and C16H3 (*Cla*I). Digests were incubated at 37° (*Apo*I at 50°) for 4 hr to overnight. After digestion, 6× loading buffer was added to each reaction, and reactions were run on a 2.5% agarose gel in a Centipede gel box (Owl, Cambridge, MA). Genotypes were determined from the gel bands, and any questionable bands were confirmed by a second scorer. Reactions in which the PCR failed or the genotype was ambiguous were repeated. The single-nucleotide polymorphisms (SNPs) used spanned most of chromosomes V and X. On chromosome V, F36H9 is 1.8 Mb from the left end, with an interpolated map distance of 2.6 map units (MU) from the left-most clone, while F38A6 is 0.2 Mb from the right telomere with an interpolated map distance of 0.3 MU from the end. For the X chromosome, Y73B3A is 36 kb, or 0.2 MU from the left end and C16H3 is 0.2 Mb or 0.27 MU from the right telomere.

Recombination and interference: To calculate the fraction of recombinant chromosomes, we determined whether each animal had zero, one, or two recombinant chromosomes. For chromosome V there were 38 animals with zero recombinant chromosomes, 102 with one, and 52 with two. For chromosome X there were 48 animals with zero recombinant chromosomes, 103 with one, and 41 with two. We compared the fraction of recombinant chromosomes to the expected value of 50%, using the chi-square test, and calculated two-tailed *P*-values using GraphPad QuickCalcs. We calculated the power of this analysis using GraphPad StatMate 2 (Graphpad Software, San Diego) At a significance level of 0.05, we had an 80% chance of detecting a change of 10% or larger.

We also determined whether the presence of an insertion altered the fraction of recombinant chromosomes. For *oxIs107*, there were 79 animals with zero recombinant chromosomes, 151 with one, and 58 with two, or 46.4% recombinant chromosomes. For *oxIs131*, there were 85 animals with zero recombinant chromosomes, 144 with one, and 59 with two, or 45.5% recombinant chromosomes. For *oxIs12*, there were 90 animals with zero recombinant chromosomes, 144 with one, and 54 with two, or 43.8% recombinant chromosomes. Two-tailed *P*-values were 0.03, 0.01, and 0.18, respectively. Thus, the two insertions on V caused a small but significant decrease in the fraction of recombinant chromosomes, suggesting that some bivalents in insertion heterozygotes fail to receive a crossover.

Construction of *oxIs107* and *oxIs131*: For *oxIs107*, the extrachromosomal array *oxEx506*, containing 5 ng/μl *rab-3*::UNC-70, 2 ng/μl *myo-2*::GFP, and 80 ng/μl *lin-15*(+), was generated by standard microinjection techniques. This array was then integrated by X-ray irradiation to generate *oxIs107*, mapped 8/38 to *dpy-11*, and outcrossed twice against N2 Bristol. *oxIs131* was constructed by a similar procedure. The starting array was *oxEx508*, containing 5 ng/μl *rab-3*::UNC-70, 20 ng/μl *unc-129*::GFP, and 80 ng/μl *lin-15*(+). The integrant mapped 0/12 to *dpy-11*. *oxIs12* was composed of similar DNA and is linked to chromosome X (McIntire *et al.* 1997).

We also mapped *oxIs107*, *oxIs131*, and *oxIs12* to intervals between SNP markers. Since all three insertions contain a dominant GFP marker, we could determine whether each progeny of a heterozygote was homozygous, heterozygous, or homozygous null for the insertion. After genotyping each progeny for each SNP on the linked chromosome, this information was used to determine the position of the insertion. For *oxIs107* and *oxIs131* mapping was done on the same progeny used for determining recombination frequencies, while *oxIs12* was mapped in a separate experiment. These experiments unambiguously positioned each insertion in an interval between adjacent SNPs, as shown in Figure 4.

Crossover suppression: To identify regions in which recombination was reduced in insertion heterozygotes, we compared the proportion of recombinants in each interval with controls using the chi-square test and calculated two-tailed *P*-values (GraphPad QuickCalcs). To calculate the minimum size of the suppressed area, we assumed that this area had zero recombination. The size of the suppressed area is then equal to the fraction of suppression in each interval multiplied by the size of the interval. When suppression was observed in two adjoining intervals (*oxIs107* and *oxIs131*) we summed the suppressed regions from the adjoining intervals. These calculations yielded 9.7 MU for the suppressed region due to *oxIs107* (5.2 + 4.5 MU), 18 MU for *oxIs131* (7.8 + 10.2 MU), and 6.8 MU for *oxIs12*. If the suppressed region has greater than zero recombination, the size of the region would be larger than these estimates to obtain the same effect.

Measuring recombination in *oxIs12* homozygotes: EG3300 was constructed by outcrossing *oxIs12* to CB4856 Hawaiian 18 times. This strain was genotyped for SNPs on the X chromosome and found to be Hawaiian for all SNPS except F11A1 and F22E10. A final outcross was done and 192 F_2 animals were singled onto individual plates. Plates that were homozygous for *oxIs12* were screened for Hawaiian DNA at F22E10 and F11A1. Animals with recombination between *oxIs12* and F22E10 were isolated and homozygosed, but no recombination events between *oxIs12* and F11A1 were recovered. Because EG3300 still contains Bristol DNA at F11A1, we measured recombination between C46F4 and F22E10. We crossed CB4856 males to EG3300 hermaphrodites and singled out 188 F_2 recombinants onto individual plates. From these, we washed the F_3 progeny into individual wells of 96-well plates for SNP genotyping using the methods described above. We obtained readable SNP genotypes for both C46F4 and F22E10 in 163 wells. Of these, we found 38 that had recombination between the SNPs (11.7 MU). We compared this recombination frequency with the recombination frequency between C46F4 and F22E10 SNPs in the Bristol/Hawaiian and *oxIs12*/Hawaiian data (13.4 and 8.0 MU, respectively). Chi-square two-tailed *P*-values were calculated with GraphPad QuickCalcs. It is notable that we did not find an increased distance for this interval in the *osIs12* homozygote corresponding to the genetic length of the insertion itself. It may be that the length of the insertion was too short to be detected in these experiments. Alternatively, the inserted DNA may be refractile to recombination because this repetitive region is assembled into heterochromatin or because it lacks *C. elegans* sequences that facilitate recombination.

