Online Supplementary Material

Crossover interference underlies sex differences in recombination rates

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Crosses, markers and distribution of recombination frequencies

Recombination frequencies were studied in backcrosses of C57BL/6JxCAST/EiJ. F1 hybrids were produced by reciprocal crosses in which either strain was female or male parent. These hybrids were then backcrossed to C57BL/6J and recombination was detected in their progeny. All parents and F1 hybrids were genotyped for three markers on each chromosome to ensure strain identity using DNA isolated from tail tips.

Mouse spleens were digested in 900 μl buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% v/v Nonidet P40, 0.45% v/v Tween 20, and 60 μg/ml proteinase K overnight with occasional shaking. After digestion, pH of the samples was adjusted by adding 100 μl of 100 mM Tris-HCl, pH 8.0. These digests were stored at -80^{o}C. Samples were diluted 20x in 10 mM Tris-HCl, pH 8.0 for genotyping. All progeny was genotyped using 28 previously described assays [1] for single nucleotide polymorphisms (SNPs) based on Amplifluor technology [2]. The average spacing of markers was 6.8±3.6 Mb. Individuals with a gap of > 20 cM or > 35 Mb between typed markers were omitted from subsequent analyses. Recombination was detected as transition

from homozygous to heterozygous genotype or vice versa. We did not detect significant differences in recombination frequencies between reciprocal crosses at this level of resolution. The distribution of recombination rates along Chr 1 is shown in Figure S1.

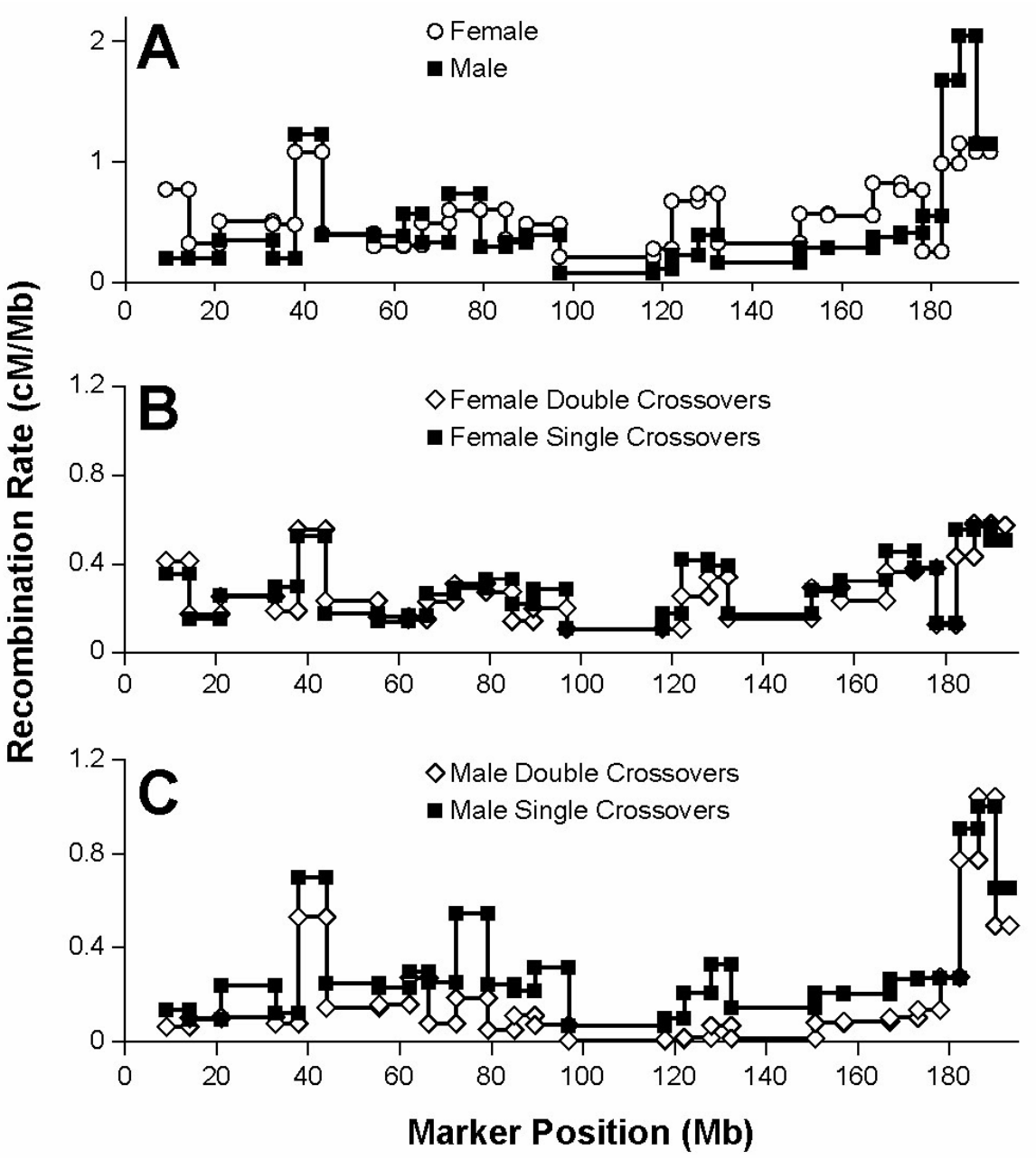

Figure S1. Distribution of relative recombination rates along Chr 1 in backcrosses of C57BL/6JxCAST/EiJ, expressed in cM/Mb. Marker positions on

the X axes begin at the centromere-proximal end. Recombination rates in each interval are represented by horizontal lines connecting two adjacent markers.

