Variation in crossover interference levels on individual chromosomes from human males

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Crossovers (COs) generated by homologous recombination ensure the proper segregation of chromosomes during meiosis. COs exhibit interference, which leads to widely spaced COs along chromosomes. Strong positive CO interference has been found in humans. However, little is known about the extent of human CO interference. In this study, variations in CO interference over the entire human genome and among individuals were analyzed by immunofluorescence combined with fluorescence in situ hybridization of testicular biopsies from 10 control men. These methods allow for direct identification of the frequency and location of COs in specific chromosomes of pachytene cells. The strength of CO interference was estimated by fitting the frequency distribution of inter-CO distances to the gamma model. Positive interference among CO on chromosomes was observed in these men, and the strength of inter-arm interference was significantly stronger than that for intra-arm CO. In addition, interference was observed to act across the centromere. Significant inter-individual and inter-chromosomal variations in the levels of interference were found, with smaller chromosomes exhibiting stronger interference. Discontinuous chromosome regions (gaps) and unsynapsed chromosome regions (splits) in chromosome 9 had both cis and trans effects on CO interference levels. This is the first report that the interference level varies significantly across the whole genome and that, at least in the human male, anomalies in chromosome synapsis play an important role in altering CO interference levels.

INTRODUCTION

Crossover (CO) formation is a crucial step in the establishment of a physical connection between homologous chromosomes during prophase I of meiosis, which facilitates proper chromosome segregation. Improper segregation of homologs (i.e. nondisjunction) results in aneuploidy, the leading cause of fetal loss and birth defects in humans (1). In humans, positive interference affects the distribution of COs along chromosomes (2–5). Positive CO interference, by definition, means that the occurrence of one CO discourages the formation of other COs in its vicinity (6). An obvious consequence of positive interference would be that COs are more evenly spaced than would be expected with random placement across chromosomes, a situation which reduces the risk of non-disjunction (7). Yeast mutants for which interference is absent show a high incidence of non-disjunction of homologous chromosomes at the first meiotic division, indicating that interference is crucial to CO control and homolog segregation (8,9). Despite the clinical importance of CO interference, the mechanisms of interference are still poorly understood (10).

CO interference has been examined by several mathematical models. Among these, the gamma model has been most commonly used for the analysis of interference.

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levels (2,11–13). The strength of interference among COs can be calculated by the interference parameter \( v \), which is estimated by fitting the frequency distribution of inter-CO distances to the gamma model. When \( v = 1 \), distribution of inter-focus distances is exponential, representing random distribution of COs along chromosomes and no interference between CO foci. When \( v > 1 \), a positive interference is indicated, and as \( v \) increases, the interference level is stronger.

Employing the gamma model, variations in interference levels across chromosomes and among individuals were found in two systematic studies of human CO interference, on the basis of the Center d’Etude du Polymorphisme Humain (CEPH)-family genotyping data (2,3). Earlier studies of human pedigree data also showed the variation of CO interference levels. For example, smaller chromosomes exhibited stronger interference than larger chromosomes (14), and the level of interference on the p-arm tended to be lower than that on the q-arm of chromosome 1 (15). Meiotic recombination and CO data in these early studies originated from the construction of linkage maps, using genetic markers rather than precisely localized genetic exchanges, so CO interference was necessarily analyzed in chromosomal segments and not at specific locations. There was no direct evidence in these studies that interference showed heterogeneity across the entire human genome.

Recently, CO positions in meiotic chromosomes have been identified precisely by immunofluorescence methods, using antibodies against mut L homolog 1 (MLH1; marks the location of COs) and synaptonemal complexes (SCs; proteinaceous structures linking homologous chromosomes during meiotic prophase I) (16–18). Results from the application of these techniques suggested that there is a variation in CO interference (as measured by inter-CO distances) in human males (19,20). In the human male, chromosome synapsis anomalies such as discontinuous regions (gaps), and unsynapsed regions (splits) in the SC (21,22) significantly altered the distribution of COs on SCs (23). These studies indicate that incompletely formed SCs may affect the levels of CO interference in the human male.