RESULTS

Crossover distribution is normal in Bristol/Hawaiian heterozygotes: Since interference can limit crossing over across whole chromosomes, a rigorous characterization of interference requires a method that can locate every crossover event in a population of meiotic chromosomes. We located crossovers by assaying SNPs in the progeny of heterozygotes between the two wild-type strains Bristol N2 and Hawaiian CB4856. This method allowed us to assign every crossover event to a particular interval on a chromosome. Polymorphisms between the Bristol and Hawaiian strains are common: they occur approximately every 3 kb in the genomes of these two strains (Wicks *et al.* 2001). Because sequence polymorphisms can reduce the frequency

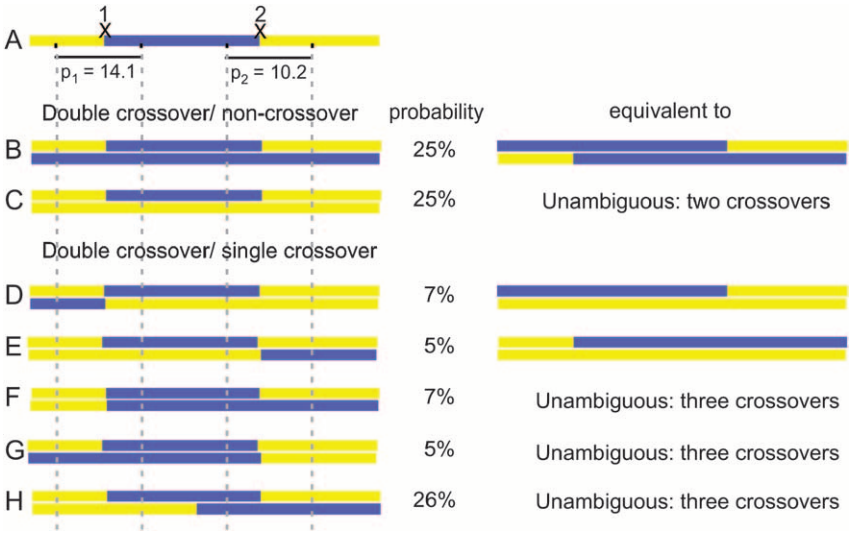

FIGURE 1.—Ambiguous chromosome configurations. A hypothetical double recombinant on chromosome 5 (A) can be missed in two ways. If it is paired with a nonrecombinant chromosome so that the telomeric SNPs are heterozygous (B) it is ambiguous because it is indistinguishable from two single recombinants. However, it can be identified if it is paired with a nonrecombinant chromosome of the same genotype as that of the telomere SNPs (C). If it is paired with a recombinant chromosome in the same SNP interval (D and E) it will appear indistinguishable from a single recombinant. The probability of each event is one-half of the probability of a crossover event in the SNP interval. If it is paired with a crossover in the same orientation (F) or with a crossover that occurred outside of both SNP intervals (G) the result is unambiguous, since all three crossovers can be resolved.

of homologous recombination (TE RIELE *et al.* 1992), we used visible markers to compare recombination in the Bristol/Hawaiian heterozygous background with that in Bristol/Bristol homozygous controls. Between *unc-60* and *dpy-11* on chromosome V, we found 13.4% recombinants (95% C.I.: 12.1–14.7) in the Bristol background and 15.7% (14.1–17.6) in Bristol/Hawaiian heterozygotes ($P = 0.03$). Similarly, between *unc-2* and *lon-2* on chromosome X we found 5.8% recombinants (4.8–7.0) in Bristol and 6.5% (5.4–7.7) in Bristol/Hawaiian heterozygotes ($P = 0.42$). These results demonstrate that strain polymorphisms do not reduce crossovers and that evolutionary divergence between the two strains has not functionally altered recombination factors.

Detecting single and double crossovers with SNPs: We used single-nucleotide polymorphisms to determine the position of crossover events in the germ line of Bristol/Hawaiian heterozygotes. Because we allowed heterozygotes to self-fertilize and determined the genotypes of their progeny, both homologs in the progeny are independent products of heterozygous Bristol/Hawaiian bivalents. Our first goal was to determine the distribution of crossovers on a chromosome. It is not necessary to assign recombination events to individual homologs to determine the frequency of recombination that occurs in each SNP interval. Our second goal was to measure interference by assaying the number of doubly recombinant chromosomes. If doubly recombinant chromosomes occur, we can detect them in two ways, depending on whether we can reconstruct the genotype of the individual homologs. First, we can reconstruct the genotype of each homolog and directly visualize double crossovers. This should compose 63% of doubly recombinant chromosomes. Second, we can detect most of the remaining 37% of doubly recombinant chromosomes by an increase in the fraction of particular recombinant genotypes. The derivation of these numbers follows.

Because *C. elegans* has very high interference, we can unambiguously determine the individual genotype of each homolog in most cases. Specifically, we can expect to detect ~63% of doubly recombinant chromosomes, if they occur (Figure 1). Our ability to detect doubly recombinant chromosomes depends on the genotype of the homolog after fertilization. Unambiguous double-recombination events can be detected when the doubly recombinant chromosome is found with a nonrecombinant homolog of the opposite genotype as the central region (for example, Figure 1C, but not Figure 1B) and in all cases when a crossover on the homolog occurs in a different interval (for example, Figure 1H). Further, unambiguous double recombination events occur in half of the cases when a crossover on the homolog occurs in the same interval (for example, Figure 1, F and G, but not Figure 1, D and E). By Mendelian segregation, these cases will compose 63% of the total.

