Crossover Distribution and High Interference for Both the X Chromosome and an Autosome During Oogenesis and Spermatogenesis in *Caenorhabditis elegans*

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ABSTRACT

Regulation of both the number and the location of crossovers during meiosis is important for normal chromosome segregation. We used sequence-tagged site polymorphisms to examine the distribution of all crossovers on the X chromosome during oogenesis and on one autosome during both oogenesis and spermatogenesis in *Caenorhabditis elegans*. The X chromosome has essentially one crossover during oogenesis, with only three possible double crossover exceptions among 220 recombinant X chromosomes. All three had one of the two crossovers in the same chromosomal interval, suggesting that crossovers in that interval do not cause interference. No other interval was associated with double crossovers. Very high interference was also found on an autosome during oogenesis, implying that each chromosome has only one crossover during oogenesis. During spermatogenesis, recombination on this autosome was reduced by ~30% compared to oogenesis, but the relative distribution of the residual crossovers was only slightly different. In contrast to previous results with other autosomes, no double crossover chromosomes were observed. Despite an increased frequency of nonrecombinant chromosomes, segregation of a nonrecombinant autosome during spermatogenesis appears to occur normally. This indicates that an achiasmate segregation system helps to ensure faithful disjunction of autosomes during spermatogenesis.

NEARLY all sexually reproducing eukaryotic organisms make gametes via the complex cell divisions known as meiosis, characterized by recombination. Recombination events are often visible as structures known as chiasmata that physically connect two homologous chromosomes during prophase I. The physical connection is generally regarded as necessary for normal segregation of meiotic homologs since chromosomes that do not cross over often fail to segregate properly (Hawley 1988; Nicklas 1997), although achiasmate segregation has been described in several organisms (Hawley 1988; Hawley *et al.* 1992, 1993; Wolf 1994; Dernburg *et al.* 1996).

Although all chromosomes typically cross over, animal chromosomes frequently have only a small number of crossovers. For example, the number of crossovers during oogenesis in humans is slightly more than two per chromosome (Broman and Weber 2000; see also Figure 5A in Hassold and Hunt 2001). In mice, the mean number of crossovers per chromosome is ~1.4 (Broman *et al.* 2002). The mean number of chiasmata in *Drosophila melanogaster* is ~1.3 per chromosome arm during oogenesis (Carpenter 1988). Clearly the number of crossovers is not distributed at random among the chromosomes since the frequency of achiasmate chromosomes is much lower than that expected from such low crossover rates (discussed in Carpenter 1988; Hawley 1988).

In addition, comparisons of genetic and physical maps in many different organisms demonstrate significant differences in recombination rate even within the same chromosome. In humans, the rate of recombination in the genome varies from zero to as many as 8.8 crossovers per megabase; the rate of recombination typically varies by a factor of 5 or more on a chromosome (Yu *et al.* 2001; Venter *et al.* 2001). Thus, both the number and the location of crossovers are controlled, but regulation of the number of crossovers may be at least partially separate from regulation of the location of crossovers (Zetka and Rose 1995; Kleckner 1996).

An especially striking example of regional differences in recombination is seen with the autosomes of *Caenorhabditis elegans*. Each of the five autosomes of *C. elegans* has a central cluster of tightly linked genes flanked by chromosome arms in which genes are more widely spaced. These regional differences on the autosomes, seen by looking at the standard genetic map, arise from both a physical effect and a meiotic effect (Barnes *et al.* 1995). In particular, the frequency of recombination (per kilobase) in the arm of an autosome is as much as fivefold higher than the frequency of recombination in the central cluster. This distinctive arrangement has been evident and commented on from the first genetic

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map generated in *C. elegans*, but its origin and possible significance remain unclear (Albertson *et al.* 1997). The distribution of crossovers is affected by the *rec-1* mutation (Zetka and Rose 1995) and by radiation (Kim and Rose 1987; Dernburg *et al.* 1998). In both cases, map distances are expanded in the central cluster and reduced in the arms; that is, the genetic map more closely resembles the physical map. Curiously, the genetic and physical maps of the X chromosome do not show this distinctive arrangement, as both coding regions and recombination events are more uniformly distributed along the length of the X chromosome (Barnes *et al.* 1995). The rate of recombination on the X chromosome is intermediate between the relatively high rate on the arms of the autosomes and the low rate in the clusters.

In addition to, or perhaps as part of, the inherent differences among certain regions in crossover frequency, the presence of one crossover on a chromosome usually reduces the probability of a second crossover occurring nearby, in the phenomenon of interference. Although interference has a long history in the literature of genetics, its mechanism is unknown. Among the ideas postulated to explain interference have been the assembly of the synaptonemal complex, the kinetics of chromosome synapsis, and tension release in the vicinity of a crossover (Sym and Roeder 1994; Heyting 1996; Zickler and Kleckner 1998). Since it can extend over long distances in terms of DNA sequence, interference may arise from or be correlated with changes in the three-dimensional structure of the chromosome (Wu and Lichten 1994; Zickler and Kleckner 1999). Chromosomes in most organisms including *C. elegans* certainly undergo dramatic changes in three-dimensional shape during meiosis, becoming greatly shorter in length and much thicker in diameter. In what is probably the most explicit hypothesis of how interference and chromosome structure might be connected, Kleckner (1996) has proposed a model in which the chromosome compaction process itself results in crossovers that "are minimal in number and are maximally spaced" as part of crossover control.