In the present study, the distribution of meiotic COs in each chromosome was identified by immunofluorescence methods combined with centromere-specific multicolor fluorescence in situ hybridization (cenM-FISH) on testicular tissue from 10 control men with normal spermatogenesis. The strength of CO interference was measured by the interference parameter \( v \) of the gamma model, and then interference heterogeneity across the entire human genome and among individuals was examined. In addition, the effects of SC formation on CO interference were determined by analyzing interference strength in chromosome 9 (the SC most frequently observed to have gaps/splits in pachytene stage meiotic cells).

RESULTS

An example of a pachytene SC spread, with identification of individual autosomes and cenM-FISH signals in the same cell, is shown in Figure 1. MLH1 foci in about 100 pachytene-stage spermatocytes were analyzed for each of the 10 men in order to determine the distribution of COs. The observed inter-CO distances (expressed as percentage of the length of the SC) were fitted to the gamma distribution. The Kolmogorov–Smirnov test was used to examine the goodness-of-fit for the observed frequency distribution of inter-CO distances to the gamma distribution. The results showed that the gamma model gives a very good fit to inter-CO distances along SC in most cases, except that some fits were less perfect because of the limited range of observable

Figure 1. Human pachytene spermatocyte with SCs shown in red, centromeres in blue and MLH1 foci in yellow (upper). Gaps in SC1 and SC9 are marked with a ‘g’. Subsequent cenM-FISH analysis permits identification of individual chromosomes so that recombination (MLH1) foci can be analyzed for each SC (lower).
inter-CO distances or local chromatin properties (12). The interference parameter \( y \), a measure of the strength of interference among COs, was then calculated from the gamma distribution. No interference parameter was estimated for chromosome 21, since it rarely has more than one CO.

Characterization of CO interference

Examples of the estimate of \( y \) for meiotic chromosomes are shown in Figure 2. The expected frequency distribution of inter-CO distances (the solid curves), obtained from the gamma model, fits well to the observed inter-CO distances (the bars). Furthermore, the fit of inter-CO distances to the gamma distribution (indicating CO interference) was significantly better than the fit to the exponential distribution (corresponding to random distribution of COs) (Fig. 2). All estimated interference parameters (\( y \)) were significantly greater than 1 (\( P < 0.0001 \), likelihood-ratio tests), providing strong evidence in favor of genome-wide positive CO interference in human males.

In an earlier study, the centromere was assumed to play a role in recombination that acted as a barrier to interference (24), but recent evidence from several species suggests that CO interference acts across the centromere (2,25–27). To verify the role of the centromere in CO interference in human males, the relationship between the distance from the centromere to the nearest CO on each arm (of chromosomes with at least one CO on each arm) was examined. The distances were expressed as percentage of SC length. Pearson correlation analysis showed a significant negative correlation between them (\( r = -0.3748 \), \( P < 0.0001 \)) (Fig. 3), suggesting that when a CO occurred near the centromere in one arm, the nearest CO in the other arm tended to be farther away from the centromere. Thus, CO interference does continue uninterrupted across the centromere in human male meiosis.

Intra-chromosomal variation in CO interference

As described earlier, CO interference acts across the centromere, indicating that COs are not formed independently in the two arms. Using pooled data for each chromosome from
the 10 individuals, the \( y \) of inter-CO distances on the p- and q-arms of a chromosome was estimated to evaluate intra-arm interference heterogeneity. Using SCs 1–7 that had at least two COs on each arm (since SCs 8–22 rarely have more than one CO on the p-arm), no significant difference in the inter-CO \( y \) was observed between the p- and q-arms (\( P = 0.84 \), one-way ANOVA) (Fig. 4A). This is consistent with previous results (3), and suggests that CO interference is constant along the chromosome arms.