Two-thirds of the remaining 37% of doubly recombinant chromosomes can be detected by their effect on the fraction of particular recombinant genotypes. In these cases (25% of the total doubly recombinant chromosomes), the doubly recombinant chromosome and its homolog appear similar to two singly recombinant chromosomes with heterozygous telomeres and two recombination events (Figure 1B). If there are no double-recombinant chromosomes, the fraction of animals with heterozygous telomeres and two recombination events will be one-sixteenth (Figure 1B, right), and the presence of doubly recombinant chromosomes would increase this number. We found 47 cases of this chromosome configuration in wild-type animals. This number is close to the expected 48 ($768 \cdot \frac{1}{16}$) cases if there were no double-recombinant chromosomes. Thus, these genotypes were scored as two single-recombinant chromosomes.

The final 12% of the total doubly recombinant chromatids cannot be detected, because a second crossover on the *trans* chromosome exactly cancels one of the

crossover events (Figure 1, D and E). Half of all double-crossover chromosomes should have a homolog containing a crossover. However, only in rare cases does a crossover on the *trans* chromosome occur in the same SNP interval as one of the double crossovers. The frequency of such an event will be $\sim p1 + p2$, where $p1$ and $p2$ are the observed crossover frequencies in the two SNP intervals containing the crossovers. In addition, only in half of the cases will the SNPs have opposite genotypes and hide each other. If the crossover occurs in the same orientation the data are unambiguous (Figure 1, F and G). The largest two SNP intervals in our data set sum to 24.3 MU, so we are unable to analyze the presence of double crossovers in just 12% of our progeny. Thus, we are able to detect 88% or more of all double crossovers that occur. Since this number includes double crossovers detected directly and via statistical means, we used the more conservative number of 63% of double crossovers that can be directly detected to adjust our measurements.

***C. elegans* has complete interference:** Previous reports have found that the percentage of double-recombinant chromosomes is <2%, showing that *C. elegans* has high crossover interference. Using visible markers, Hodgkin *et al.* (1979) found 0% double recombinants on the X chromosome. Using a denser set of polymorphisms, Meneely *et al.* (2002) found 1.4% double recombinants on chromosome X and 0% on chromosome III, while most recently Nabeshima *et al.* (2004) found 0.4% on chromosome X and 0.6% on chromosome I. Animals homozygous for the *him-3*(*me80*) mutation have reduced interference, suggesting that interference is an active, regulated process (Nabeshima *et al.* 2004).

We tested whether each bivalent received a single crossover in two ways. First, we scanned each individual chromosome for double recombinants. On average, each double-crossover bivalent produces one double-crossover chromosome (of four) in the absence of chromatid interference (see below). Thus, the frequency of double-crossover chromosomes should represent one-quarter the frequency of double-crossover bivalents. We found 0 unambiguous double recombinants out of 768 wild-type chromosomes (Figure 1; Figure 2, A and B). Because we were able to analyze 97.1% of the map length of chromosome V and 99.5% of the map length of chromosome X (on the basis of interpolated genetic positions of the SNP markers), we assume that few if any crossover events occurred outside of our assayed intervals. These data place a strong restriction on theoretical frequencies of double-crossover events. At a 95% confidence limit, there must be <0.6% double-crossover chromosomes formed. It is possible that some double crossovers were hiding in the ambiguous cases; however, it is unlikely that many such cases exist since the fraction of ambiguous cases is exactly as predicted by the existence of only single crossovers. Nevertheless, to be conservative, we have expunged all ambiguous cases from our data to arrive at a theoretical limit of double crossovers. Because we could miss 37% of double crossovers using our methods, this limit must be adjusted by 1.59 ($1/(1 - 0.37)$), and thus double-crossover chromosomes must represent no more than 1.0% of meiotic products (indicating that <4% of bivalents experience two crossovers).

Second, we examined the fraction of recombinant chromosomes. Because crossing over takes place at the four-strand stage we expect exactly half of our chromosomes to be recombinant if every bivalent receives a single crossover. For chromosome V, 206 of 384 chromosomes were recombinant (53.6%, 95% C.I.: 49–59) (Figure 3). For chromosome X, 185 of 384 chromosomes were recombinant (48.2%, 95% C.I.: 43–53) (Figure 3). Together, these data confirm the previous observations that interference in *C. elegans* is essentially complete.

Evidence against chromatid interference in *C. elegans*: In *chromosome* interference (discussed in the previous section and usually called simply "interference") the presence of a crossover between two homologs interferes with the formation of a second crossover. In *chromatid* interference a crossover does not eliminate a second crossover from occurring between the homologs, but a crossover on a particular chromatid will eliminate it from participation in the second crossover (Lindegren and Lindegren 1942). Instead, the other two nonrecombinant chromatids will cross over and thus chromatid interference will lead to an excess of four-strand double crossovers in the paired homologs. Although four-strand double crossovers cannot be detected by searching for doubly recombinant chromatids, our data do provide a statistical test for their prevalence. Chromatid interference would have led to >50% recombinant chromosomes. Again, 53.6% of chromatids were recombinant for chromosome V, and 48.2% of chromatids were recombinant for chromosome X. Since we did not detect an increased fraction of recombinant chromosomes (one-tailed $P = 0.08$ and 0.25, respectively), it is unlikely that a significant number of double crossovers are masked by chromatid interference.

Together, these data suggest that meiotic bivalents receive exactly one crossover with a high degree of fidelity and that interference is essentially complete. We observed complete interference for both oogenesis and spermatogenesis in hermaphrodite animals and for the entire lengths of both an autosome and the X chromosome.

