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Domain-Specific Regulation of Recombination in *Caenorhabditis elegans* in Response to Temperature, Age and Sex

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ABSTRACT

It is generally considered that meiotic recombination rates increase with temperature, decrease with age, and differ between the sexes. We have reexamined the effects of these factors on meiotic recombination in the nematode *Caenorhabditis elegans* using physical markers that encompass >96% of chromosome III. The only difference in overall crossover frequency between oocytes and male sperm was observed at 16°. In addition, crossover interference (CI) differs between the germ lines, with oocytes displaying higher CI than male sperm. Unexpectedly, our analyses reveal significant changes in crossover distribution in the hermaphrodite oocyte in response to temperature. This feature appears to be a general feature of *C. elegans* chromosomes as similar changes in response to temperature are seen for the X chromosome. We also find that the distribution of crossovers changes with age in both hermaphrodites and females. Our observations indicate that it is the oocytes from the youngest mothers—and not the oldest—that showed a different pattern of crossovers. Our data enhance the emerging hypothesis that recombination in *C. elegans*, as in humans, is regulated in large chromosomal domains.

MEIOTIC recombination establishes a physical link between homologs that helps ensure segregation to opposite poles during the first meiotic division. Thus, failure to recombine can lead to chromosome missegregation and aneuploid gametes. Accordingly, crossover formation is tightly regulated to ensure that each chromosome (chr) receives at least one crossover, known as the obligate crossover. In many organisms, including *Caenorhabditis elegans*, chromosome pairs receive the obligate crossover and very few additional crossovers. When additional exchanges do occur, they tend to be widely distributed and evenly spaced, a phenomenon known as crossover interference (CI).

Meiotic crossovers are induced by programmed double-strand breaks (DSBs) catalyzed by the topoisomerase-like protein, Spo11. DSBs occur nonrandomly along chromosomes and chromatin architecture plays a fundamental role in determining the break sites. (reviewed in de Massy 2003). The physical positions of crossovers are regulated locally, with hotspots and coldspots corresponding to DNAse-sensitive, open chromatin and DNAse-insensitive, closed chromatin, respectively (Robine *et al.* 2007). Exchanges are thought to occur preferentially in chromatin loops (Blat *et al.* 2002) away from the chromosome axis that is involved in both synaptonemal complex formation and chromatin cohesion (Glynn *et al.* 2004). Chromatin loops may also contribute to the crossover landscape as population studies in yeast, mice, and humans indicate that exchanges fall into large, coordinately regulated chromosomal blocks (Baudat and Nicolas 1997; Borde *et al.* 1999; Gerton *et al.* 2000; Daly *et al.* 2001; Gabriel *et al.* 2002). In addition, telomeres and centromeres establish recombinationally repressed regions (Stern 1926; Lambie and Roeder 1986; Blitzblau *et al.* 2007). How these crossover domains are established and regulated remains an outstanding question (Dorman *et al.* 2007; Fukuda *et al.* 2008).

As in other organisms, the organization of the genes on the *C. elegans* chromosomes has supported the suggestion that recombination domains exist, although concrete evidence for their existence has been lacking. The central region of each autosome is gene rich and relatively "cold" with close to a fivefold lower frequency of crossovers/kilobase than the chromosome arms. In contrast, the X chromosome has a more uniform distribution of genes and recombination frequencies (Barnes *et al.* 1995). The gene-dense clusters appear to have an inherent property that makes them refractory to recombination as exchanges were repressed even when these domains were relocated closer to the end of the chromosome (Hillers and Villeneuve 2003).

C. elegans has been thought to demonstrate an extreme example of CI, with each chromosome having just one crossover in almost all meioses (Hodgkin *et al.* 1979; Hillers and Villeneuve 2003). Hillers and

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Villeneuve (2003) elegantly showed that end-to-end chromosome fusions comprising nearly half the genome (three chromosomes) still received approximately one crossover per meiosis. Furthermore, genetic analyses suggest that there is only a single pathway for crossover formation in *C. elegans*, making it an attractive system to understand crossover regulation (Kelly *et al.* 2000).

Recent work in *C. elegans* has provided insight into CI. The chromosome fusion experiments described above indicated that CI acts chromosome-wide and depends on a contiguous chromosome axis (Hillers and Villeneuve 2003). This work has been supported by the identification of mutations in axial element components that abrogate interference (Hillers and Villeneuve 2003; Borner *et al.* 2004; Fung *et al.* 2004; Nabeshima *et al.* 2004). Furthermore, mutations in *dpy-28*, a dosage compensation complex subunit that resembles a condensin subunit, lead to double and triple crossovers implicating chromatin structure as a major determinant of CI (Tsai *et al.* 2008). Another determinant of CI is prophase progression. Mutations in *him-8* prevent pairing of the X chromosome and cause meiotic nuclei to stall the cell cycle during early pachytene, the time at which DSBs are made. This delay results in an increased level of double crossovers (DCOs) and a loss of CI on the (paired) autosomes (Carlton *et al.* 2006).

In addition to chromatin, chromosome context and CI, recombination rates are also affected by parental age (Stern 1926) and sex (reviewed in Lenormand and Dutheil 2005), as well as temperature (Plough 1917; Lamb 1969; Rose and Baillie 1979; Saleem *et al.* 2001), radiation (Mavor and Svenson 1923; Muller 1925; Kovalchuk *et al.* 1998), and other stresses (Schewe *et al.* 1971; Barth *et al.* 2000). In most cases, the effects of these factors on recombination have been determined for specific intervals on a chromosome with different chromosome regions responding differently to each stress. How these factors influence CI and exchanges along an entire chromosome are only now beginning to be analyzed in the genetic systems (Barth *et al.* 2000; Paigen *et al.* 2008).

In part, such studies have been hampered by the fact that they require analysis of large populations to determine how recombination rates across the population are influenced by different environmental conditions. With the advent of new genotyping technologies for mapping single nucleotide polymorphisms (SNPs), the ability to genotype hundreds of animals at tens (to hundreds) of positions along a chromosome is making it feasible to obtain such population data under varying conditions. Indeed a recent study in mice pinpointed CI as a major factor driving sex-specific differences in recombination rates (Petkov *et al.* 2007). This is unlikely to be the case in *C. elegans* where CI is extremely high, although sex differences in CI have been reported (Meneely *et al.* 2002). Instead, the analyses of sex, temperature, and age effects on recombination in *C. elegans* have led to the suggestion that domain-specific changes in recombination rates underlie many of the differences (Zetka and Rose 1990).