C. elegans may offer a good organism to test this idea. Chromosomes in *C. elegans* are extremely compact during meiosis (Albertson *et al.* 1997). Furthermore, interference has been postulated to be quite high. On the basis of the length of the genetic map as measured from two-factor data, Herman and Kari (1989) proposed that there is probably only one crossover on the X chromosome. Hodgkin *et al.* (1979), in the only published analysis of interference during oogenesis in *C. elegans*, found no double crossovers on the X chromosome. No comparable experiments have been done for the autosomes during oogenesis but the length of the genetic map of the autosomes also suggests that interference is very high (Barnes *et al.* 1995).

Interestingly, in most organisms, the number and location of crossovers differ between spermatogenesis and oogenesis, typically having fewer crossovers in the male (discussed more fully in Singer *et al.* 2002). Even leaving aside the extreme example of dipteran insects such as Drosophila in which males have no crossing over, the sex difference can be significant. For example, in mice, the male genetic map is ~63% of the length of the female genetic map (Broman *et al.* 2002). In humans, the rate of recombination in males is ~55% of that in females, but is highly variable for different regions (Yu *et al.* 2001). In zebrafish, the male genetic map is only ~37% of the length of the map in females, with most of the map compression occurring in centromeric regions. The male map is slightly expanded near telomeres (Singer *et al.* 2002). This further suggests that the number of crossovers is regulated separately from their location.

In *C. elegans*, the standard genetic map is based on recombination in hermaphrodites, which represents a composite of the recombination rate during oogenesis and hermaphrodite spermatogenesis; recombination during male spermatogenesis in *C. elegans* has been much less studied. The most systematic work indicated that recombination on LGI occurs less during male spermatogenesis than during oogenesis and that the distribution of crossovers is slightly different. Zetka and Rose (1990, 1995) found that double crossovers did occur on LGI during male spermatogenesis with no apparent interference. Hodgkin *et al.* (1979) found that double crossovers occurred on linkage group IV during male spermatogenesis, although with ~55% interference. This result was in contrast to their results with the X chromosome during oogenesis. It has not been clear if the occurrence of double crossovers on autosomes during male spermatogenesis reflects a difference between the X chromosome and the autosomes generally, a difference between spermatogenesis and oogenesis, or some other difference. Each of these studies used three markers; thus, the double crossover events could not be distinguished from gene conversion events at the center marker.

Strain polymorphisms provide an attractive alternative set of markers to study crossover distribution in *C. elegans* since they are dominant, easy to score, widely dispersed, and compatible with male mating (Williams *et al.* 1992). In this study, we use sequence-tagged site (STS) polymorphisms to reexamine the number and distribution of crossovers in *C. elegans*. As inferred or reported in previous analyses, we find that interference on the X chromosome is very high during oogenesis, 100% for all but one interval; in that interval, no interference is observed. Interference is likewise very high, and possibly complete, on LGIII during oogenesis. There is significantly less recombination on LGIII during spermatogenesis than during oogenesis although the distribution of the remaining crossovers is not greatly altered. In contrast to previous studies using morphological mark-

ers on LGI and LGIV, we find no double crossovers on LGIII during male spermatogenesis. Commensurate with the lower rate of recombination and the high interference, we find many noncrossover chromosomes during male spermatogenesis. These noncrossover chromosomes appear to segregate normally, and no evidence for high rates of autosomal nondisjunction during spermatogenesis was found. This suggests that some form of achiasmate segregation functions for the autosomes during male spermatogenesis. Finally, we use these results and other tests to determine that most if not all of the spontaneous X chromosome loss that occurs in *C. elegans* probably happens during spermatogenesis in the hermaphrodite.

MATERIALS AND METHODS

Standard methods were used for growing and handling worms. All strains were grown and all matings were done at 20°. For progeny counts, a single L4 hermaphrodite was picked and transferred daily to a fresh plate until no more eggs were laid.

Bergerac strains were obtained from the Caenorhabditis Genetics Center (originally RW7000 but renamed FH1050 for our studies) or from David Pilgrim (DP13). Although both of these are ostensibly wild type, each has a recognizable recessive uncoordinated phenotype that was useful for distinguishable cross-progeny from self-progeny. The Bergerac strains differ in the presence or absence of some of the polymorphisms. In particular, *eP64* on LGIII is missing from FH1050 but present in DP13, so DP13 was used for all of the analysis on LGIII.

The multiplex PCR assay for STS polymorphisms was done as described (Williams *et al.* 1992; Williams 1995). We typically used a primer mix at a concentration of 10–15 pmol/μl and the *Tc1* primer 618 at 25 pmol/μl. Worms to be assayed were picked onto plates with no bacteria for a few hours before being picked individually into 2.5 μl of the lysis mix (including proteinase K) in the cap of a PCR tube. Reaction volumes of 25 μl were used, and all experiments were done in a Perkin-Elmer 480 machine. Results were assayed on a 6% polyacrylamide gel stained with ethidium bromide after electrophoresis. In many cases and in all cases involving suspected double crossovers, an additional aliquot of the reaction sample was run on a separate gel to confirm the presence or absence of particular markers.