Insertion heterozygotes display normal crossover interference: A physical break in the DNA of one homolog in a bivalent partially disrupts interference (in a heterozygote containing a compound chromosome with two homolog recognition regions) (Hillers and Villeneuve 2003). This disruption of interference might be due to a lack of local chromosome alignment at the

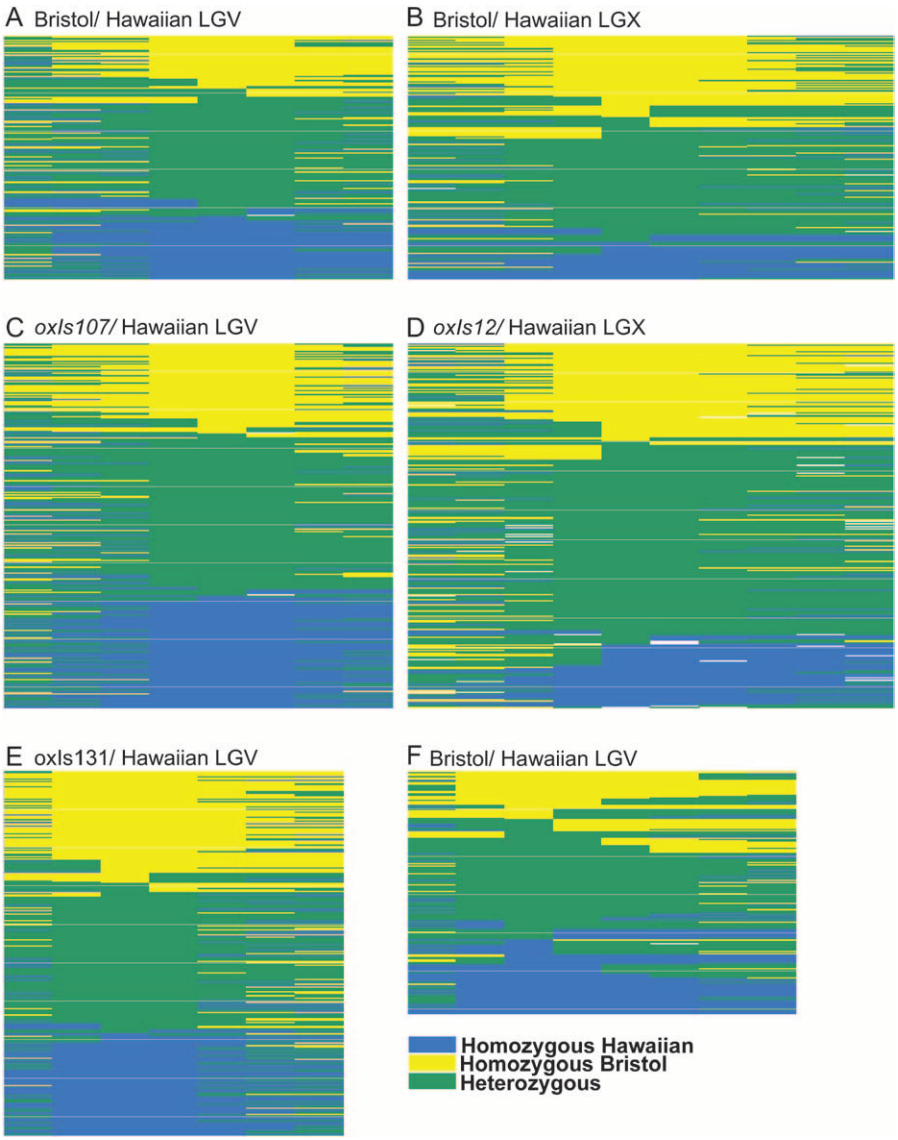

FIGURE 2.—Mapping crossovers using SNP genotypes. Each block shows all chromosomes analyzed from the indicated heterozygous genotype. Two control experiments (Bristol/Hawaiian, chromosomes V and X: A, B, and F) and three insertion heterozygotes (C–E) are shown. Within each block, each horizontal line represents the genotype of a single progeny of the indicated heterozygote. Each column represents a SNP, depicted in order from left to right. The genotype of the animal at each particular SNP is indicated by color. Because *C. elegans* are hermaphrodites all F_2 self progeny will contain two potentially recombinant chromosomes (the equivalent of a cross between sibling heterozygotes). Crossover events are visible as color changes along each horizontal line, which indicate crossing over in the interval between the flanking SNPs. *oxIs107* (C) and the corresponding control (A) were sorted by their SNP genotype at the closest SNP marker, F57G8. *oxIs12* (D) and the corresponding control (B) were sorted by their SNP genotype at the closest SNP marker, F11A1. *oxIs131* (E) and the corresponding control (F) were sorted by their SNP genotype at the closest SNP marker, VC5.

discontinuity or due to the physical break in one homolog. To attempt to distinguish between these two models, we used megabase-scale insertions to disrupt sequence identity along the chromosome without breaking the chromosome. We generated extrachromosomal arrays of transgenic DNA and integrated them into the Bristol genome, producing insertions that could not align with a wild-type chromosome in meiosis. Insertions are typically in the range of 800 kb to 3 Mb (STINCHCOMB *et al.* 1985). *C. elegans* chromosomes are between 13.9 Mb and 20.9 Mb long. We used three transgene insertions: *oxIs107* and *oxIs131*, on chromosome V, and *oxIs12*, on chromosome X. Unlike other chromosomal rearrangements, insertions do not delete sequences or disrupt chromosomal continuity but disrupt only continuous sequence identity.

Reduced interference in these heterozygous animals would be visible as increases in both double recombinants and the percentage of chromosomes that are recombinant. To directly measure the effect on crossing over due to the insertions, we determined the genotype at each SNP of 288 progeny from each of three genotypes: *oxIs107*/Hawaiian heterozygotes (LGV), *oxIs131*/Hawaiian heterozygotes (LGV), and *oxIs12*/Hawaiian heterozygotes (LGX) (Figure 2, C–E). We examined the meiotic products of insertion heterozygotes for evidence of double crossovers. Among 1728 chromosomes, we found 1 double recombinant. At a 95% confidence limit, these data suggest that there must be $<0.37\%$ double crossovers. After adjusting this number by a factor of 1.59 (see above), we conclude that there can be no more than 0.6% double crossover chromosomes in the insertion heterozygotes.

