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Crossover interference underlies sex differences in recombination rates

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In many organisms, recombination rates differ between the two sexes. Here we show that in mice, this is because of a shorter genomic interference distance in females than in males, measured in Mb. However, the interference distance is the same in terms of bivalent length. We propose a model in which the interference distance in the two sexes reflects the compaction of chromosomes at the pachytene stage of meiosis.

Introduction

Meiosis consists of two consecutive cell divisions after a single round of DNA replication, thereby ensuring reduction of the chromosome number to produce haploid gametes. This reduction occurs in the first meiotic division, when homologous chromosomes are joined together in prophase to form bivalents and eventually separate in anaphase. In mammals, higher plants and yeast, chromosome recognition and formation of the synaptonemal complex is initiated by double-strand breaks on one chromatid. These breaks are repaired by homologous recombination, leading to genetic crossing over and/or gene conversion when a non-sister chromatid is used as a template. Given the segregation of chromatids into haploid gametes, only half of the genetically recombinant chromosomes that result from molecular recombination events will be detected.

Crossover events are not randomly spaced along chromosomes. Instead, the presence of one crossover event on a chromosome reduces the possibility of a second event nearby [1–3], a phenomenon known as crossover interference. In many species, recombination rates differ in the two sexes. The female recombination map is 1.7 times longer than that of males in humans [4,5] and 1.3 times longer in mice [6]. Several mechanisms have been proposed to play important roles: haploid selection [7]; different epistatic interactions among genes expressed during male and female meiosis [8]; presence of X-linked modifiers [9]; and regional differences in the chromatin structure of male and female gametocytes [10]. However, experimental evidence in support of these suggestions has remained elusive. Here we show that crossover interference in meiosis is the main factor underlying sex differences of recombination rates, and that the average intercrossover distance is the same in both sexes when measured in micrometers of synaptonemal complex length.

Distribution of recombination events along mouse chromosome 1

Recombination rates in each sex were measured in backcrosses of C57BL/6JxCAST/EiJ F1 male and female mice to C57BL/6J. The entirety of mouse chromosome 1 (Chr 1) was examined at ~7 Mb resolution, which ensured the detection of virtually all crossovers taking into account the strong positive interference in mouse recombination [11]. In total, we detected 2715 recombination events in 2762 progeny of female F1 parents and 1509 recombination events in 1881 progeny of male F1 parents. The average recombination rates were 0.51 cM per Mb (cM/Mb) in

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Table 1. Distribution of crossover classes along mouse Chr 1 in progeny of female and male F1

		Zero crossovers	Single crossovers	Double crossovers	Triple crossovers	Average number of chiasmata per bivalent
Female	Number	742	1367	611	42	1.97
	Relative Frequency	0.27	0.49	0.22	0.015	
Male	Number	657	941	281	2	1.60
	Relative Frequency	0.35	0.50	0.15	0.001	

females and 0.41 cM/Mb in males, which corresponds to a female-to-male ratio of 1.23. In these parameters, Chr 1 did not differ significantly from the genome wide sex-averaged recombination rate of 0.55 cM/Mb and female-to-male ratio of 1.3.

There was a maximum of three recombination events on an individual chromosome in both crosses; however, the relative frequencies of single, double and triple crossovers were markedly different in male and female meiosis ($p = 10^{-16}$, by a χ^2 test). No crossovers were found in 27% of the progeny of F1 females and 35% of F1 males. Single crossovers were found in ~50% of the progeny of both sexes, which is consistent with the expectation of an obligate crossover on each chromosome (see Online Supplementary Material). The difference lay in the frequencies of multiple crossovers (Table 1). The progeny of female F1 had ~1.5 times higher frequency of double crossovers and ~14 times higher frequency of triple crossovers than the progeny of male F1. Triple crossovers were extremely rare in male meiosis; only two such events were found in all male F1 progeny compared with 42 in the progeny of females. Note that chromosomes lacking a crossover are not the product of a meiosis in which no chiasmata (the cytologically visible manifestations of crossing over) formed on Chr 1; there must be at least one chiasma on every chromosome for a successful meiosis. Because chiasmata formation involves only two of the four available chromatids, one-half of the chromosomes are non-crossover when there is a single chiasma and one-quarter when there are two. Using the data in Table 1 and assuming equal probability for chiasma formation between any two non-sister chromatids (no chromatid interference), we estimated (by maximum likelihood) that for Chr 1 in female meiosis, 11.9% of bivalents will have three chiasmata, 72.4% two chiasmata and 15.6% a single chiasma. For the male meiosis, these figures are 0.8%, 58.6% and 40.5%, respectively. It is evident that female bivalents form two or three chiasmata more frequently than male bivalents.