To test for dominance of the markers, homozygous DP13 hermaphrodites were compared to heterozygous Bristol/Bergerac hermaphrodites. No obvious difference in the strength of the signal was detected among >300 worms assayed. We also assayed a very large number of homozygous Bergerac hermaphrodites and heterozygous Bristol/Bergerac hermaphrodites to test the penetrance of each marker. Although exact counts were not kept, >2500 worms were assayed for these and related experiments and in preparing this assay for use in a teaching laboratory. In the control worms in which all markers are expected, we found no examples that were missing one or a few polymorphisms. That is, the worm either had all of the polymorphisms that we assayed in that experiment or had none of the polymorphisms (Williams 1995). There are variable and sometimes high numbers of failed reactions in which no PCR products are observed from Bergerac worms. Since the success rate was higher with purified genomic Bergerac DNA than with lysed worms, it appears that variability in the lysis procedure contributed to the failed reactions.

Because an absence of products is also the expected result with a nonrecombinant Bristol chromatid, we considered only recombinant chromatids and nonrecombinant Bergerac chromatids (which have all of the PCR products) in our calculations of map distances, particularly for distances on the X chromosome. We observed 220 recombinant X chromosomes, so our calculations are based on 440 chromosomes. The data indicating that recombinant and nonrecombinant chromatids are equally frequent are described below. To calculate the expected number of double crossovers, we calculated the expected number of double crossovers for each pair of genetic intervals separately and summed the results over all intervals on the chromosome.

RESULTS

The STS polymorphisms for studying meiosis: Two wild-type isolates of *C. elegans*, Bristol (strain N2) and Bergerac (strains FH1050 and DP13), differ in the number and location of the transposable element Tc1. As previously described (Williams *et al.* 1992; Williams 1995), these strain differences provide convenient genetic markers for mapping. Primers have been made corresponding to genomic locations adjacent to a Tc1 element in the Bergerac strain but with no adjacent Tc1 element in the Bristol strain. Each of these primers can be used in combination with a primer from within Tc1 (referred to as primer 618) to generate a PCR product of a defined size from a Bergerac worm. Significantly, a cocktail of genomic primers can be used together with the Tc1 primer 618 in a multiplex PCR assay to generate a series of products from the Bergerac genome. For convenience, we refer to these Bergerac alleles as STS polymorphisms. However, since no Tc1 is adjacent to these genomic sites in Bristol, the corresponding Bristol worm gives no PCR products (Figure 1).

This assay has several advantages for the analysis of meiosis. First, unlike most markers in *C. elegans*, the STS polymorphisms do not compromise male mating ability or fertility. Second, individual worms can be scored for many different markers simultaneously and many markers are available, making it possible to assay nearly the entire length of a chromosome for crossovers. On LGIII, the most terminal polymorphisms are 680 kb from the left end and 730 kb from the right end, thus spanning 90% of the chromosome. On the X chromosome, the most terminal polymorphisms are 570 kb from the left end and 170 kb from the right end and thus span >95% of the chromosome (http://www.wormbase.org). Third, the markers are dominant and highly penetrant. Because of the complexity of the multiplex PCR assay, we were initially concerned about the penetrance of the polymorphic phenotype. To test this, in the course of these and related experiments we have assayed several thousand worms homozygous or heterozygous for a Bergerac chromosome. With very rare exceptions (⩽1% of the cases), if the reaction was successful for one marker, all of the markers were visible (Williams *et al.* 1992; Williams 1995). The two exceptions that may affect

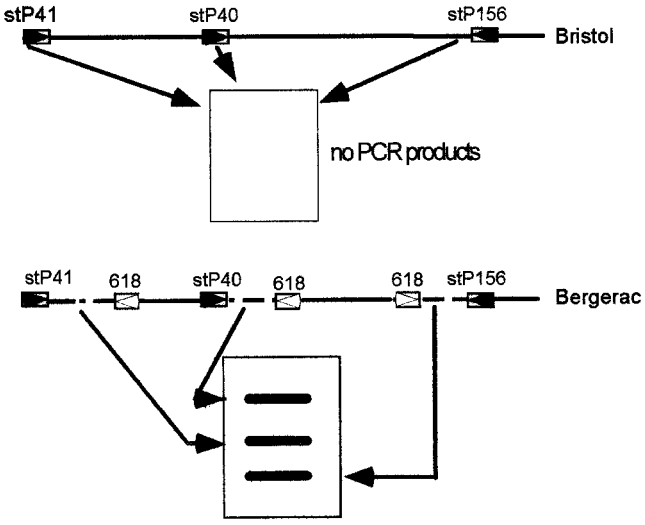

FIGURE 1.—The strategy of the multiplex PCR STS assay. PCR primer 618 corresponds to a sequence within the transposable element Tc1. The other primer is a cocktail of primers from genomic sequences located next to a Tc1 element in the Bergerac strain but not in the Bristol strain. The Tc1 element is shown as a dashed line, with the primer 618 represented with an open box and arrow. Boxes with solid arrows represent the genomic primers. A portion of the X chromosome is illustrated.

the interpretation of our data are discussed in more detail below.