In addition, we found that in *oxIs107*, *oxIs131*, and *oxIs12*, 46.4, 45.5, and 43.8% of chromosomes were recombinant, respectively, demonstrating that they still had an average of one crossover per homologous pair. Thus, even with a disruption in chromosome alignment,

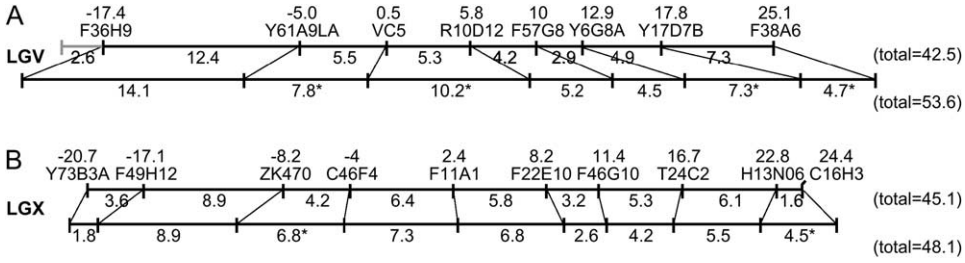

FIGURE 3.—Effect of SNPs on crossover frequency. A comparison of the interpolated genetic distances (A and B, top line) between SNPs on LGV (A) and LGX (B) and our measured distances (A and B, bottom line) is shown. The *C. elegans* genetic map, deposited at www.wormbase.org (release WS120), contains interpolated genetic positions for each SNP. However, actual map distance between these SNPs has never been measured. We compared our distances for each SNP interval to the interpolated Wormbase distances. There is no significant difference in total chromosome length and no systematic distribution of differences. We found that in most cases our observations agreed with Wormbase's predictions, but that some intervals were significantly different. Some of these differences could be due to the low density of cloned genes near chromosome ends, providing few landmarks to anchor the physical map and resulting in inaccurate interpolated predictions. Alternatively, making multiple comparisons may have resulted in our overestimating of significance. However, it is possible that both Wormbase and our experiments are correct and that a few intervals in Bristol/Hawaiian heterozygotes recombine at different frequencies than the Bristol/Bristol homozygote.

C. elegans chromosomes maintain only one crossover per bivalent. Since crossovers occurred on either side of each insertion site, interference may act across the insertion. Alternatively, alignment on one side excludes alignment on the other side (see DISCUSSION).

Insertions reduce local crossing over: In addition to assessing the number of crossovers formed, we also determined the effect that insertions had on the distribution of crossovers. We compared map distances in insertion animals with those in controls on an interval-by-interval basis. Surprisingly, we found that although insertion heterozygotes did not increase the frequency of double-crossover chromosomes, the insertions did affect the distribution of crossovers along the chromosomes. In all three cases, the interval containing the insertion received significantly fewer crossovers than controls (47% of normal for *oxIs107*, 34% for *oxIs131*, and 27% for *oxIs12*, $P < 0.05$) (Figure 4). This was a local effect, since in all cases crossover frequencies were nearly normal for more distant intervals on both sides of the insertion (Figure 4). In two cases (*oxIs107* and *oxIs131*), crossover frequency was also reduced in an interval adjacent to the one containing the insertion (50% of normal for *oxIs107* and 60% for *oxIs131*, $P < 0.05$) (Figure 4). We estimated the minimum sizes of the three suppressed regions and found them to be between 5 and 10 MU (see MATERIALS AND METHODS) (STINCHCOMB *et al.* 1985).

It is possible that the nature of the inserted sequences could interfere with crossing over. Repetitive sequences cause formation of heterochromatin, which might spread to nearby regions and be a source of reduced crossovers in paired DNA (YAMAMOTO and MIKLOS 1978; WU and LICHTEN 1994; KAGAWA *et al.* 2002). To test this possibility, we introgressed the *oxIs12* insertion into the Hawaiian background, which allowed us to measure crossover frequency in the *oxIs12* homozygous state. If the repetitive nature of the insertion interferes with crossing over, insertion homozygotes should have suppression of crossing over similar to that of heterozygotes. However, we found that crossovers were not suppressed across the interval spanning the insertion in *oxIs12* homozygotes. The map distance of the interval containing the insertion was greater in *oxIs12* homozygotes (11.7 MU) than in *oxIs12*/+ heterozygotes (8.0 MU, $P = 0.08$). Moreover, the distance of this interval in the *oxIs12* homozygote was not significantly different from that in the wild-type control Bristol/Hawaiian heterozygotes (13.4 MU, $P = 0.50$) across this same interval. However, the length of the interval was not increased as one might expect if crossovers were occurring in the inserted DNA. These data suggest that insertions suppress local crossovers in heterozygotes due to lack of sequence identity between homologs, rather than due to the *cis*-acting effects of the inserted DNA.

Insertions redistribute crossovers toward the homolog recognition region: Despite a local depression of crossing over around the insertion, the total number of crossovers across the whole chromosome was normal. Thus, the crossovers missing from regions near the insertion had to be redistributed elsewhere on the chromosome. The simplest possibility would be for the crossover frequency to increase uniformly outside the suppressed region. However, we observed a consistent pattern in which crossover frequency increased on one side of the insertion and decreased on the other side (Figure 4). *C. elegans* chromosomes are asymmetrical, since one end of each chromosome contains the homolog recognition region (MCKIM *et al.* 1988; HERMAN and KARI 1989). At least one homolog must contain one of these regions for proper segregation (VILLENEUVE 1994). Significantly, in all three insertions the polarity of redistribution corresponded to the location of the chromosome's homolog recognition region (Figure 4). Crossing over was increased on the side of the insertion containing the homolog recognition region and normal or reduced on the distal side (MCKIM *et al.* 1988, 1993).