The distribution of recombination events along the entirety of Chr 1 showed similar trends in both female and male meioses, with elevated recombination rates

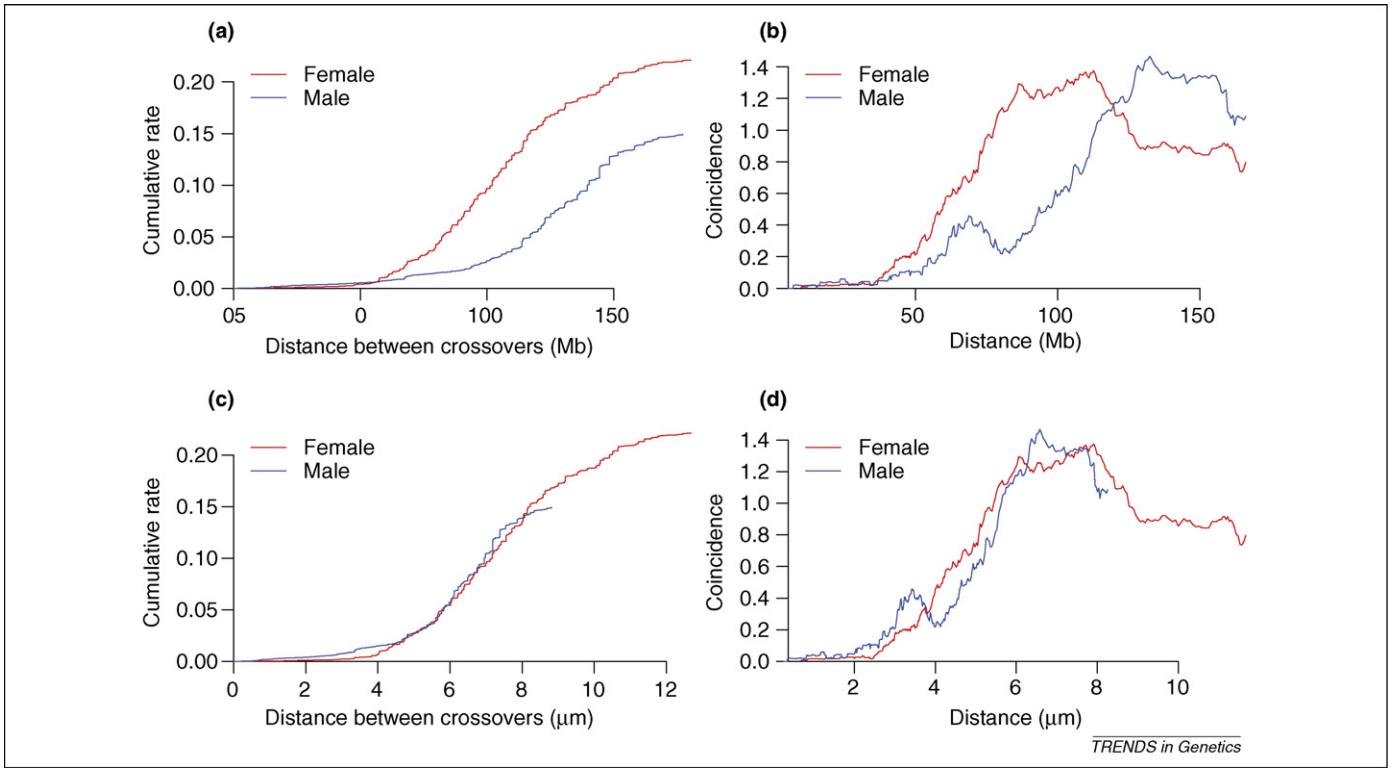

Figure 1. Interference as a function of intercrossover distances. **(a)** Cumulative rates of double crossovers in female and male meioses. Intercrossover distances are measured in megabases of DNA length. **(b)** Coefficient of coincidence as a function of distance between two crossovers in female and male meioses in a sliding window of 30 intervals. Intercrossover distances are measured in megabases of DNA length. **(c)** Cumulative rates of double crossovers in female and male meioses. Intercrossover distances are expressed in physical length of pachytene bivalents (μm), calculated from [13]. **(d)** Coefficient of coincidence as a function of distance between two crossovers in female and male meioses in a sliding window of 30 intervals. Intercrossover distances are expressed in physical length of pachytene bivalents (μm). A comparison between panels (a) and (c), as well as (b) and (d) shows that interference in both sexes is the same in terms of bivalent length (μm) rather than genomic length (Mb). In panels (a–d): female, red line; male, blue line.

around 40 Mb, 75–80 Mb, 120–130 Mb, 165–170 Mb and in the 10 Mb region near the centromere-distant telomere (Figure S1a). Relative recombination rates (cM/Mb) were higher in males, compared with females, in the centromere-distant telomeric region between 40 Mb and 80 Mb. The reverse trend was observed in most of the remaining regions of the chromosome. Although the ratio of recombination events involved in single and double crossovers did not vary along the chromosome in female meiosis (Figure S1b), this was not true in males. Except for the 10 Mb region that borders the telomere, crossovers involved in double recombinants were significantly reduced along the rest of the chromosome (Figure S1c).

Figure 1a shows the cumulative rates of double crossovers as a function of the intercrossover distance, expressed in Mb. On average, the distance between the two events was 102 Mb in females and 122 Mb in males. Only ~2% of double crossovers were spaced closer than 40 Mb apart in females and closer than 57 Mb apart in males.