We have explored this question in more detail by building a detailed recombination map of chr III at three temperatures from both male sperm and hermaphrodite oocytes. In addition, we further explore whether results we obtain for chr III hold true for the X chromosome and whether the effects of temperature can be generalized to other stresses, specifically to aging.

MATERIALS AND METHODS

Genetic crosses: Strains were grown according to the standard procedures (Brenner 1974). Strains used were: Bristol N2; Hawaiian strain CB4856; EG1285 *lin-15(n765) oxIs12 [Punc-47::GFP; lin-15(+)]*X; *dpy-18(e364)* III; *unc-45(e286) dpy-18(e364)* III; *dpy-18(e364) unc-64(js115)* III; and *tra-2(q122gf)* II. To measure recombination in oocytes: *dpy-18* hermaphrodites were crossed with CB4856 males to obtain non-DPY heterozygous N2/Hawaiian hermaphrodites. These F_1 progeny were crossed to *unc-47::GFP* (X) males and GFP-positive, L4 hermaphrodites were collected, individually plated, grown to starvation, and harvested for genomic DNA according to established protocols (Wicks *et al.* 2001). The F_1 animals were plated individually and clonally expanded prior to isolation of genomic DNA. This expansion step was necessary to obtain the quantities of DNA needed for genotyping multiple SNPs, but does not change the representation of each SNP in the lysate (Wicks *et al.* 2001).

For recombination rates from male sperm, Bristol N2 hermaphrodites were crossed with Hawaiian males. The heterozygous male offspring were crossed with *dpy-18* hermaphrodites and non-DPY L4 cross progeny were grown for genomic DNA as described above. All crosses were done at the temperatures being tested: 16°, 20°, or 23°. For both oocytes and male sperm, the L4 cross progeny collected were from the first 4–4.5 days of egg laying. During this time >95% of all eggs are laid.

To determine whether there is a difference between the genetic and SNP map of chromosome III, we assayed markers that span 96% of the chromosome by crossing *unc-45 dpy-18* hermaphrodites to Hawaiian or N2 males, collecting non-Unc non-Dpy cross progeny. These heterozygous cross progeny were selfed and transferred every 2 days until the extinction of egg laying. All progeny were scored for wild-type, Dpy, Unc, and Dpy Unc phenotypes. Total progeny and map size were calculated according to Brenner (1974).

For analysis of recombination rates in oocytes of young and old hermaphrodites, crosses were set up as described above, but the hermaphrodites were moved to new plates 24 hr after the onset of egg laying (as ascertained by visual inspection). These plates became the source of the day 0/1 samples. On day 6, fresh males were provided to increase progeny production (Hughes *et al.* 2007). Adults were removed after 24 hr and this plate of progeny became the day 6/7 samples. Since very few eggs are laid in both of these time periods, ~15 crosses with four hermaphrodites and seven males each were set up to enable the collection of sufficient cross progeny. For females, we used *tra-2(q122gf)* females instead of *dpy-18* hermaphrodites in the first set of crosses. Lysates were made from hermaphrodite cross progeny of the second cross: *tra-2/Hawaiian* females *X GFP*$^+$ *(X)* males.

SNP analysis: Most polymorphisms were analyzed by real-time PCR procedure of Wang *et al.* (2005) with slight modi-

TABLE 1

Recombination frequencies and map size for chr III

	Segregation from *unc-45 dpy-18/+ +*			Segregation from *dpy-18 unc-64/+ +*		
Genotype	Recombination frequency	Map size (MU)	χ^2	Recombination frequency	Map size (MU)	χ^2
N2/N2	3318/9593	44.5	0.4*	580/4421	14.1	0.2*
N2/Hawaiian	2243/6572	43.6		531/3969	14.4	

*$P > 0.5$.

fications described below. In brief, allele-specific PCR primers were designed with unique 6-mer or 14-mer GC-rich tails to discriminate PCR products on the basis of differences in melting temperature. Primers for real-time PCR were designed using the *C. elegans* SNP database (http://genome.wustl.edu/genome/celegans/celegans_snp.cgi) based on described specifications (Wang *et al.* 2005). For a list of all primers used, see supplemental Table 1.

Real-time PCR mixes were as follows: 1 μl of lysate to 14 μl of PCR mix (0.3 μl of each primer (10 mm), 1.5 μl real-time buffer (0.1 m Tris pH 8.0, 0.4 m KCl, 0.25 m $MgCl_2$), 0.075 μl 10 mm dNTPs, 0.3 μl 10× SYBR Green, 0.3 μl Rox reference dye, 2.0 μl 25% dimethyl sulfoxide (DMSO), 0.37 μl 100% glycerol, 5.88 μl H_2O and 2.0 μl Stoffel conjugate (1.8 μl 10× Stoffel buffer and 0.2 μl AmpliTaq DNA polymerase, Stoffel fragment). PCR reaction setup was done in 96-well low-profile multiplates and sealed with Microseal "B" adhesive film. Product was initially heat activated at 95° for 12 min and followed by 40 cycles of DNA amplification (20 sec at 95°, 1 min at 60°, and 30 sec at 72°) in a MT Research PTC-225 Peltier thermal cycler. Melting curve analysis was performed from 70° to 95° using the DNA engine Opticon continuous fluorescence detector. For primer sets chr III P20 and chr X P5, dilution buffer for JumpStart *Taq* antibody, JumpStart *Taq* antibody, and AmpliTaq DNA polymerase, Stoffel fragment in the ratio of 8:4:1 was incubated at room temperature for 10 min before 2 μl of conjugate were added to the PCR mix. For primer sets chr III P1, chr III P6, and chr III P16, a 5:1 ratio of Hawaiian forward primer to N2 forward primer was used in the reaction. Primers from cosmid W06F12 (Davis *et al.* 2005) at physical position 13.72 Mb were used in lieu of chr III P20 for the aging analysis.