DISCUSSION

We found that a large nonhomologous insertion caused a polar redistribution of crossovers, with higher crossover frequency near the homolog recognition region and lower frequency distal to the insertion point. A similar effect was observed by McKim *et al.* (1993) on the X chromosome, using insertion of chromosomal fragments, rather than a transgenic array. In yeast and mice, paracentric inversions and translocations can lead to synapsis of nonhomologous regions, called heterosynapsis (Ashley 1988; Dresser *et al.* 1994). Our results are consistent with insertions also causing heterosynapsis or possible synapsis failure in *C. elegans*. Villeneuve (1994) proposed that heterosynapsis in *C. elegans* causes complete crossover suppression distal to the homolog recognition region in translocation heterozygotes. This model proposes that the homolog recognition region may have two functions, perhaps accomplished by a single molecular process: homolog recognition and initiation of synapsis. After initiating at the homolog recognition region, synapsis then proceeds unidirectionally to the distal end, even through regions of nonhomology (Figure 5). Such regions of nonhomology lead to heterosynapsed regions in which crossovers

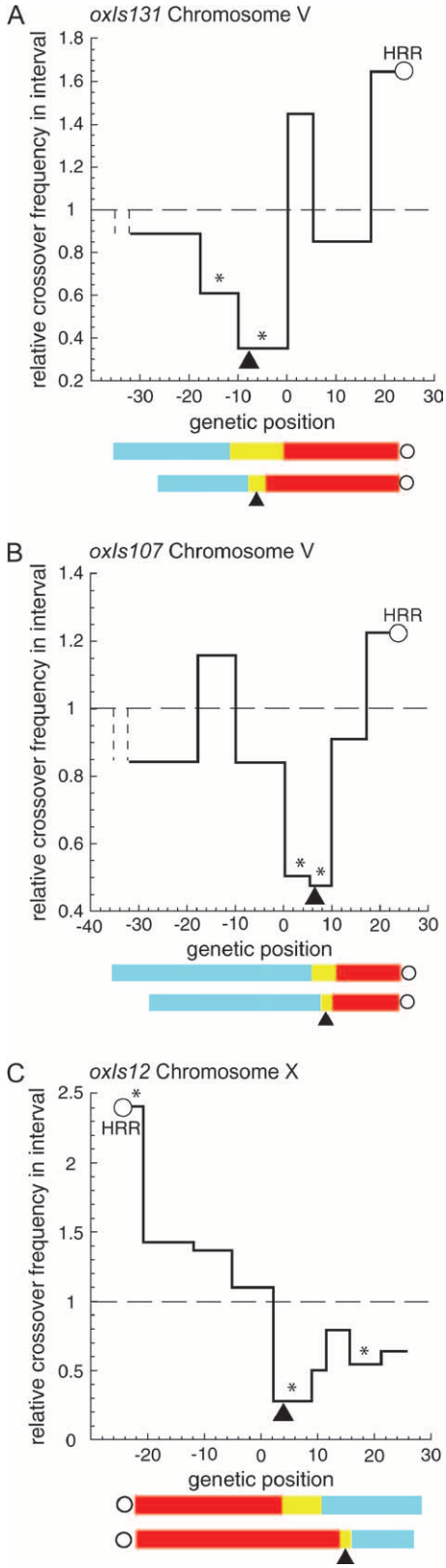

Figure 4.—Insertions alter the distribution of crossovers. (A–C) The amount of crossing over in each SNP interval in an insertion heterozygote, normalized to controls, is shown. The width of each interval corresponds to the map distance as determined by the control experiment. Triangles indicate the position of insertions; asterisks indicate intervals with significantly different crossover frequencies; HRR indicates the end with the homolog recognition region. The dashed line indicates wild-type levels of crossing over. The vertical dashed lines at the edge of chromosome V represent the interval near the telomere for which we had no SNP and thus no crossover data. A summary of genetic lengths of wild-type and insertion chromosomes is shown beneath each graph. Circles indicate HRR, triangles indicate location of insertions. Red, proximal to HRR; yellow, insertion-containing region; blue, distal to HRR.

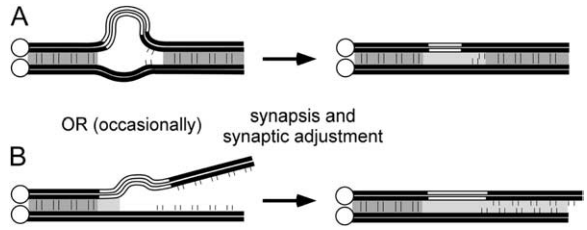

Figure 5.—Model for chromosome pairing in insertion heterozygotes. Homologs pair at the homolog recognition regions (circles) and zipper up along regions with sequence identity. Sometimes the chromosomes can pair distal to the sequence discontinuity (A). Pairing is not stable between homologs near the insertion site and these regions are not shown as paired. In other cases, the sequence discontinuity initiates heterosynapsis, which continues into the distal region (B). After synaptic adjustment, the chromosomes contain only a short region of heterosynapsis in the first case, but extensive heterosynapsis in the second case. Circles, homolog recognition regions; solid bars, normal chromosome sequences; open bars, insertion sequences; double lines, internal pairing sites; dark shading, homosynapsed synaptonemal complex; light shading, heterosynapsed synaptonemal complex.

do not occur. This model predicts that disrupting the continuity of homolog alignment should have a polar effect on crossover distribution, with crossover frequency increasing near the homolog recognition region. We found that in insertion heterozygotes, crossover frequency increased only on the side of the insertion containing the homolog recognition region. These data are consistent with pairing initiating primarily at the homolog recognition region end of each chromosome.