The coefficient of coincidence (Z) is a traditional measure of interference [1,2]. It is expressed as a ratio of the frequency with which crossovers occur in a pair of intervals relative to the marginal frequencies for crossover events in the two intervals. Plotting the coefficient of coincidence as a function of intercrossover distance, we found substantial differences between female and male meioses (Figure 1b). In female meiosis, the interference was complete up to 40 Mb and then faded away between 40 and 77 Mb, with Z = 0.5 at 62 Mb; in males, complete interference was found up to 57 Mb fading away between 57 and 112 Mb with Z = 0.5 at 95 Mb.

The difference in interference distances explains why triple crossovers are common in female meiosis but very rare in males. To position three crossover events that are not subjected to strong interference (Z>0.5) in female meiosis requires at least 124 Mb, which is quite possible within the 197 Mb span of Chr 1. However, to accommodate three such events in males requires spacing that approaches the entire length of the chromosome.

Interference correlates with the length of bivalents at the pachytene stage of meiosis

The recombination events begin by the initiation of double-strand breaks in the leptotene stage of meiosis I, and the repair process that resolves intermediates into crossovers or convertants is completed in the pachytene stage. Differences in bivalent lengths between female and male meioses have been reported in both humans [12] and mice [13]. De Boer *et al.* [13] measured the average length of bivalent 1 in the pachytene stage of meiosis I in mice of mixed B6/129 genetic background to be 13.7 μm in females and 10.2 μm in males. Using these estimates, the distribution of double crossovers over interference distances in females and males is not different in terms of physical length of pachytene bivalents, with a minimum interference distance of 2.8 microns in both sexes (Figure 1c and 1d). The fact that the average numbers of chiasmata per bivalent calculated from our data in female and male meioses (1.97 and 1.60; see Table 1) are in striking agreement with the cytological data inferred from mutL homolog 1 (MLH1) foci (1.85 and 1.54) [13] lends support to this interpretation, assuming that MLH1 foci mark >95% or virtually all crossovers [14].