Statistical analysis: Because the analysis is based on SNP mapping, all crossover frequencies were calculated using raw data. Chi-square tests were performed to test for significant changes in frequency and position of crossovers between sexes and temperature. Whole chromosomal map units (MU) for each sex and temperature were calculated using the formula MU = (no. of single crossovers (SCOs) + 2(no. of DCOs))/sample size × 100. MU for specific intervals in a chromosome were calculated using the formula MU = no. of COs in interval/no. of COs in chromosome X MU for chromosome.

To calculate interference, we first calculated the coefficient of coincidence (COC) for any two intervals using the formula COC = observed no. of DCOs/expected number of DCOs. Interference (I) was calculated as I = 1 − COC. The expected number of DCOs for any two intervals was calculated as

$$E(\text{DCO}) = \left(\frac{(\text{MU for interval 1}) \times (\text{MU for interval 2})}{100}\right) \times \text{sample size}.$$

Class I has two crossovers that occur in interval 0.22–5.43 Mb and 5.43–13.44 Mb, respectively. Class II has two crossovers that occur in interval 6.64–10.54 Mb and 10.54–13.44 Mb, respectively. Class III has two crossovers that occur in interval 0.22–1.33 Mb and 1.33–3.92 Mb, respectively. The MU used to calculate *E*(DCO) for all classes and temperatures are summarized in supplemental Table 2.

RESULTS

Chromosome-wide mapping of recombination in *C. elegans*: We measured crossover frequencies on chr III from male sperm and hermaphrodite oocytes at three different growth temperatures, 16°, 20°, and 23°. *C. elegans* grow optimally at 20° (Lewis and Fleming 1995). Fecundity at higher or lower temperatures is decreased, although significant numbers of progeny are attained at 16° and 23°. This contrasts with 13° and 26° at which *C. elegans* growth and fecundity are significantly impacted (Hirsh *et al.* 1976). We reasoned that these temperatures would allow us to examine how temperature and sex affect recombination without the confounding effect of severe stress to the organism. Heterozygotes of the two polymorphic wild-type strains were outcrossed to marked (N2) strains to obtain cross progeny. These animals are genotyped to determine whether they have acquired any of the Hawaiian strain polymorphic markers from the heterozygous sperm or oocyte (see materials and methods).

We found that the method of genotyping with Tm-shift primers (Wang *et al.* 2005) was amenable to large-scale genotyping in 96-well plates and was cheaper than other methods described for *C. elegans* (Wicks *et al.* 2001). This method requires PCR amplification with three primers, a common primer for both N2 and Hawaiian and two allele-specific primers, one of which is designed with a 6-mer tag, the other with a 14-mer tag. The PCR products are quantified in a real-time PCR machine, which can discriminate the melting temperatures of the two products and which gives a readout of corresponding peaks. This method can be adapted for use at almost any polymorphism and thus we were able to design primers that extended close to the ends of the chromosome.

Previous studies determined that strain polymorphisms between Bristol (N2) and Hawaiian did not interfere with crossover formation for a small region of the X chromosome (Wicks *et al.* 2001). We confirmed that this held true for the whole of chr III using genetic markers that span ~93% of the chromosome (Table 1). Thus, we have confirmed that the differences between

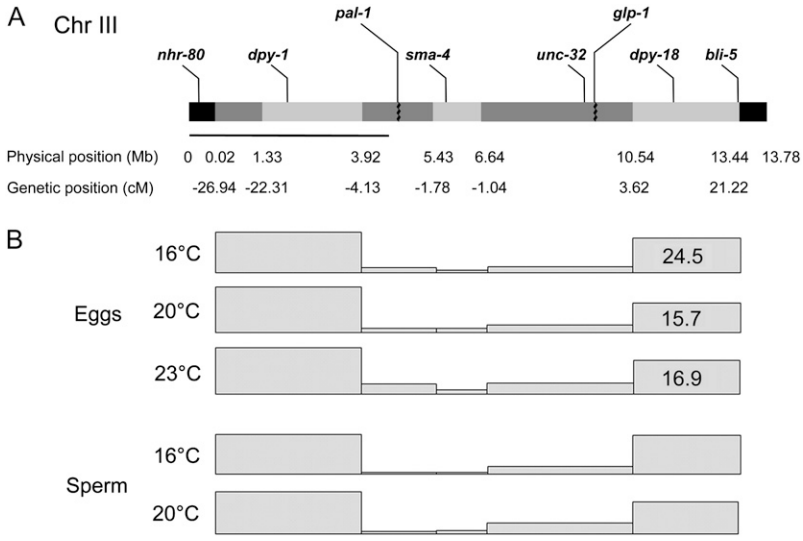

FIGURE 1.—Positions of crossovers on chr III differ with sex and temperature. (A) Superimposition of the physical and genetic map of chr III. The locations of the genetic markers across the chromosome are shown above the chromosome. *pal-1* and *glp-1* mark the ends of the central, gene-rich cluster. The physical markers (Mb) that we used in this study are shown below the chromosome with different colored shading demarcating the regions analyzed. The pairing center is demarcated by the line under the chromosome. (B) Single and double crossover positions have been mapped to five intervals on chr III. The size of the genetic map on the basis of crossover distribution is depicted by shaded squares. The map size for intervals that differ significantly with temperature are written in the respective boxes.

the Bristol and Hawaiian strains, which include single nucleotide polymorphisms, insertions, and deletions, do not interfere with recombination between the strains.