Most of the X chromosome exhibits complete interference during oogenesis: Previous direct and indirect evidence has suggested that there is only one crossover on the X chromosome during oogenesis (HODGKIN *et al.* 1979; HERMAN and KARI 1989; BARNES *et al.* 1995). We confirmed this by two different crosses. In the first experiments, males with a Bergerac X chromosome were mated to a *dpy-6 (e14) unc-3(e151)* hermaphrodite to produce hermaphrodite offspring heterozygous for all X-linked Bergerac markers *in trans* to *dpy-6* and *unc-3*. A heterozygous hermaphrodite was mated to a wild-type male and the recombinant Dpy non-Unc and the non-Dpy Unc male offspring were picked singly and prepared for PCR analysis. Since *dpy-6* and *unc-3* are ~18.5 map units apart (HODGKIN and MARTINELLI 2001), Dpy non-Unc and non-Dpy Unc males are not rare; 63 Unc recombinants and 41 Dpy recombinants were used. (For unknown reasons, the Dpy recombinants lysed more poorly than the Unc recombinants.) Each of these recombinants was assayed for the location of the crossover and for additional X-linked recombination events by using the STS polymorphisms.

In all cases, the crossover between *dpy-6* and *unc-3* could be unambiguously located, and the observed pattern of STS polymorphisms agreed with the expected pattern for a recombination event in this interval. For example, the Dpy non-Unc recombinants had none of the STS polymorphisms located to the left of *dpy-6* but had some subset of the STS polymorphisms defined by *stP129*, *stP61*, and *stP72*, and all of them had *stP2* (Figure 2). In all examples of Dpy non-Unc recombinants, the pattern of polymorphisms could be explained by a single crossover event between *dpy-6* and *unc-3*.

For the non-Dpy Unc recombinants, a single crossover could unambiguously account for all but one of the worms. The exceptional worm had inherited *stP40*, *stP156*, *stP33*, *stP103*, and *stP129*, but not *stP41*, *stP61*, *stP72*, and *stP2*. Thus, there was clearly a crossover between *stP129* and *stP61* (Figure 2). The absence of *stP41* in this worm could indicate that a second crossover had occurred between the site of *stP41* on the Bristol chromosome and the site of *stP40* on the Bergerac chromosome. Alternatively, since only one marker is involved in this event, it could also be explained by a failed single PCR reaction involving *stP41*, an excision of the Tc1 element adjacent to *stP41* on the Bergerac chromosome, a gene conversion, or some other event at this site. We tentatively conclude that this may have been a double exchange, but the evidence is equivocal.

A second assay for X chromosome recombination mated a Bristol/Bergerac heterozygous hermaphrodite to wild-type Bristol males, with no genetic markers on the Bristol chromosome. This method allows us to assay single, double, and multiple crossover events no matter where they occurred on the X chromosome. In principle, it would also allow us to detect nonrecombinant chromosomes. However, many of these experiments were done while reaction conditions were being established, so we did not directly determine the number of nonrecombinant chromosomes (either with all polymorphisms or with no polymorphisms). In one test for which all chromosomes were recorded, we observed 47 recombinant chromosomes and 25 chromosomes with all of the polymorphisms. This indicates that about half the chromatids were recombinant, as expected for single crossovers at the four-chromatid stage of meiosis I. (This assumes that, in addition to 25 chromosomes with all of the polymorphisms, a similar number had none of the polymorphisms). Overall, we observed 116 recombinant chromosomes in these experiments. Of these 116 recombinant chromosomes, 114 could be explained by a single crossover that we could readily locate, as shown (Figure 2). In most intervals, the expected number of crossovers based on the standard genetic map (HODGKIN and MARTINELLI 2001) agrees very well with the observed number ($P < 0.05$ on χ^2 tests), indicating that our assay with the interstrain hybrids gives recombination results comparable to known map data. The correspondence of our map distances based on oogenesis with the standard reference map distances (HODGKIN and MARTINELLI 2001) suggests that the rate of recombination is similar in both hermaphrodite germ lines.

However, two of the 116 recombinants could not be easily explained by a single crossover and were most likely due to a double crossover. In one worm, *stP40*,

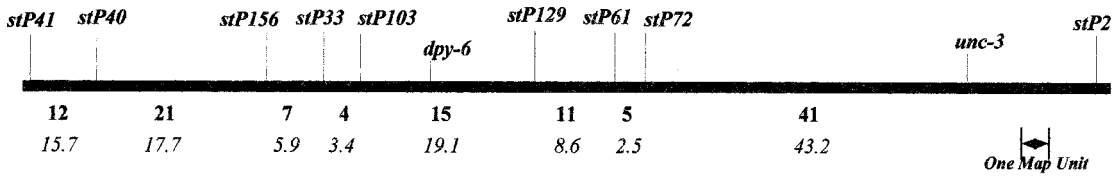

FIGURE 2.—The X chromosome map resulting from these experiments. The locations of the genetic markers *dpy-6* and *unc-3* are included for reference. The number of recombinants observed in each interval is shown in boldface type. The total number of recombinant chromosomes assayed was 116. The predicted number of recombinants based on the closest genetic markers on the standard genetic map (HODGKIN and MARTINELLI 2001) is shown in italics. No significant difference was seen between the observed and the expected number for any interval.

stP156, and *stP33* were absent while the other polymorphisms were present. In the other worm, *stP40* and *stP156* were absent, while the other polymorphisms were present. Thus, each appeared to have a crossover between *stP41* and *stP40* and a second crossover elsewhere on the chromosome. We did observe 10 other worms in which the only crossover on the X chromosome was between *stP40* and *stP41*.