Our data also suggest that extensive heterosynapsis may occur in only a fraction of meioses. If extensive heterosynapsis always occurred, all intervals distal to an insertion would receive zero crossovers. However, despite the polar redistribution of crossovers, we observed near-normal levels of crossing over distal to the insertions. This suggests that when homologous synapsis is interrupted by a break in sequence identity due to an insertion, two outcomes can occur (Figure 5). Some chromosomes may be unable to reestablish homologous synapsis after the insertion (Figure 5B). In these heterosynapsed chromosomes, crossovers are restricted to the correctly aligned and synapsed region proximal of the insertion point. Other chromosomes are able to reestablish correct registry after the insertion, and in these chromosomes crossovers can occur on both sides of the insertion (Figure 5A). The ability of some chromosomes to reestablish homologous alignment suggests that pairing or synapsis may be reinitiated at internal sites or from the other telomere, albeit with a lower frequency of success (Rosenbluth *et al.* 1990; McKim *et al.* 1993). These pairing sites may also be similar to the pairing sites that have been proposed to limit the range of crossover suppression caused by a DNA break in Drosophila (Hawley 1980). Recent evidence in Drosophila suggests that these sites may not function in the large-scale physical pairing of chromosomes, since pairing defects are not detectable by cytology when these sites are deleted (Sherizen *et al.* 2005). Alternatively, homologous sequences distal to the insertion may become aligned through a process of synaptic adjustment. Synaptic adjustment is a poorly understood phenomenon in which differences in chromosome length in rearrangement heterozygotes are minimized, so that aberrations visible in early pachynema become invisible by late pachynema. Such adjustment could also result in sequences proximal to the insertion attaining an out-of-register configuration (Moses and Poorman 1981; Moses *et al.* 1982). Future experiments to directly address chromosome pairing, such as fluorescence *in situ* hybridization, would be necessary to directly test our heterosynapsis model.

Despite the local inhibition of crossing over in insertion heterozygotes, interference was still essentially complete. If homologous sequences on both sides of the insertion are aligned and synapsed simultaneously in a substantial fraction of meioses, this result would indicate that the crossover interference mechanism can act across large-scale breaks in sequence identity. However, we cannot rule out the alternative possibility that any given chromosome pair is correctly aligned only on one side of the insertion.

If insertions disrupt local crossing over without affecting interference, they must disrupt a process important only for crossing over. During meiotic recombination, sister chromatids are first organized onto axial elements. Then the homologs synapse and the synaptonemal complex forms between the paired chromosomes (Page and Hawley 2004). One possibility is that insertions may disrupt local crossovers by disrupting the synaptonemal complex. The synaptonemal complex in *C. elegans* is essential for crossing over (Colaiacovo *et al.* 2003). However, in yeast, interference precedes formation of the synaptonemal complex and the interference signal is likely to be transmitted by axial elements (Hunter and Kleckner 2001; Borner *et al.* 2004). Since insertions should not affect axial element formation, our data are consistent with the idea that axial elements transmit interference in *C. elegans* and that the synaptonemal complex is dispensable for this process. Significantly, a mutation in HIM-3, a protein component of the *C. elegans* meiotic axial element, disrupts interference (Nabeshima *et al.* 2004). In contrast to that in *C. elegans*, interference in yeast (de los Santos *et al.* 2003; Argueso *et al.* 2004; Malkova *et al.* 2004), mammals (Housworth and Stahl 2003), and Arabidopsis (Copenhaver *et al.* 2002; Higgins *et al.* 2004) is not complete and affects only a specific class of crossovers.

Although in our case the insertions were generated in the laboratory, chromosome insertions may play an important role in biology. It has been proposed that local suppression of recombination resembles an early step in the evolution of heteromorphic sex chromosomes (Muller 1918; Charlesworth and Charlesworth 2000). Reduction of recombination on chromosomes bearing sex-determining genes allows initially indistinguishable chromosomes to maintain a heterozygous state. A lack of crossing over and gene conversion favors the buildup of further mutations that will further strengthen the recombination suppression until the nascent sex chromosomes become readily distinguishable as full-fledged heteromorphic chromosomes. Examples of steps along this evolutionary trajectory have been found in a number of species (Griffin *et al.* 2002; Kondo *et al.* 2004; Liu *et al.* 2004; Peichel *et al.* 2004). Our transgene insertions similarly reduce crossing over in a local area and may serve as a model for understanding early changes that allow chromosomes to start down the path to becoming sex chromosomes. Along the same lines, models of speciation have been proposed in which reduced local recombination rates favor the formation of new species (Felsenstein 1981; Trickett and Butlin 1994; Rieseberg 2001).

We thank the Caenorhabditis Genetics Center for strains. This work was supported by grant 9733685 from the National Science Foundation to E.M.J.

LITERATURE CITED

Argueso, J. L., J. Wanat, Z. Gemici and E. Alani, 2004 Competing crossover pathways act during meiosis in *Saccharomyces cerevisiae*. Genetics **168:** 1805–1816.

Ashley, T., 1988 G-band position effects on meiotic synapsis and crossing over. Genetics **118:** 307–317.

Barnes, T. M., Y. Kohara, A. Coulson and S. Hekimi, 1995 Meiotic recombination, noncoding DNA and genomic organization in *Caenorhabditis elegans*. Genetics **141:** 159–179.

Borner, G. V., N. Kleckner and N. Hunter, 2004 Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell **117:** 29–45.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Charlesworth, B., and D. Charlesworth, 2000 The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. **355:** 1563–1572.

Colaiacovo, M. P., A. J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo *et al.*, 2003 Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev. Cell **5:** 463–474.

Copenhaver, G. P., E. A. Housworth and F. W. Stahl, 2002 Crossover interference in Arabidopsis. Genetics **160:** 1631–1639.

de los Santos, T., N. Hunter, C. Lee, B. Larkin, J. Loidl *et al.*, 2003 The Mus81/Mms4 endonuclease acts independently of double-Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics **164:** 81–94.

Dresser, M. E., D. J. Ewing, S. N. Harwell, D. Coody and M. N. Conrad, 1994 Nonhomologous synapsis and reduced crossing over in a heterozygous paracentric inversion in *Saccharomyces cerevisiae*. Genetics **138:** 633–647.

Felsenstein, J., 1981 Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evol. Int. J. Org. Evol. **35:** 124–138.

Griffin, D. K., S. C. Harvey, R. Campos-Ramos, L. J. Ayling, N. R. Bromage *et al.*, 2002 Early origins of the X and Y chromosomes: lessons from tilapia. Cytogenet. Genome Res. **99:** 157–163.

Hawley, R. S., 1980 Chromosomal sites necessary for normal levels of meiotic recombination in *Drosophila melanogaster*. I. Evidence for and mapping of the sites. Genetics **94:** 625–646.