An overlay of the genetic and physical maps of chr III with the marker positions used in this study is shown in Figure 1A. The physical markers used in this study encompass 96% of chr III, more than any of the previous studies (ZETKA and ROSE 1990; MENEELY *et al.* 2002; DAVIS *et al.* 2005; HAMMARLUND *et al.* 2005). Given the repression near the telomeres (see below), the domains that we analyzed actually harbor 99% of all crossovers. We attribute our ability to detect a significant number of DCOs to the comprehensive coverage of the chromosome. Like all the *C. elegans* autosomes, chr III has a central gene-rich cluster that is recombinationally suppressed. This cluster, which extends from near the *pal-1* gene at 4.81 Mb to *glp-1* at 9.09 Mb, occupies only 2.61 cM of the genetic map. This is flanked on both sides by ~4.7 Mb of gene-poor sequence, which has elevated recombination and encompasses ~24 cM on each side (WICKS *et al.* 2001). Thus, the rates of recombination on the arms *vs.* in the cluster differ almost 10-fold (~5.11 MU/Mb *vs.* ~0.54 MU/Mb, respectively). Extending from both arms toward the telomere are more gene dense regions, which are also recombinationally active (WICKS *et al.* 2001). Since telomeric sequences in other systems are known to repress recombination (BLITZBLAU *et al.* 2007), we examined the 60–360 kb from the right end of chr III. This region has 6-fold fewer crossovers than expected (supplemental Table 3), supporting the conclusion that the telomeres establish a domain repressive for crossover formation. Further analysis of the recombination data for the interval 20–500 kb from the IIIL telomere between *par-2* and *unc-45* (http://www.wormbase.org) revealed that this interval is also recombinationally suppressed. Thus, it appears that telomeric suppression of recombination is conserved in *C. elegans*. The organization of the autosomes into gene-rich cluster and gene-poor arms is poorly understood.

Sex-specific difference in genetic map: Our data for crossover frequencies on chr III are shown in Table 2. We obtained a map size of ~54 MU in oocytes and ~52 MU in sperm at 20°. Our results are in good agreement with previous analyses with regard to the overall map size

TABLE 2

Effects of temperature on crossover frequencies in eggs and sperm on chr III

		Noncrossover		Single crossover		Double crossover			
		No.	%	No.	%	No.	%	Total	Map size (MU)
Eggs	16°	215	44.0	272	55.6	2	0.4	489	56.4
	20°	297	47.1	325	51.6	8	1.3	630	54.1
	23°	121	46.5	131	50.4	8	3.1	260	56.5
Sperm	16°	286	54.9	232	44.5	3	0.6	521	45.7
	20°	91	50.6	85	47.2	4	2.2	180	51.7
	23°	223	49.0	225	49.5	7	1.5	455	52.5

We observed an overall difference in the genetic map size of chr III between sperm developed at 16° and 23° [$\chi^2(1, N = 462) = 8.41$, $P < 0.005$] and between eggs and sperm developed at 16° [$\chi^2(1, N = 524) = 24.14$, $P < 0.005$].

TABLE 3

Crossover distribution on chr III

		Interval (Mb)				
		0.02–3.92	3.92–5.43	5.43–6.64	6.64–10.54	10.54–13.44
Eggs	16°	29.4 (144)	0.8 (4)	0.2 (1)	1.4 (7)	24.5 (120)
	20°	34.9 (220)	0.6 (4)	0.5 (3)	2.4 (15)	15.7 (99)
	23°	34.6 (90)	1.9 (5)	0.4 (1)	2.7 (7)	16.9 (44)
Sperm	16°	22.1 (115)	0.2 (1)	0.2 (1)	2.5 (13)	20.7 (108)
	20°	22.2 (40)	0.6 (1)	0.6 (1)	1.1 (2)	27.2 (49)
	23°	27.2 (124)	0.2 (1)	0.4 (2)	4.0 (18)	20.6 (94)

Numbers shown are MU (no. of crossover in interval). No statistical differences between sperm at any temperature are observed except for eggs developed at 16° and 20° [$\chi^2(5, N = 276) = 29.55$, $P < 0.005$] and for eggs developed at 16° and 23° [$\chi^2(5, N = 147) = 16.34$, $P < 0.01$].

and interference levels in the hermaphrodite (Zetka and Rose 1990, 1995; Meneely *et al.* 2002). However, the similarity between the oocyte and male sperm maps at 20° (and 23°) contrasts with previous studies (Zetka and Rose 1990; Meneely *et al.* 2002) which observed a significantly smaller genetic map in sperm (~31 MU). The differences between our data and those of Zetka and Rose (1990) can be explained if different autosomes have dramatically different map sizes, as they analyzed chr I and we have analyzed chr III. Differences in the recombination rates between autosomes have been observed in *C. elegans*, albeit in mutant backgrounds with altered recombination rates (Hodgkin *et al.* 1979; Carlton *et al.* 2006; Tsai *et al.* 2008). The differences between our observations and those of Meneely *et al.* (2002) can be explained by the size of their data set. A random sampling of even 90 animals can falsely lead to an inaccurate map if that set of animals were predominantly nonrecombinant (smaller map) or recombinant (expanded map). We observed such skewing during the course of data collection. Alternatively, the difference between this study and others may be explained by position and coverage of the markers used.

Previous studies of crossover frequencies in oocyte and male sperm observed more recombination as temperatures were raised. We wanted to determine whether the temperature-dependent changes were due to an effect on the overall frequency of crossing over, which is reflected in the size of the genetic map. Therefore, we compared the genetic map of chr III from oocytes and sperm at 16°, 20°, and 23° (Table 2). The sperm developed at 16° had the smallest map size (45.7 MU). This differed significantly from sperm at 20° and 23° (51.7 and 52.5), indicating that sperm respond to higher temperatures by increasing the total number of crossovers per chromosome.

The temperature-dependent changes in map size observed in male sperm are not seen in oocytes. Rather, it appears that the genetic map is stable in oocytes, 56.4 MU at 16° compared to 56.5 MU at 23°. At the higher temperatures, this map size is not statistically different from that in sperm, suggesting the two germ lines may regulate recombination frequencies in the same manner at these temperatures. The differences between the genetic maps of oocytes and sperm at 16°, however, suggests that at the lower temperature the mechanism for regulating recombination frequencies is different between the germ lines. Thus, the change in the genetic map in sperm and not in oocytes suggests that recombination rates in the two germ lines respond differentially to changes in temperature.

Temperature globally affects recombination in sperm: The observed temperature-dependent change in the map size of male sperm (45.7 MU at 16° to 52.5 MU at 23°) could be due to a chromosome-wide effect on crossover formation, to the local activation of a recombination hotspot at higher temperatures, or to the formation of a coldspot at lower temperatures. The latter two models predict that the changes in recombination frequency could be mapped to a single chromosomal domain whereas the former predicts that the temperature-dependent changes would be shared across the chromosome.