In summary of our X-linked recombination data, all but 3 of 220 recombinant chromosomes had only one crossover, which we could locate unequivocally. As expected from previous experiments, interference is very high. On the basis of our map data, we expected to observe 12.2 double crossover chromosomes, and we found 2 or at most 3. Thus, for the entire X chromosome, interference is between 75 and 85%.

However, this type of calculation may obscure a more interesting aspect of our results. All three of the double crossover cases had one of the crossovers in the interval between *stP41* and *stP40* and a second crossover elsewhere on the X chromosome. Thus, the region between *stP41* and *stP40* appears to confer no interference (2.8 double crossovers are expected), and all other X-linked regions exhibited 100% interference (9.4 double crossovers are expected and none were observed). More precisely, there is a 95% certainty that the number of double crossovers is 3 or less for these intervals; thus the coefficient of coincidence is at most 0.32 (3/9.4) and interference is >68%.

It is worth noting that HODGKIN *et al.* (1979) observed 100% interference using the three X-linked markers, *dpy-3*, *lon-2*, and *unc-7*. Most of the interval between *stP41* and *stP40* is to the left of *dpy-3* and thus would not have been examined in their experiment. Thus, for the remaining intervals on the X chromosome, our data agree with theirs and no double crossovers have been observed.

Linkage group III also exhibits complete interference during oogenesis: The absence of appropriate genetic markers has previously prevented assays for interference on an autosome during oogenesis, although the genetic map implies that each autosome has very high interference. Because the STS markers are dominant yet do not prevent male mating, we can use them to assay this directly. The STS polymorphisms on LGIII were chosen for this analysis.

In these experiments, Bristol males were mated to DP13 Bergerac hermaphrodites, and cross-progeny hermaphrodite progeny were picked. The cross-progeny can be distinguished from self-progeny since DP13 Bergerac has a recessive uncoordinated phenotype. The Bergerac/Bristol hermaphrodites were then mated to Bristol males, and the male cross-progeny were picked and assayed for recombination. Such animals have LGIII that arose during oogenesis from the Bergerac/Bristol hybrid hermaphrodite. In this case, all chromosome III data were tabulated (summarized in Figure 3). Of 177 chromosomes assayed, 80 (45.2%) were recombinant. The expected recombination distance between the STS polymorphisms *eP64* and *stP17* is ~44 map units, as determined by summing two-factor data for nearby genes (HODGKIN and MARTINELLI 2001), so the observed frequency of recombinant chromosomes during oogenesis agrees well with the expected number. This suggests that few, if any, of the nonrecombinant chromosomes arose from an achiasmate meiosis; rather, they are likely the nonrecombinant chromatids from a chiasmate meiosis. In 79 of these 80 recombinant chromosomes, exactly one crossover was found (shown in Figure 3). For all intervals examined the observed number of crossovers agreed very well with the expected number ($P < 0.05$ on χ^2 tests), demonstrating that the strain differences between Bristol and Bergerac do not notably alter the map distances on LGIII.

One worm gave an ambiguous result, since it had all of the STS polymorphisms except *stP127*. Several explanations could account for this pattern of alleles. First, there may have been a double crossover, with one crossover in the small interval between *stP120* and *stP127* and a second crossover in the larger interval between *stP127* and *stP17*. Alternatively, this chromosome could represent a failure of the *stP127* polymorphism to show up in a nonrecombinant Bergerac chromatid, possibly because of an excision of the Tc1 element on the Bergerac chromosome, a gene conversion at the *stP127* site, or a failure of this single reaction in the multiplex mix. We have no way to discriminate among these possibilities. This is the only example of chromosome III that