Herman, R. K., and C. K. Kari, 1989 Recombination between small X chromosome duplications and the X chromosome in *Caenorhabditis elegans*. Genetics **121:** 723–737.

Higgins, J. D., S. J. Armstrong, F. C. Franklin and G. H. Jones, 2004 The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in Arabidopsis. Genes Dev. **18:** 2557–2570.

Hillers, K. J., and A. M. Villeneuve, 2003 Chromosome-wide control of meiotic crossing over in *C. elegans*. Curr. Biol. **13:** 1641–1647.

Hodgkin, J., H. R. Horvitz and S. Brenner, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

Housworth, E. A., and F. W. Stahl, 2003 Crossover interference in humans. Am. J. Hum. Genet. **73:** 188–197.

Hunter, N., and N. Kleckner, 2001 The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell **106:** 59–70.

Kagawa, N., K. Nagaki and H. Tsujimoto, 2002 Tetrad-FISH analysis reveals recombination suppression by interstitial heterochromatin sequences in rye (*Secale cereale*). Mol. Genet. Genomics **267:** 10–15.

Kondo, M., I. Nanda, U. Hornung, M. Schmid and M. Schartl, 2004 Evolutionary origin of the medaka Y chromosome. Curr. Biol. **14:** 1664–1669.

Lindegren, C. C., and G. Lindegren, 1942 Locally specific patterns of chromatid and chromosome interference in Neurospora. Genetics **27:** 1–24.

Liu, Z., P. H. Moore, H. Ma, C. M. Ackerman, M. Ragiba *et al.*, 2004 A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature **427:** 348–352.

Malkova, A., J. Swanson, M. German, J. H. McCusker, E. A. Housworth *et al.*, 2004 Gene conversion and crossing over along the 405-kb left arm of *Saccharomyces cerevisiae* chromosome VII. Genetics **168:** 49–63.

McKim, K. S., A. M. Howell and A. M. Rose, 1988 The effects of translocations on recombination frequency in *Caenorhabditis elegans*. Genetics **120:** 987–1001.

McKim, K. S., K. Peters and A. M. Rose, 1993 Two types of sites required for meiotic chromosome pairing in *Caenorhabditis elegans*. Genetics **134:** 749–768.

McIntire, S. L., R. J. Reimer, K. Schuske, R. H. Edwards and E. M. Jorgensen, 1997 Identification and characterization of the vesicular GABA transporter. Nature **389:** 870–876.

Meneely, P. M., A. F. Farago and T. M. Kauffman, 2002 Crossover distribution and high interference for both the X chromosome and an autosome during oogenesis and spermatogenesis in *Caenorhabditis elegans*. Genetics **162:** 1169–1177.

Moses, M. J., and P. A. Poorman, 1981 Synaptosomal complex analysis of mouse chromosomal rearrangements. II. Synaptic adjustment in a tandem duplication. Chromosoma **81:** 519–535.

Moses, M. J., P. A. Poorman, T. H. Roderick and M. T. Davisson, 1982 Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. Chromosoma **84:** 457–474.

Muller, H. J., 1918 Genetic variability, twin hybrids and constant hybrids in a case of balanced lethal factors. Genetics **3:** 422–499.

Nabeshima, K., A. M. Villeneuve and K. J. Hillers, 2004 Chromosome-wide regulation of meiotic crossover formation in *Caenorhabditis elegans* requires properly assembled chromosome axes. Genetics **168:** 1275–1292.

Page, S. L., and R. S. Hawley, 2004 The genetics and molecular biology of the synaptonemal complex. Annu. Rev. Cell Dev. Biol. **20:** 525–558.

Peichel, C. L., J. A. Ross, C. K. Matson, M. Dickson, J. Grimwood *et al.*, 2004 The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Curr. Biol. **14:** 1416–1424.

Rieseberg, L. H., 2001 Chromosomal rearrangements and speciation. Trends Ecol. Evol. **16:** 351–358.

Rosenbluth, R. E., and D. L. Baillie, 1981 The genetic analysis of a reciprocal translocation, eT1(III; V), in *Caenorhabditis elegans*. Genetics **99:** 415–428.

Rosenbluth, R. E., R. C. Johnsen and D. L. Baillie, 1990 Pairing for recombination in LGV of *Caenorhabditis elegans*: a model based on recombination in deficiency heterozygotes. Genetics **124:** 615–625.

Sherizen, D., J. K. Jang, R. Bhagat, N. Kato and K. S. McKim, 2005 Meiotic recombination in Drosophila females depends on chromosome continuity between genetically defined boundaries. Genetics **169:** 767–781.

Stinchcomb, D. T., J. E. Shaw, S. H. Carr and D. Hirsh, 1985 Extrachromosomal DNA transformation of *Caenorhabditis elegans*. Mol. Cell. Biol. **5:** 3484–3496.

Sturtevant, A. H., 1913 The linear arrangement of six sex linked factors in *Drosophila*, as shown by their mode of association. J. Exp. Biol. **14:** 43–59.

te Riele, H., E. R. Maandag and A. Berns, 1992 Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. Proc. Natl. Acad. Sci. USA **89:** 5128–5132.

Trickett, A. J., and R. K. Butlin, 1994 Recombination suppressors and the evolution of new species. Heredity **73**(4): 339–345.

Villeneuve, A. M., 1994 A *cis*-acting locus that promotes crossing over between X chromosomes in *Caenorhabditis elegans*. Genetics **136:** 887–902.

Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston and R. H. Plasterk, 2001 Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. Nat. Genet. **28:** 160–164.

Wu, T. C., and M. Lichten, 1994 Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science **263:** 515–518.

Yamamoto, M., and G. L. Miklos, 1978 Genetic studies on heterochromatin in *Drosophila melanogaster* and their implications for the functions of satellite DNA. Chromosoma **66:** 71–98.

Zetka, M. C., and A. M. Rose, 1992 The meiotic behavior of an inversion in *Caenorhabditis elegans*. Genetics **131:** 321–332.

Communicating editor: A. Villeneuve