To distinguish between these alternative models, we assayed whether crossover position changes in response to temperature by mapping the positions of all single and double crossovers from our SNP analyses (Figure 1, Table 3). As described previously (Barnes *et al.* 1995), we also observed that the middle of chr III is repressed for recombination and the terminal ~4 Mb of each chromosome share ~90% of all crossovers at 20°. Comparison of five different intervals showed no statistically significant change in crossover distribution between sperm at all three temperatures. However, we noted that some intervals could account for the increase in map size more than other intervals. For example, the interval between 0.02 and 3.92 Mb shows a difference of ~5 MU ($P < 0.07$) between 16° and 23°. A larger sample size could resolve whether the bias in this region is biologically relevant. Nevertheless, since the overall pattern of recombination is the same in sperm at all temperatures, we suggest that the increased

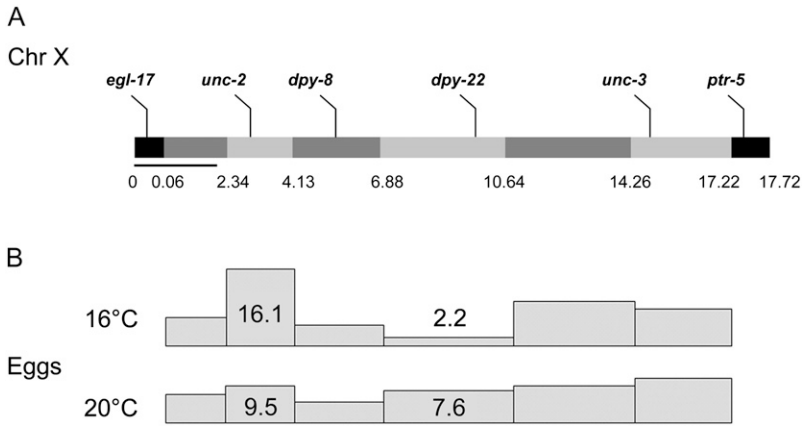

FIGURE 2.—Positions of crossovers on the X chromosome differ with temperature. (A) Superimposition of the physical and genetic map of the X chromosome. The locations of the genetic markers across the chromosome are shown above the chromosome. The physical markers (Mb) that we used in this study are shown below the chromosome with different colored shading demarcating the regions analyzed. The pairing center is demarcated by the line under the chromosome. (B) Single and double crossover positions have been mapped to six intervals on X for oocytes at 16° and 20°. The relative map size for each interval on the basis of crossover distribution is depicted by shaded squares. The map size for intervals that differ with temperature are written in the respective boxes.

map size at 20° and 23° is due to a global effect on the chromosome.

Crossover distribution changes with temperature in oocytes: Although we observed no temperature-dependent change in the map size in oocytes, previous work had suggested that recombination frequencies in some chromosomal intervals increased with temperature (ROSE and BAILLIE 1979). We reasoned that chromosomal domains may be differentially sensitive to changes in temperature so that a loss of crossovers in one domain would appear to be compensated for by an increase in other domains. Thus, we examined crossover positions from oocytes reared at 16°, 20°, and 23°. In contrast to sperm, oocytes display dramatic changes in crossover distribution in response to temperature (Figure 1, Table 3). Crossovers are nearly equally distributed on the right and left ends of the chromosome in oocytes developed at 16°, with a slight bias toward the right end (8.4 MU/Mb on IIIR *vs.* 7.9 MU/Mb on IIIL). In contrast, at 20° and 23°, almost two-thirds of all crossovers on chr III occur near the left end of the chromosome. These results suggest that in *C. elegans* oocytes chromosomal domains may be differentially sensitive to changes in temperature.

The temperature-dependent differences in crossover distribution could be explained by the activation of coldspots or hotspots (depending on the reference temperature). To determine whether a coldspot/ cold domain is activated on the right end of the chromosome at temperatures >16° (interval 10.54–13.44 Mb), we further analyzed the crossover distributions in each of the four other intervals on chr III. Since the map size of the chromosome does not change with temperature, the crossovers that are lost by activation of a coldspot should be redistributed proportionally to the remainder of the chromosome. In effect, the rest of the chromosome would become "hotter" with each interval receiving a proportionate increase in the crossovers relative to the map at the lower temperature. When we analyzed the oocyte 20° and 23° data in this manner, they revealed that crossovers are redistributed across the chromosome with values near to proportional (see supplemental Table 4). Therefore, it seems likely that the domain at the right end of chr III is repressed for crossover formation at higher temperatures.

Changes in crossover distribution are a general feature of *C.elegans* chromosomes in oocytes: The loss of crossovers on the right end of chr III and redistribution toward the left end is striking because the pairing center (PC) localizes to the left end of the chromosome (see Figure 1). The PC is a specialized chromosomal domain required to both establish and maintain homolog alignment. Synapsis starts at the PC and proceeds processively down the chromosome. One tantalizing possibility for the shift toward the pairing center is that crossovers adjacent to the PC could stabilize homolog interactions that would be more labile at higher temperatures. To determine whether temperature-dependent changes in crossover distribution are a general feature of all chromosomes and influenced by the PC, we extended our studies to the X chromosome. The sex chromosome has several features that distinguish it from autosomes, including differences in transcriptional states in the germ line, recombination patterns, chromatin organization, and gene distribution (GOLDSTEIN 1982; ZETKA and ROSE 1990; GARVIN *et al.* 1998; REINKE *et al.* 2000; KELLY *et al.* 2002). Figure 2A shows the genetic and physical maps with the marker positions used in the study. The PC is on the left end of the X chromosome. Our analysis of X chromosome recombination focused on oocytes developed at 16° and 20° since the changes we observed in recombination distribution on chr III occurred only in oocytes between these two temperatures (Figure 1B).

Similar to previous observations, crossovers on the X chromosome are more uniformly distributed across the chromosome (BARNES *et al.* 1995; Figure 2B, Table 5), reflecting the inherent differences in the genetic map between the X and autosomes (BRENNER 1974). As with chr III, the map size of the X chromosome in oocytes remains the same at different temperatures, ~51 MU (Table 4). Also similar to chr III, X chromosome crossover distribution changes with temperature. We observed a cold region from 2.34–4.13 Mb (Figure 2B, Table 5).