Subsequently, inter- and intra-arm interference \( y \) were compared to further assess the heterogeneity of intra-chromosomal interference. Inter-arm CO interference \( y \) values were estimated by fitting trans-centromere inter-CO distances to the gamma model, from SCs with at least one CO on each arm. For evaluating intra-arm CO interference, the analysis was restricted to chromosomes with at least two COs in the same arm, using pooled inter-CO distances across the p- and q-arms for each chromosome. The fitted curves for the estimated \( y \) from inter- and intra-arm are shown in Figure 4B. A significantly higher interference level was found for inter-arm COs than for intra-arm COs (\( P < 0.0001 \), one-way ANOVA), providing strong evidence in favor of non-constant interference across the whole chromosome. Furthermore, short chromosomes exhibited higher interference levels for inter-arm COs when compared with longer chromosomes, whereas intra-arm interference strength was at similar levels to those of the longer chromosomes (Fig. 4B). Therefore, the overall interference strength for short chromosomes was stronger than for longer chromosomes.

**Inter-chromosomal and inter-individual variation in CO interference**

The \( y \) for each chromosome was estimated by pooled data for the frequency distribution of inter-CO distances from all individuals. A significant difference in the levels of interference over the entire human genome was observed, with smaller chromosomes exhibiting higher levels of interference than larger chromosomes, with the exception of chromosome 22 (Fig. 5). Furthermore, the heterogeneity of inter-chromosomal interference levels (Fig. 5) is remarkably parallel to that of inter-arm interference levels (Fig. 4), suggesting that the difference in inter-arm interference levels may be the main contributor to variation in inter-chromosomal interference levels.

Inter-individual variation in interference was assessed by estimating \( y \) for each chromosome for each donor. Significant differences in \( y \) among individuals were found for chromosomes 1, 3, 5–8, 13 and 15–20 (Fig. 6). In addition, the degree of variation in interference levels was lower in the larger chromosomes than in smaller chromosomes (\( P < 0.05 \) for chromosomes 1, 3, 5–8, 15 and 17, whereas \( P < 0.0001 \) for chromosomes 13, 16, 18, 19 and 20).

**The role of the mature SC in CO interference**

To evaluate the relationship between abnormal synapsis and CO interference, the effect of gaps/splits on the interference levels of chromosome 9 and other chromosomes was examined. The inter-arm \( y \) for normally synapsed, gapped and split SC 9 is shown in Table 1. The interference levels for gapped and split SC 9 were significantly elevated compared to normally synapsed chromosomes (for gapped SC 9, \( P < 0.0001 \); for split SC 9, \( P < 0.05 \)), indicating that gaps/splits have a cis effect on chromosome 9’s interference pattern. Furthermore, \( y \) values in Table 2 indicate that gaps/splits also exerted a trans effect on the interference pattern of other chromosomes. SCs 13, 14, 16 and 18 in cells with split SC 9 and SC 20 in cells with gapped SC 9 were both significantly increased compared to normally synapsed chromosomes, and SC 19 showed a significant reduction in the CO interference level in cells with a gapped SC 9.

To examine a possible trans effect among chromosomes, correlation analysis between the \( y \) for each chromosome from the 10 individuals was performed. Interestingly, significant positive correlations between the \( y \) of all the chromosomes except chromosomes 2, 4, 6 and 8 were found (Fig. 7, \( P < 0.05 \), Pearson’s correlation analysis). Furthermore, this correlation always occurred among chromosomes with similar SC length. For example, among longer SCs, \( y \) for chromosome 1 was positively correlated with chromosomes 3 and 5, and \( y \) for chromosome 3 was positively correlated with chromosomes 5 and 12. Among shorter chromosomes, chromosome 16 was positively correlated with chromosomes 15 and 17, and chromosome 19 was positively correlated with chromosomes 17 and 20 (Fig. 7).