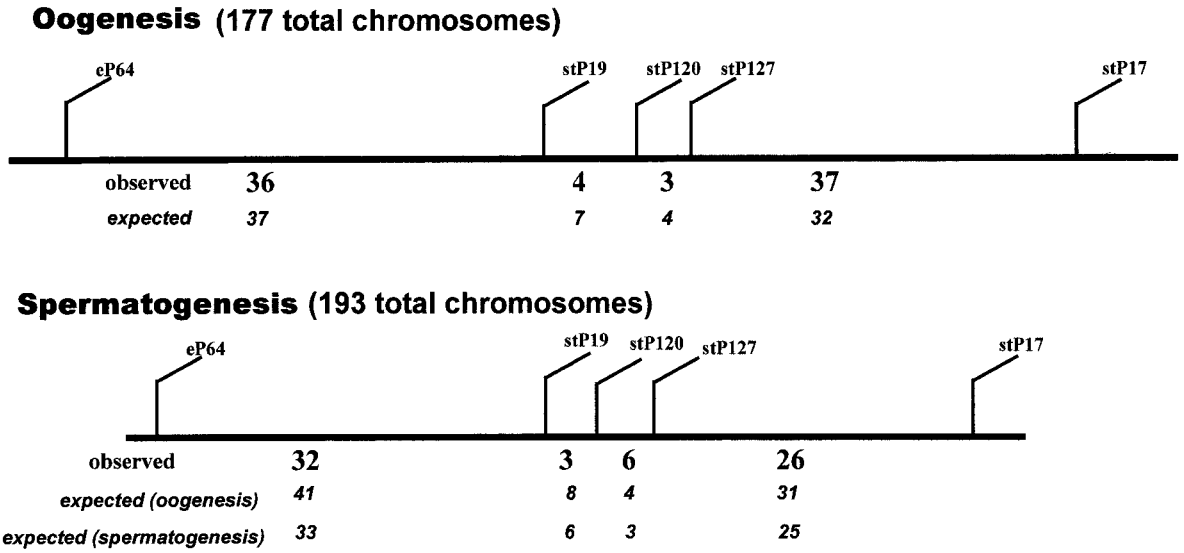

FIGURE 3.—The LGIII map resulting from these experiments. At the top is the map arising from crossovers during oogenesis. The observed number of crossovers in each interval is shown in boldface type and the predicted number based on the standard genetic map (HODGKIN and MARTINELLI 2001) is shown in italics. The differences are not significant for any interval. At the bottom is the map arising from crossovers during spermatogenesis. Note that the map length is shorter, reflecting the large number of achiasmate chromosomes. The observed number of crossovers in each interval is shown in boldface type, and the predicted number from the standard map is in italics. "Expected oogenesis" refers to the number of recombinants expected if oogenesis and spermatogenesis have similar rates of recombination. "Expected spermatogenesis" is the number expected if each interval on the chromosome is reduced proportionally and there is no change in the distribution of residual crossovers.

may have had a double crossover. If we assume no interference for any interval on this chromosome, we would have expected 10.5 double crossovers; at most we observed 1, and this one is equivocal. Thus, we conclude that interference on LGIII during oogenesis is at least 60% (with 95% confidence) and may be 100% for the intervals we tested.

Spermatogenesis has less recombination than oogenesis and very high interference for LGIII: To assay recombination on LGIII during spermatogenesis, Bristol males were mated to DP13 hermaphrodites, and the male cross-progeny was picked. These males were mated to a hermaphrodite homozygous for a recessive Bristol marker [*dpy-17 (e164)*], and the cross-progeny males and hermaphrodites were picked singly for analysis of recombination during spermatogenesis (Figure 3).

Of 193 chromosomes sampled, 67 were recombinant, representing a total map distance of 34.7 map units. On the basis of the map distances observed during oogenesis, we expected 87 recombinants among the 193 chromosomes. This distance (67/193) represents significantly fewer recombinants than expected ($\chi^2 = 8.37$, $P < 0.005$). This demonstrates that there is substantially less recombination during male spermatogenesis than during oogenesis. The reduction in recombination appears to be uniform across the chromosome since, in three of the four intervals, the observed numbers do not differ significantly from the expected number; *i.e.*, all map distances are reduced proportionally. However, in the interval from *stP127* to *stP17*, fewer crossovers were observed than expected on the basis of oogenesis data ($\chi^2 = 4.9$, $P = 0.02$). From these data, we conclude that the overall recombination frequency for LGIII during spermatogenesis is lower than that for oogenesis, but that the location of the exchanges changes only slightly.

A single crossover could explain all of these recombinant chromosomes. No double crossovers or possible double crossovers were observed, although seven double crossover chromosomes were expected. From this we conclude that interference for LGIII during spermatogenesis is very high and may in fact be 100%.

Meiotic stability of the X chromosome and the autosomes: The recombination data suggest that, on average, there is one or possibly slightly more than one crossover per chromosome during oogenesis. Since the overall map is shorter during spermatogenesis, there may be less than one crossover per chromosome during spermatogenesis—that is, some chromosomes apparently have no exchanges during spermatogenesis. Since nonexchange chromosomes might be lost, we assayed directly for spontaneous loss of an autosome during spermatogenesis and oogenesis and for loss of an X chromosome during oogenesis.

Autosomal nondisjunction is expected to result in inviable monosomics and thus will not have a distinct and easily recognized phenotype (HODGKIN *et al.* 1979). To investigate the spontaneous loss of autosomes, we used *him-6 (e1423)* strains that produce gametes nullisomic and disomic for each chromosome (HODGKIN *et*

al. 1979; Haack and Hodgkin 1991). These can be detected when *him-6* males are mated with *him-6; dpy; unc* hermaphrodites, where *dpy* and *unc* are any of several unlinked recessive autosomal markers, by looking for Dpy non-Unc or non-Dpy Unc offspring.