TABLE 4

The effects of temperature on crossovers on the X chromosome in eggs

		Noncrossover		Single crossover		Double crossover			Map size
		No.	(%)	No.	%	No.	%	Total	(MU)
Eggs	16°	130	47.6	141	51.7	2	0.7	273	52.4
	20°	180	48.9	188	51.1	0	0.0	368	51.1

No difference between samples: $\chi^2(1, N = 275) = 0.31$, $P > 0.5$.

However, the increases in crossovers on the rest of the chromosome to compensate for this cold domain were not proportional as on chr III. Instead there appears to be a bias toward the 6.88- to 10.64-Mb interval, which is further away from the X chromosome PC (Figure 2, supplemental Table 5). Although we cannot rule out that the X chromosome and autosomes might regulate crossovers differently, this result suggests that it is unlikely that the PC regulates the temperature-dependent changes in crossover distribution. Furthermore, changes in crossover distribution in oocytes are a shared feature of multiple chromosomes and the underlying mechanism appears to operate on large chromosomal domains.

Regulation of double crossovers differs between the sexes: Domain specific regulation of crossovers has been proposed to explain recombination maps in multiple systems (International Hapmap Consortium 2007; Coop *et al.* 2008; Fukuda *et al.* 2008). One of the governing principles for crossover regulation in most systems is CI. Previous studies have suggested that CI is almost 100% in *C. elegans* (Hodgkin *et al.* 1979; Hillers and Villeneuve 2003), meaning that one and only one crossover occurs on each chromosome. When multiple crossovers are seen, they follow the "rules" of CI in mapping to distant chromosomal domains (Hillers 2004). Since we were able to screen >96% of chr III, we observed an unprecedented number of DCOs in both oocyte and sperm. As shown in Table 2, we observed as many as 3.1% of all crossovers manifest as DCOs on chr III in oocytes when hermaphrodites were raised at 23° and almost 1.5% of crossovers as DCOs in sperm. We note that due to the rare occurrence of these events within such a large sample size that we cannot conclude whether the number of DCOs achieved at any temperature between or within germ lines is statistically significant. Nevertheless, the appearance of DCOs in our assays allowed us to ask whether CI is regulated in the same fashion in the two germ lines.

CI can be reflected in two components: the number of crossovers per chromosome and the position of crossovers (the crossover landscape). When we examined crossover position along the chromosome, we observed that DCOs in sperm can be more closely spaced than in oocytes (Figure 3, Fisher's exact test, $P < 0.02$). At least 4 of the 14 mapped DCOs in sperm occurred in adjacent intervals (classes II and III, as summarized in Figure 3), whereas no such DCOs were observed in oocytes (Figure 3). Instead, in oocytes, all DCOs fell into class I, with one crossover mapping to each half of the chromosome. Thus, values for interference along the chromosome are lower in sperm (Table 6). These results reinforce that recombination is regulated differentially in oocytes and sperm and suggests that the mechanisms that control crossover position along the chromosome can be regulated independently of the mechanisms that determine crossover number.

Almost all instances of DCOs are rare, with different chromosomes showing different levels of CI. In our experiments, chr III is more permissive to DCOs than the X (compare Table 2 to Table 4), which is in line with previous studies (Brenner 1974; Hodgkin *et al.* 1979; Zetka and Rose 1990; Meneely *et al.* 2002). Further, recent analysis of chr IV suggests that ~10% of all recombinants had DCOs (J. Henzel and K. Hillers, unpublished data). It is intriguing to speculate that the differences between the X and the autosomes may reflect the inherent differences in the chromatin organization of these chromosomes. In the germ line, the X is maintained in a predominantly silent, heterochromatic state (Kelly and Fire 1998; Kelly *et al.* 2002; Bender *et al.* 2004, 2006) whereas the autosomes are transcriptionally active. Although there is no known functional link between the transcriptional state and recombination in *C. elegans* as in other organisms (Nicolas 1998; Coop *et al.* 2008), it may be that the

TABLE 5

Crossover distribution on the X chromosome

		Interval (Mb)					
		0.06–2.34	2.34–4.13	4.13–6.88	6.88–10.64	10.64–14.26	14.26–17.22
Eggs	16°	8.1 (22)	16.1 (44)	6.6 (18)	2.2 (6)	10.6 (29)	9.5 (26)
	20°	7.3 (27)	9.5 (35)	6.5 (24)	7.6 (28)	9.8 (36)	10.3 (38)

Numbers shown are MU (no. of crossovers in interval). The change in crossover positions with temperature is statistically significant: $\chi^2(6, N = 141) = 23.35$, $P < 0.005$.

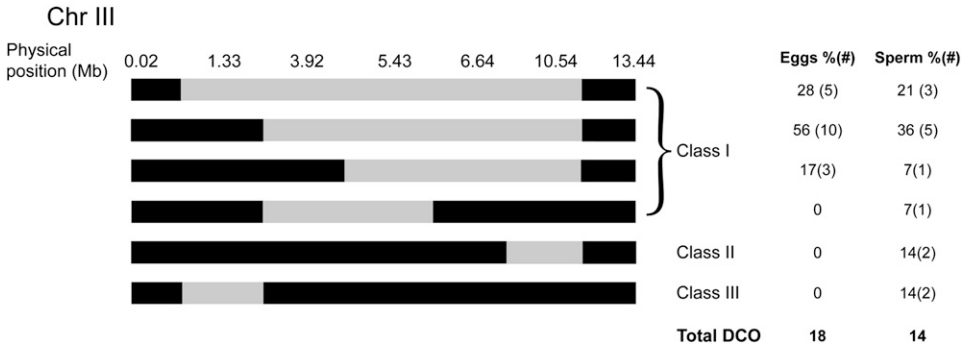

FIGURE 3.—Positions of double crossovers on chromosome III. The physical markers analyzed are shown at the top. The patterns of observed DCOs are indicated by the altered shadowing in each graphic. The percentage (and number) of DCOs with each pattern in oocyte and male sperm are shown at right. Classes of crossovers are used to calculate interference as described in the MATERIALS AND METHODS.

chromatin status regulates both transcriptional activity levels and crossover levels.