**DISCUSSION**

Our results provide the first direct evidence for significant variation in the levels of CO interference across chromosomes and among human males. Variation in the inter-arm interference levels is by far the largest contributor to variation in inter-chromosomal CO interference. The heterogeneity of
inter-chromosomal interference also demonstrated a negative correlation between SC length and the global (chromosomal) level of interference, indicating that the strength of CO interference might be modulated by the total length of the SC (28,29). In other words, if CO interference acts over a relatively fixed length of SC, short SCs will have fewer COs and higher levels of interference than longer SCs.

Based on the comparison of inter- and intra-arm CO distances in pachytene cells from two men, Codina-Pascual et al. (19) reported that the level of trans-centromere interference was higher than that of intra-arm interference. In the same paper, they also proposed that the first CO in nonacrocentric chromosomes is formed in subtelomeric regions of each arm, and exerts interference on the next CO to be established along the SC arm towards the centromere. They further proposed that when the interference from both sides of the centromere coincides, it would result in a higher trans-centromere interference level than that for intra-arm. However, this hypothesis may not completely explain the heterogeneity seen in intra-chromosomal interference. First, although subtelomeric regions are hot regions for recombination (5,19), it is not yet certain that CO formation is actually initiated in subtelomeric regions and proceeds proximally toward the centromere. Second, since pericentromeric regions are usually heterochromatic and characterized by repetitive DNA, reduced recombination frequencies, few protein-coding regions and gene silencing (30), the inhibitory effect of pericentromeric heterochromatin on recombination cannot be excluded. Therefore, a complete explanation of the heterogeneity seen in intrachromosomal interference has not yet been elucidated.

Our results suggest that the mature SC plays an important role in CO interference in the human male. The SC is a tripartite structure with two parallel lateral elements [corresponding to the protein, synaptonemal complex protein 3 (SCP3)] and a central element held together by transverse filaments (corresponding to the protein SCP1) (31). It is not clear which of the SC components play a major role in regulating CO interference levels. In this study, interference levels for inter-arm COs were found to be significantly increased in both gapped and split SC 9 compared with controls (Table 1). In addition, there was no significant difference in the interference levels between SCs with gaps and those with splits (Table 1; \( P = 0.71 \)). The effects of gaps and splits on interference levels on chromosomes exhibited both a cis and a trans effects.

To date, the molecular mechanisms of interference action and variations are poorly understood. Over the past few years, several models have been proposed to explain CO interference. One of the main models has been the ‘counting’ model, in which recombination precursors mature to COs in such a way that a fixed number of non-CO events occurs.
Inter-individual variation in \( y \) (± SE) for each chromosome. For each plot, the horizontal dashed line corresponds to the mean \( y \) from 10 individuals. The \( P \)-values exhibit the significance of variance among individuals for each chromosome (one-way ANOVA). Note that there are no \( y \) for chromosomes 21 and 22 due to insufficient data from each individual. Significant variations in \( y \) among individuals were found on chromosomes 1, 3, 5–8, 13 and 15–20.

**Figure 6.** Inter-individual variation in \( y \) (± SE) for each chromosome. For each plot, the horizontal dashed line corresponds to the mean \( y \) from 10 individuals. The \( P \)-values exhibit the significance of variance among individuals for each chromosome (one-way ANOVA). Note that there are no \( y \) for chromosomes 21 and 22 due to insufficient data from each individual. Significant variations in \( y \) among individuals were found on chromosomes 1, 3, 5–8, 13 and 15–20.
Figure 6. Continued
between any two adjacent COs (32). As a consequence, the distribution of CO precursors is obtained by counting early recombination nodules and interference strength is assumed to be constant along the chromosomes (33). However, a phenomenon called ‘CO homeostasis’ [where both CO frequency and CO interference remain at wild-type levels when the numbers of double-strand breaks (DSBs) are reduced] has been demonstrated in yeast (34). These findings indicate that CO frequency does not decrease, as expected by the counting model, in order to maintain a fixed number of non-CO events. The data from the current study, as well as those observed in Arabidopsis thaliana Chromosome 4 (35), have shown that CO interference acts variably across chromosomes.