This assay was used to test for spontaneous nondisjunction of the autosomes by mating *him-6* males to *unc-24 (e138) IV; dpy-11 (e224) V* hermaphrodites or *dpy-10 (e128) II; unc-36 (e251) III* and looking for Unc non-Dpy or non-Unc Dpy progeny. None were seen among >30,000 offspring examined for autosomes IV and V or among 18,000 offspring examined for autosomes II and III. This indicates that the disomic ova arise very rarely (if at all) spontaneously. To test for nullisomic sperm, wild-type males were mated to *him-6; unc-24; dpy-11* hermaphrodites or *him-6; dpy-10; unc-36* hermaphrodites. No non-Unc Dpy and Unc non-Dpy progeny were seen among >25,000 offspring for autosomes IV and V and >14,000 offspring for autosomes II and III, indicating that nullisomic sperm also occur spontaneously very rarely (if at all). From similar assays with each parent homozygous for *him-6*, autosomal loss is found at the rate of 2–5/1000 animals (16 Unc-24 and 29 Dpy-11 among 6342 offspring), so we know that disomic ova and nullisomic sperm can be recovered in this assay (Haack and Hodgkin 1991). Thus, we have been unable to detect any spontaneous loss of an autosome during either spermatogenesis or oogenesis. This genetically confirms results that have been previously observed cytologically (Villeneuve 1994; Dernburg *et al.* 1998; Zalevsky *et al.* 1999).

Spontaneous loss of the X chromosome is common during hermaphrodite self-fertilization (Hodgkin *et al.* 1979). From among 8806 F_1 self-progeny of 33 individual hermaphrodites, 16 X0 males were found, a frequency of 1 male in 550 offspring. These males probably arise from meiotic nondisjunction of the X rather than mitotic loss since only 1 of the 33 broods had 2 males, and none had >2 males. The absence of broods with >1 spontaneous male also suggests that most of the loss occurs at meiosis II rather than at meiosis I. However, the loss could be occurring during hermaphrodite spermatogenesis, during oogenesis, or during both processes.

Events during oogenesis can be measured by mating the hermaphrodite with males and examining the cross-progeny for the patroclinous inheritance of the X chromosome. Hermaphrodites homozygous for the X-linked recessive mutation *unc-3* or for *dpy-6 unc-3* were mated with wild-type males, and all of the cross-progeny offspring were counted. If X chromosome loss occurs during oogenesis, then some ova are expected to be nullo-X, which would result in a patroclinous non-Unc male. We examined 15,562 cross-progeny offspring, of which 7887 were Unc males and 7675 were non-Unc hermaphrodites. No patroclinous non-Unc males were seen. On the basis of the rate of X chromosome loss observed during cross-fertilization (16/8806), we estimate that >28 gametes from these hermaphrodites were nullo-X. Since no patroclinous males have been observed, the rate of X chromosome loss during oogenesis is not nearly high enough to account for the observed rate of loss during self-fertilization. Thus, although we cannot directly measure meiotic events during hermaphrodite spermatogenesis, we infer that most if not all of the observed X chromosome loss during self-fertilization occurs during hermaphrodite spermatogenesis.

DISCUSSION

Normal chromosome segregation during meiosis I and II involves controlling both the location and the number of crossovers at prophase I (Koehler *et al.* 1996; Lamb *et al.* 1996). Despite the clear evidence for crossover control, the molecular mechanisms underlying it are quite obscure. *C. elegans* may offer several advantages for studying this problem not only because detailed genetic and physical maps are available but also because the number of crossovers in *C. elegans* is quite low.

In *C. elegans*, there appear to be extremely high and nearly 100% interference for both the X chromosome and one autosome during oogenesis. In agreement with previous direct and indirect evidence (Herman and Kari 1989; Barnes *et al.* 1995; Albertson *et al.* 1997), we find exactly one crossover over most chromosomal regions during oogenesis. Our assay examined >95% of the X chromosome and 90% of LGIII, which enabled us to monitor double crossovers and interference directly. However, the apparent exceptions on the X chromosome may be as interesting as the intervals with no double crossovers. Results from two worms are best explained as arising from a double crossover, and a third worm may also have had a double crossover. All of these worms had an X chromosome with one crossover in the interval between *stP41* and *stP40*, near the left end of the X chromosome. Although not conclusive, the observation that all double crossover chromosomes included one crossover in this interval suggests that crossovers in this region have a unique inability to cause interference.

The interval between *stP41* and *stP40* (which comprises >1 Mb) has also been implicated in other unusual meiotic properties. For example, duplications of this end of the chromosome recombine with the normal X chromosome at a very high rate in both X0 and XX animals (Herman and Kari 1989). Such duplications also cause X chromosome nondisjunction in XX animals (Herman and Kari 1989). Both of these properties appear to be unique to duplications of this region of the X chromosome and are not seen with duplications of other parts of the X chromosome. In addition, a rearrangement in this region greatly reduces recombination along the length of the X chromosome (Villeneuve 1994). Furthermore, mutations in autosomal genes *him-5* and *him-8* greatly reduce recombination in all other intervals of the X chromosome but have increased re-