A. Comparison between total female and male recombination rates.

B. Comparison between the rates of recombination events involved in single and double crossovers in female meiosis.

C. Comparison between the rates of recombination events involved in single and double crossovers in male meiosis.

There is significant regional variation in sex-specific recombination rates along the chromosome. Single and double crossovers are distributed more equally in female than in male meiosis.

Distribution of crossovers as products of different chiasmata numbers

Every single recombination event in meiosis involves only two of the available four chromatids. As a result, every crossover event results in two crossover and two non-crossover chromatids. Single crossover events on one bivalent might involve any two chromatids. Crossover events can be detected as MLH1-positive foci at the pachytene stage of meiosis I or as cytologically visible chiasmata at the diplotene stage; however, it is difficult to identify individual chromatids participating in the exchange by cytological methods. There is presently no evidence that the chromatids participating in one recombination influence the choice of chromatids for another recombination on the same bivalent; this is commonly termed no chromatid interference (NCI).

A single chiasma will result in 50% non-crossover and 50% single crossover chromatids. Two chiasmata will result in the following types of chromatids depending on the number of chromatids involved:

Two chromatids – two non-crossovers and two double-crossovers

Three chromatids – one non-crossover, two single crossovers and one double crossover.

Four chromatids – four single crossovers.

Under no chromatid interference model, these possibilities together result in 25% non-crossovers, 50% single crossovers and 25% double crossovers.

Calculations for three chiasmata follow the same logic. Table S1 shows the expected frequencies of crossovers in bivalents with different chiasma numbers:

Table S1.

	Frequency of chromatids with crossover number			
Number of chiasmata per bivalent	0	1	2	3
0	1			
1	0.5	0.5		
2	0.25	0.5	0.25	
3	0.125	0.375	0.375	0.125

Both cytological and genetic data point out that there must be at least one chiasma per bivalent for successful meiosis [3,4]. Our data did not detect more than three crossovers on the same chromosome; the number of chiasmata per bivalent can conceivably be limited to between 1 and 3. In the absence of achiasmatic bivalents, those with either one or two chiasmata will produce 50% single crossovers whereas the frequencies of non-crossovers and double crossovers will vary depending on the relative frequencies of bivalents with one and two chiasmata. Occurrence of bivalents with three chiasmata will reduce the

frequency of single crossovers; however, the effect will not be significant if the frequency of three chiasmata is relatively low. For example, 12% bivalents with three chiasmata will reduce single crossovers to 48.5% (0.88x0.5 + 0.12x0.375=0.485).

If we make the assumption of NCI, the distribution of the total number of chiasmata per bivalent can be estimated from the observed number of crossovers on a single chromatid. Let *m* denote the total number of chiasmata on a random bivalent, and let *n* denote the number of crossovers on a single random chromatid. Under NCI, $n \mid m \sim \text{binomial}(m, ½)$. Let $p_i = \Pr(m = i)$. Then

$$\Pr(n = j) = \sum_{i=j}^{\infty} p_i \binom{i}{j} \left(\frac{1}{2}\right)^i$$

The p_i were estimated by maximum likelihood [5].

Calculation of the coefficient of coincidence

We have used the following procedure to estimate the coefficient of coincidence:

1. Calculate the observed frequency of recombinants in each interval, p_i.
2. Calculate the expected frequency of double recombinants for each pair of intervals (assuming no interference), $e_{ij} = p_i \times p_j$
3. Calculate the observed frequency of double-recombinants for each pair of intervals, o_{ij}.
4. Calculate the physical distance in Mb between each pair of intervals, $d_{ij,}$
5. Calculate physical distance between each pair of intervals in micrometers of bivalent length using the estimates from de Boer *et al*., 2006 (See ref [13] in main text) , of 13.7 μm for female and 10.2 μm for male bivalents.