Table 1. Estimated interference parameters (v) in normally synapsed, gapped and split SC 9

<table>
<thead>
<tr>
<th>Chromosome type</th>
<th>Estimated inter-arm v</th>
<th>No. of intervals</th>
<th>v</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>502</td>
<td>14.6</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Gapped</td>
<td>229</td>
<td>21.2*</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>33</td>
<td>23.2**</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.0001, **p < 0.05 compared to normally synapsed chromosomes.

Table 2. Estimated interference parameters (v) of affected SCs in cells with normally synapsed, gapped and split SC 9

<table>
<thead>
<tr>
<th>SC</th>
<th>Chromosome type</th>
<th>No. of intervals</th>
<th>v</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Normal</td>
<td>493</td>
<td>11.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Gapped</td>
<td>214</td>
<td>13.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>30</td>
<td>23.3*</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Normal</td>
<td>487</td>
<td>11.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Gapped</td>
<td>207</td>
<td>13.6</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>29</td>
<td>21.2**</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Normal</td>
<td>547</td>
<td>11.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Gapped</td>
<td>232</td>
<td>11.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>34</td>
<td>24.5*</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Normal</td>
<td>420</td>
<td>16.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Gapped</td>
<td>187</td>
<td>13.1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>30</td>
<td>27.2**</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Normal</td>
<td>491</td>
<td>16.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Gapped</td>
<td>208</td>
<td>11.2**</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>26</td>
<td>11.2</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Normal</td>
<td>467</td>
<td>18.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Gapped</td>
<td>193</td>
<td>30.3*</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Split</td>
<td>31</td>
<td>12.1</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001, **p < 0.05 compared to normally synapsed chromosomes.

More recently, another interference model, known as the mechanical stress model (36,37), proposes that the expansion of chromatin against some constraining elements brings...
Figure 7. The relationship between the interference parameters for each chromosome from 10 individuals. Significant positive correlations on $v$ were found between all chromosomes, indicating that there is inter-chromosomal effect on CO interference. A significant positive correlation between the $v$ for chromosome 1 and for chromosome 14, and for the distances from the MLH1 focus to the centromere in chromosome 21 was also found (Fig. 7H). These distances were expressed as percentage of the length of q-arms of chromosome 21.
about mechanical stress, which promotes the development of COs among DSBs. The occurrence of CO results in local stress relief at the affected site, which then spreads outwards in both directions along the chromosome axes with decreasing strength, thereby preventing the formation of a new CO nearby as it travels. Moreover, SC nucleation and spreading would be promoted by the stress effect as well. The model predicts that (i) a chromatin-constraining structure is required for generating interference and conducting the interference signal; (ii) CO development events among nearby recombination intermediates are not favored and (iii) the interference strength will decrease as the physical size of the SC increases in a distance-dependent manner. In the current study, significant variation in inter-chromosomal interference was observed, lending support to some features of the stress model, such as the distance-dependent manner of interference. It also provides, as does the Codina-Pascual et al. (19) study, some evidence to support the prediction in the stress model that CO interference might be modulated by SC length. Evidence from Arabidopsis thaliana has also demonstrated that the interference level between COs separated by a fixed genetic distance is a function of physical distance (38). Further experiments are needed to support/refute these models or generate new models for the characterization of CO interference and its variation.