The effect of age on oocyte recombination: The observations described above suggest that crossover distribution is influenced by growth environment. Growth at high or low temperatures is thought to exert stress upon the organism, which responds not only by altering patterns of recombination (shown here, and (ROSE and BAILLIE 1979) but also by affecting the aging process and the ability to respond to other stresses (KLASS 1977; LITHGOW *et al.* 1994). The links between the stress and aging pathways led us to reexamine whether the effects of age on recombination parallel the effects of temperature. Previous experiments analyzing chr I in hermaphrodites suggested that recombination rates decline dramatically with age in *C. elegans* (ROSE and BAILLIE 1979). However, changes either in the total number of crossovers (the genetic map size) or in the position of the crossovers could have led to the observed decrease in crossing over in the intervals tested.

Since SNP analysis allows us to simultaneously address crossover frequency and position for a given chromosome, we were able to determine whether the number or the pattern of recombination is changed in young *vs.* old mothers. *C. elegans* are fertile for almost 9 days under ideal growth conditions and good mating (HUGHES *et al.* 2007). During the first 24 hr of egg laying (days 0/1) at 20°, ~2% of eggs are laid; during days 6/7, <1% of eggs are laid (J. LIM and J. YANOWITZ, unpublished data). We thus compared day 0/1 to day 6/7 at the onset and decline of fertility. As shown in Table 7, the map size in young and old oocytes is statistically indistinguishable, 50.8 *vs.* 48.2 MU in hermaphrodites. Thus, at least on *C. elegans* chr III, age-related changes in nondisjunction are not due to the inability to establish crossovers.

When crossover positions from young and old mothers were analyzed, a dramatic change in crossover position was observed. The oocytes from young mothers showed a pattern of recombination similar to that described above for sperm at 20° with an equal distribution of crossovers to the left and right ends of chr III (Figure 4, Table 8). In the oocytes from old mothers, the pattern appeared more representative of the hermaphrodite pattern of crossovers at 20° (described above, Figure 1) with over two-thirds of crossovers occurring on the left end of the chromosome (Figure 4, Table 8). This altered crossover distribution with age may reflect an inherent change in the regulation of recombination in aging oocytes. For example, age-dependent changes in chromatin organization could affect accessibility or activity of the Spo11 cleavage complex. Alternatively, the change in crossover distribution may be due to the switch from the sperm mode to the oocyte mode of development in the hermaphrodite germ line early in development.

To distinguish between these possibilities, we reexamined the aging phenomenon in *C. elegans* females—XX hermaphrodites whose germ line has been feminized by

TABLE 6

Interference between sexes and temperatures

		Class I[a]			Class II[a]			Class III[a]		
		Expected DCO	Observed DCO	Interference	Expected DCO	Observed DCO	Interference	Expected DCO	Observed DCO	Interference
Eggs	16°	39	2	0.9	2	0	1.0	—	—	—
	20°	42	8	0.8	2	0	1.0	—	—	—
	23°	19	8	0.6	1	0	1.0	—	—	—
Sperm	16°	27	1	1.0	3	1	0.6	5	1	0.8
	20°	12	4	0.7	1	0	1.0	1	0	1.0
	23°	31	5	0.8	4	1	0.7	5	1	0.8

[a] Refer to Figure 3 and MATERIALS AND METHODS for descriptions of classes.

TABLE 7

Effects of age on crossover frequency on *C. elegans* chr III

		Noncrossover		Single crossover		Double crossover			Map size
		No.	%	No.	%	No.	%	Total	(MU)
Hermaphrodite	Day 0/1	230	49.5	234	50.3	1	0.2	465	50.8
	Day 6/7	193	52.3	174	47.2	2	0.5	369	48.2
Female	Day 0/1	146	52.3	133	47.7	0	0.0	279	47.7
	Day 6/7	133	48.4	136	49.5	6	2.2	275	53.8

a dominant gain-of-function allele of *tra-2*. No statistical differences in map size or crossover distribution were seen between hermaphrodites and female oocytes at either age (Tables 7 and 8, Figure 4). Thus, the change in crossover distribution between young and old oocytes suggests that the first oocytes laid fundamentally differ from older oocytes.

DISCUSSION

We have used a set of SNPs spanning >96% of *C. elegans* chr III to study how temperature, sex, and age affect recombination rates. We show that the germ lines respond to temperature differently. In male sperm, temperature alters the ratio between noncrossover and crossover chromosomes; whereas in oocytes, the position of crossovers changes with temperature. Further, the two germ lines show differential crossover interference. Male sperm have DCOs that are spaced more closely, often in adjacent intervals. These results suggest that different pathways regulate how each germ line affects crossover frequencies.

Differences in recombination rates between sexes has been reported in many systems, but the extent and direction of the difference are species specific (Lenormand and Dutheil 2005). Recently, genomic mapping of humans and mice has led to the conclusion that differences between males and females occur at all genomic scales (International Hapmap Consortium 2007; Coop *et al.* 2008). Globally, differences in chromatin loop organization have been suggested to explain differences in CI between the sexes (Petkov *et al.* 2007). More locally, differences in hotspot usage can explain variation between individuals of both sexes (Coop *et al.* 2008). In *C. elegans*, we observe differences in CI between the sexes, suggesting that CI may be a linchpin for sexual dimorphism in recombination.

We also observed that the sexes respond differently to varying environmental conditions. Although temperature and stress have long been known to affect recombination rates (Plough 1917; Mavor and Svenson 1923), this is the first study to directly compare chromosome-wide recombination rates for both sexes under different growth conditions. We observed that the size of the genetic map changes in male sperm in response to temperature. The increased frequency of crossovers at higher temperatures is not due to the activity of a recombination hotspot, as the distribution of crossovers across the chromosome was strikingly similar at all temperatures analyzed. Rather, one possible explanation is that the complexes that exert a choice between the crossover and noncrossover pathways may be regulated differently in each germ line by temperature. For example, the components required for the crossover pathway may be more highly expressed in sperm at higher temperatures, thereby increasing the number of these complexes on chromosomes. Alternatively, this complex may be more stable or more active at higher temperatures. However, since we know little about how the noncrossover/crossover decision is made, particularly in *C. elegans*, distinguishing between these possibilities will need to await further characterization of these pathways.