5. Sort the pairs of intervals from closest to farthest.

6. Smooth each of o_{ij} and e_{ij} by averaging 30 intervals that are adjacent in length.

7. Calculate the coincidence as the ratio, o_{ij} / e_{ij}, using the smoothed versions.

The coincidence function is the same for chiasma locations on the bivalent and crossover locations on a random meiotic product. If the rates of chiasmata (on the bivalent) in the intervals are r_1 and r_2, then the rates of crossovers in those intervals are $r_1/2$ and $r_2/2$. If the chance of chiasmata occurring in both intervals is $r_{12,}$ then the chance of crossovers occurring in both intervals is $r_{12}/4$ (see Table S1). Thus the coincidence for chiasmata (Z_{ch}) is

$$Z_{ch} = r_{12}/(r_1\ r_2),$$

and for crossovers (Z_{xo}) is

$$Z_{xo}= r_{12}/4(\ r_1/2 \times\ r_2/2) = r_{12}/(r_1\ r_2)$$

Determination of confidence bounds of the coincidence curve (Figure S2)

Pointwise confidence bounds on the coincidence curve were estimated by a nonparametric bootstrap. We sampled, with replacement, from the available data set to create a new data set of the same size, but with some individuals randomly duplicated and some omitted. The coincidence curve was estimated from the resampled data, and the process was repeated for a total of 1000 bootstrap replicates. At each point, the confidence bounds were estimated as the 2.5 and 97.5 percentiles of the coincidence values from the bootstrap replicates.

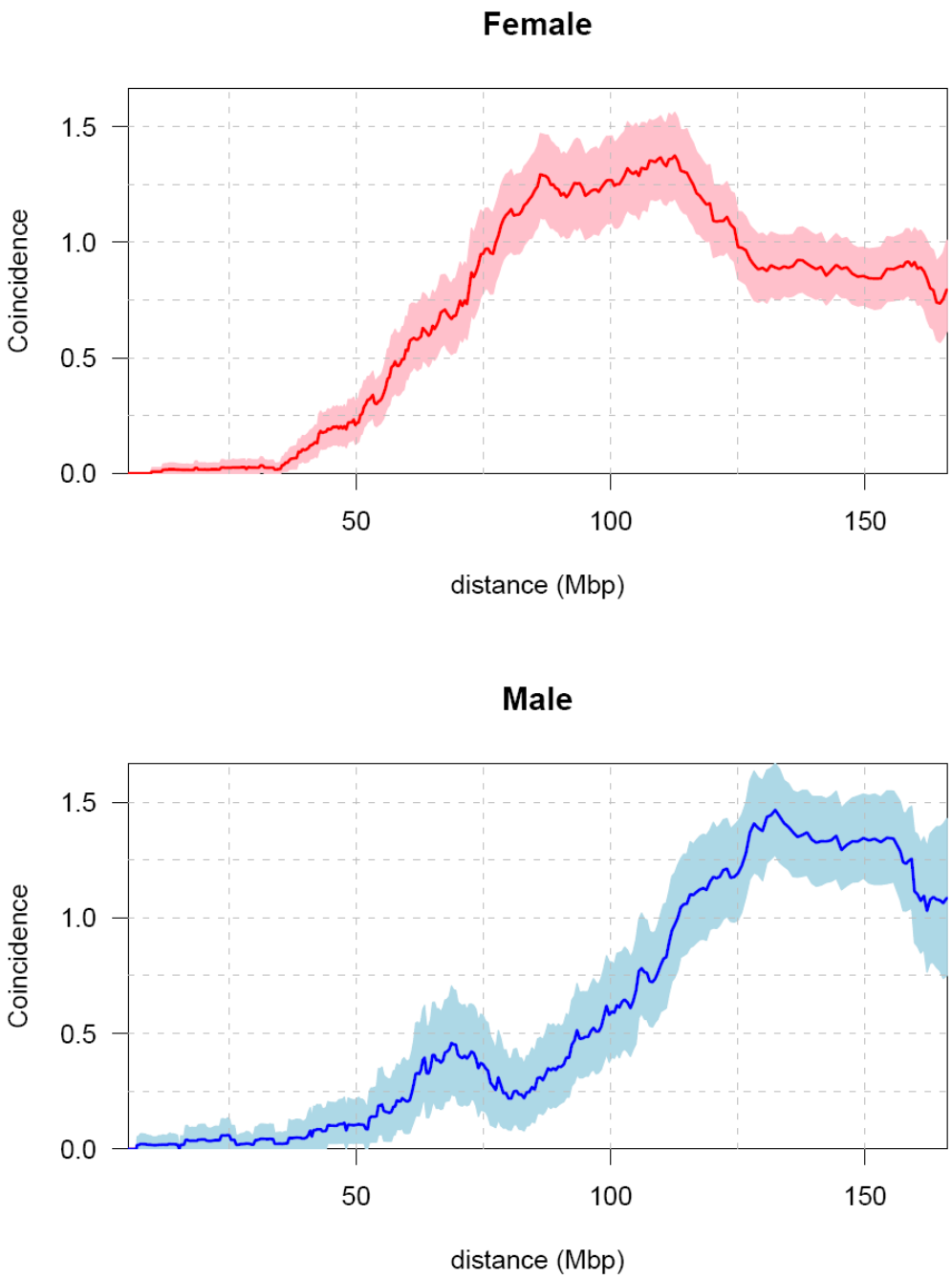

Figure S2. Bootstrap-Based Confidence Bounds of Coincidence. Red line – experimental female data; pink area – confidence bounds of female data; blue line – experimental male data; light blue area – confidence bounds of male data.

Coefficient of coincidence goes gradually up from 0 and exceeds 1, after which goes down to around or below 1. Confidence bounds confirm that this trend is real.

References

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