Our data provide genetic evidence that the crucial parameter in the relative positioning of crossovers along bivalents is the interference distance, which is, in turn, related to physical distance along the synaptonemal complex. A correlation of synaptonemal complex length with interchiasma distance has already been suggested by Tease and Hultén [12], who did not detect significant differences in mean distances between adjacent MLH1 foci that mark chiasmata in female and male germ cells. However, although it is likely that all MLH1 foci mark sites of crossing over, there remains some uncertainty whether some crossovers are processed through an alternative, non-MLH1 pathway [14] and how the latter will influence interference. Our genetic data provide the opportunity to measure interference between inherited double crossovers and to describe how the strength of interference varies as a function of distance.

As evident in Figure 1b, the coefficient of coincidence exceeds unity for distances between 75 and 120 Mb in female meiosis and then drops to around or below unity for distances above 120 Mb; in male meiosis, it exceeds unity for distances above 112 Mb. Such dependence has been reported [15] using *Drosophila* data for nine loci. Confidence bounds on the coincidence curves (Figure S2) indicate that this trend is real and not because of sampling variation. Values of Z above unity simply reflect that, under strong positive crossover interference, crossovers tend to be more evenly spaced than random.

Investigations in yeast have shown that two groups of genes are important for crossover interference. Mutations in the genes of the first group, which consists of ZIP1 [16], MER3 [17] and MSH4 [18], reduce the frequency of crossing over in addition to abolishing crossover interference, whereas mutations among the second group, NDJ1/TAM1, TID1 and DMC1, abolish interference without reducing crossing over [19]. It has been shown in mice that mutations in axial elements of the synaptonemal complex do not affect interference [20], whereas mutations in proteins of the central element result in absence of completed crossing over [21,22], which points to the possibility that as yet unknown proteins might affect both interference distance and synaptonemal complex length. If so, these proteins must be arranged linearly along the chromosome or bivalent to explain interference action over distances on the order of microns, either by creating a physical barrier or by controlling mechanical stress along the bivalents [23]. An additional, interesting candidate has been suggested by a recent study that found an entirely new class of short piRNA molecules of ~30 nt in length that are abundantly expressed in zygotene and/or pachytene of male meiosis [24]. Some of them are implicated in maintaining transposon silencing in the germline genome [25], but other classes could conceivably have a function in regulating recombination and/or interference.

The relationship between bivalent length at the pachytene stage and interference distances raises a significant issue. The decision about which double-strand

breaks will become crossovers and thus the interference pattern is made early in meiosis, prior to the leptotene-zygotene transition [20] when proteins involved in double-strand break repair and Holiday junction resolution localize between the aligned cores of the two homologous chromosomes [26]. The correlation between interference and bivalent length implies a relationship between chromosomal organization at the time of double-strand break formation and what is seen at the pachytene stage. This could be the case if double-strand breaks are initiated at sites that eventually will be aligned between the two homologous chromosomes. It has been shown that males have longer chromatin loops than females at the pachytene stage of meiosis [12], which in turn suggests that they have fewer interloop regions involved in synapsis. If recombination occurs only in interloop regions but not in loops, factors that determine loop size at earlier stages (before the leptotene-zygotene transition) would affect both inter-crossover distance and synaptonemal complex length in a similar manner.

Crossover interference and other factors affecting sex differences in recombination: concluding remarks

Our data do not preclude the possibility that additional factors, such as substantial differences in sex specific rates of recombination on regional scale and at the level of individual recombination hotspots [27], X-linked modifiers [9], or postmeiotic events, such as gametic selection [7], might influence sex differences in recombination rates. However, the major factor underlying genome-wide sex differences in recombination rates is crossover interference, which acts on physical rather than genomic distances. In this sense, the fundamental processes that regulate positioning of multiple crossovers along the mammalian chromosomes appear to be the same in female and male meiosis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tig.2007.08.015.

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