combination here (Broverman and Meneely 1994). We also inferred previously (Broverman and Meneely 1994) that some crossovers in this region do not confer meiotic stability. This combination of unusual properties has led to the suggestion that this part of the X chromosome is involved in homolog recognition or pairing (McKim *et al.* 1993; Wicky and Rose 1996) although currently available cytological evidence does not support this (Albertson *et al.* 1997). The "region" as defined by these various experiments is large and several different or independent effects may be occurring. Nonetheless, further exploration of this region and the possible connection with reduced interference might lead to a common explanation. At the very least, the interval between *stP41* and *stP40* represents a region in which crossover regulation is distinct from that of the rest of the chromosome. Two types of crossovers have been shown in *C. elegans*: the predominant ones that require the MutS-related gene *him-14* and the rare others that do not require *him-14* (Zalevsky *et al.* 1999). One suggestion is that the *him-14*-dependent crossovers result in 100% interference, whereas the *him-14*-independent ones (which for some reason may be more common in this region of the X chromosome) do not cause interference.

We also found very high interference for LGIII during oogenesis, with at most one double crossover among 193 chromosomes sampled. Although no previous direct data existed for interference on an autosome during oogenesis, our results were consistent with inferences drawn from the genetic map (Barnes *et al.* 1995; Albertson *et al.* 1997). The length of the genetic map of each of the autosomes, as well as the additive distances in two-factor mapping experiments, had suggested that very high interference would be observed during oogenesis. Our data for LGIII confirm this directly; we saw exactly one crossover on every recombinant chromosome, in the locations predicted from two-factor data. Since the other autosomes are also 50 map units long, very high or complete interference is likely to be true for other autosomes, although none have been tested yet. Taken together, our results with the X chromosome and LGIII strongly imply that there is one crossover on each chromosome during oogenesis, or a total of six crossovers overall.

Our results with recombination and interference during spermatogenesis were somewhat more unexpected. Similar to the results on LGI (Zetka and Rose 1990), we find that spermatogenesis has fewer crossovers than does oogenesis; the overall map of LGIII during spermatogenesis is about two-thirds as long as the map during oogenesis. As before (Zetka and Rose 1990), we found that the reduction in recombination frequency did not occur uniformly for all regions, although the differences between regions were modest. However, our results with interference were unexpected. Hodgkin *et al.* (1979) had shown that double crossovers do occur during spermatogenesis on LGIV, albeit with ~55% interference; Zetka and Rose (1990) found double crossovers in a region on LGI at a frequency that suggested no interference during spermatogenesis. On the basis of these previous findings, we expected to find numerous double crossovers on LGIII and were therefore surprised to find none. This difference probably reflects differences between chromosomes since preliminary tests with the STS assay found double crossovers on LGIV (P. Meneely and S. Taylor, unpublished data), confirming the work of Hodgkin *et al.* (1979). Our data show that the difference in interference is not solely between oogenesis and male spermatogenesis or even between the X chromosome and the autosomes. Rather, some chromosomes receive two crossovers occasionally and some only one during male spermatogenesis.

Our data about crossover frequency during male spermatogenesis lead to the postulate that some autosomes might also receive no crossovers. Indeed, we found ~65% noncrossover autosomes during male spermatogenesis. Rather surprisingly, these chromosomes appear to segregate normally. We have two lines of evidence that support this. First, we saw no increase in inviable (indicating trisomic or monosomic) offspring despite the high rate of noncrossover chromosomes. In addition, no sperm nullisomic for an autosome were found in a direct search that would have allowed such offspring to survive. Our results thus provide direct evidence for achiasmate segregation of the autosomes during male spermatogenesis, as suggested previously from indirect data (Hawley and Theurkauf 1993).

These data lead to the following summary for crossover control in *C. elegans*. During oogenesis, each chromosome has one crossover and segregation occurs via chiasmate mechanisms. Oocytes with achiasmate chromosomes either do not occur spontaneously or do not mature, since we recovered no ova disomic for an autosome and no ova nullisomic for the X chromosome. On the other hand, oocytes with more than one crossover are also rare, suggesting that approximately six crossovers occur during oogenesis, one per chromosome.

The situation in spermatogenesis is different, and several separate mechanisms may be at work. First, the X chromosome in an X0 male is routinely unpaired during meiosis I and yet segregates normally, implying some form of specialized segregation that does not rely on either pairing or chiasmata.

For the autosomes during male spermatogenesis, two different segregation systems appear to operate. Many of the chromosomes receive one or even two crossovers, indicating that normal chiasmate segregation operates. However, an achiasmate mechanism also appears to govern the segregation of the autosomes during spermatogenesis. Since the genetic map of an autosome during spermatogenesis is shorter than 50 map units, the average number of crossovers per chromosome is less than one, and the total number of crossovers in a male may

be less than five. Therefore, an appreciable fraction of the autosomes are presumably paired but achiasmate (Dernburg *et al.* 1998). Nonetheless, achiasmate autosomes appear to segregate normally during male spermatogenesis.

It has been suggested (Dernburg 2001) that high interference is related to the holocentrism of nematode chromosomes. In meiosis in organisms with monocentric chromosomes, sister chromatid cohesion is lost distal to a crossover and only cohesion at and near the centromere holds chromatids together. With holocentrism, cohesion is retained only in regions remote from the crossover; thus, it may be that only a system that prevents second crossovers is compatible with normal sister chromatid separation.

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