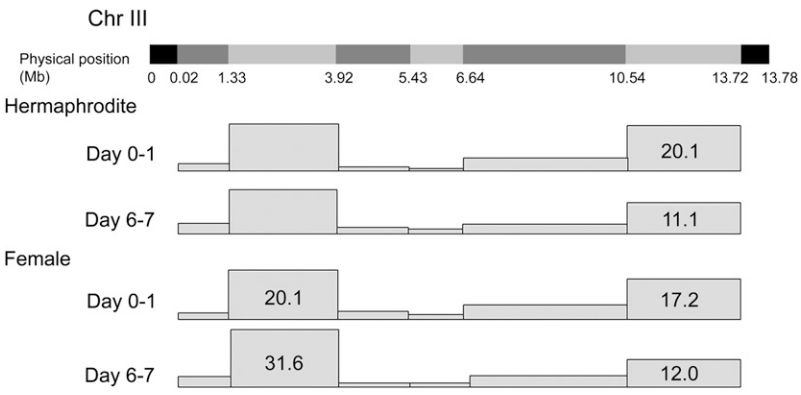

Figure 4.—Effects of aging on recombination in oocytes. Physical markers on chromosome III are shown at the top. (Bottom) A pictograph of the map size for each interval for oocytes from the first 24 hr of egg laying (day 0/1) to near the final days of egg laying (day 6/7).

TABLE 8

Crossover distribution in aging worms on chr III

		Chr III interval (Mb)					
		0.02–1.33	1.33–3.92	3.92–5.43	5.43–6.64	6.64–10.54	10.54–13.72
Hermaphrodite	Day 0/1	4.5 (21)	21.4 (100)	0.9 (4)	0.2 (1)	3.4 (16)	20.1 (94)
	Day 6/7	6.9 (26)	24.6 (93)	1.9 (7)	0.5 (2)	2.1 (8)	11.1 (42)
Female	Day 0/1	3.9 (11)	20.1 (56)	1.4 (4)	0.4 (1)	4.7 (13)	17.2 (48)
	Day 6/7	5.8 (16)	31.6 (87)	0.7 (2)	0.7 (2)	2.9 (8)	12.0 (33)

Numbers shown are MU (no. of crossover in interval). The change in crossover positions with age is statistically significant for hermaphrodite eggs [$\chi^2(6, N = 178) = 30.83$, $P < 0.005$] and female eggs [$\chi^2(6, N = 133) = 26.19$, $P < 0.005$].

One of the most striking observations that we made is that crossover positions change dramatically in oocytes in response to temperature and age (Figures 2, 3, and 4). Since earlier studies used genetic markers to map recombination in specific intervals, they would not have been able to detect a change in the pattern of crossing over along the chromosome (Rose and Baillie 1979; Zetka and Rose 1990). Consequently, region-specific differences would have been seen as increases or decreases in crossover frequencies. Thus, we believe that the apparent differences between our data and others can be resolved by invoking a global mechanism for chromosome-wide changes in crossover distribution in response to temperature, age, and sex.

The regulation of crossovers in large chromosomal domains is shared across many organisms, including humans (International Hapmap Consortium; Coop *et al.* 2008), suggesting that the mechanisms that establish these domains may be conserved. We envision that domain-specific regulation of crossovers is mediated by large-scale chromatin packaging mechanisms. Recent work from the Meyer lab (Tsai *et al.* 2008) has suggested that components of the *C. elegans* dosage compensation complex, which resemble the condensin complex, play important roles in regulating crossover interference and crossover position. In the *dpy-28* mutant, they observed an increase in crossovers in the same domain where we observed a temperature-dependent increase in crossovers on the X chromosome (Figure 2). It will be interesting to determine whether we could observe differences in condensin complex localization at different temperatures. Recent work in *Saccharomyces cerevisiae* identified condensin binding sites every 10–50 kb—which is two orders of magnitude more frequent than crossovers (Wang and Strunnikov 2008). Thus, additional levels of regulation would need to be imposed on condensin (or any of the known chromatin complexes) to regulate a chromosome-wide phenomenon at megabase distances.

At the center of an emerging picture for higher order chromosome organization are the nuclear envelope and lamina. During meiosis, attachment of telomeres or PCs to the nuclear periphery facilitates pairing (Harper *et al.* 2004; Scherthan 2007) and their release promotes proper meiotic progression (Chikashige *et al.* 2006). The nuclear lamina has been shown to interact with numerous transcription and replication factors, chromatin-associated proteins, and RNA processing proteins and accordingly is thought to play a central role in organizing hetero- *vs.* euchromatin (reviewed in (Sexton *et al.* 2007; Wagner and Krohne 2007). Recently, the association between the nuclear envelope and DNA silencers in Drosophila (Dorman *et al.* 2007) suggested that chromatin loops may be organized into larger domains by association with the nuclear periphery. We find it tempting to speculate that such foci at the nuclear periphery could establish crossover domains.

In humans, it has been suggested that the more distal crossovers are responsible for the increase in nondisjunction rates as women age (Hassold *et al.* 2000). Our data suggest in *C. elegans*, crossover position does not contribute significantly to the increased nondisjunction frequencies. Rather, we suggest that other molecular pathways play a more fundamental role in age-related meiotic dysfunction. These may include the integrity of the spindle checkpoint (Lacefield and Murray 2007), or the strength of cohesion (Hodges *et al.* 2005), or as yet unknown pathways that act on the homologs at late meiotic stages.

Our data show that the major difference in crossover regulation in *C. elegans* oocytes is seen between the youngest mothers (first day of egg laying) and the oldest mothers. Recent observations have shown that these first oocytes transit more rapidly through prophase I than older oocytes (Jaramillo-Lambert *et al.* 2007). Together, these data suggest that *C. elegans* young adults may represent a juvenile period of development that was heretofore unappreciated. It will be interesting to determine whether the changes in cell cycle progression are responsible for the differences in crossover distribution.

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