In summary, our study provides the first detailed analysis of heterogeneity in the level of CO interference over the entire human genome. Unambiguous evidence for significant inter-individual, inter- and intra-chromosomal variation in interference levels was observed in human males, with inter-arm interference levels providing the major contribution to interchromosomal CO interference. In addition, SC length modulates the interference strength at the genome-wide level, since (i) incomplete synapsis (gaps/splits in chromosome 9) had a cis and trans effects on the levels of CO interference, (ii) the interference levels of one chromosome were similar to levels of non-homologs with a similar SC length and (iii) the SC transverse filament protein SCP1 played a major role in regulating interference levels among COs. The results in this study could help to further understanding of current CO interference models and the role of the SC in CO interference. Finally and most importantly, a better understanding of CO interference may provide valuable insight into chromosomal non-disjunction, which, when elucidated, may have far-reaching clinical benefits.

MATERIALS AND METHODS

Testicular sample collection

Testicular samples were obtained from non-chemotherapy patients undergoing orchiectomy for testicular or prostate cancer (n = 3, age range: 47–81, Calgary, Canada) and vaso-vasostomy (n = 7, age range: 35–61, University of California, CA, USA). These patients were ascertained for reasons unrelated to meiotic defects or infertility. Testicular tissues were kept in phosphate buffered saline (pH 7.4) until use, and transferred on ice to Calgary by air courier where appropriate. We have previously demonstrated that cold storage of testicular tissue does not affect recombination frequencies (39). Histo-

logical examination showed normal spermatogenesis in these 10 donors (ages: 47–81 years). There was no significant difference in the frequency of recombination or meiotic abnormalities in cancer patients compared to men undergoing vasectomy reversals (20). Donors gave informed consent, and this study received ethical approval from the institutional review boards at the University of Calgary and at the University of California, CA, USA.

Fluorescence immunostaining and cenM-FISH

Slides with chromosome spreads were subjected to immuno-fluorescence staining as described previously (5). Primary antibodies against the following proteins were used: SCP1 (transverse filament proteins of the SC, 1:1000 dilution, a gift from P. Moens, York University), SCP3 (lateral element proteins of the SC, 1:250 dilution, a gift from T. Ashley, Yale University), MLH1 (marks the site of COs, 1:100 dilution, Oncogene, San Diego, CA, USA) and CREST (Calcinosis, Raynaud’s phenomenon, Esophageal dysfunction, Sclerodactyly, Telangiectasia, marks the centromere; 1:100 dilution, a gift from M. Fritzler, University of Calgary). These primary antibodies were detected using a cocktail of secondary antibodies (donkey antiserum) conjugated with different fluorochromes: blue 1-amino-4-methylcoumarin-3-acetic acid (AMCA) and red Cy3 (1:100 dilution, Jackson Immuno-research, West Grove, PA, USA), green Alexa 488 and red Alexa 555 (1:125 dilution, Molecular Probes, Eugene, OR, USA). Primary and secondary antibodies were incubated overnight at 37°C, and for 90 min at 37°C, respectively. Slides were examined on a Zeiss Axioshot epifluorescence microscope equipped with propidium iodide (PI), fluorescein isothiocyanate (FITC) and 4',6-diamidino-2-phenylindole (DAPI) filters and a cooled charged coupled device (CCD) camera. Three fluorescent images (red, green, and blue) of the SC, CO sites, and CREST locations, respectively, were captured using Applied Imaging Cytovision 3.1 software (Applied Imaging Corporation, Santa Clara, CA, USA). Spreads were localized using a gridded finder slide.

Each pachytene-stage nucleus used for analysis met the following criteria: (i) the correct numbers of bivalents (22 autosomes and 1 sex body) were present, (ii) the SCs were not overlapped or bent back on themselves, allowing all foci to be scored and (iii) background was fairly low, allowing the SCs to be distinguished from background noise and from each other. MLH1 signals were scored if they were distinct and localized on an SC. SCs were classified as normally synapsed if they were completely linear, without any obvious bubbles, forks, loops or irregularities.

After the analysis of the captured immunofluorescence images, cenM-FISH was carried out on the same spermatocytes. This technique allowed simultaneous identification of each autosome. Techniques developed by Nietzel et al. (40) and Oliver-Bonet et al. (41) were modified to make use of the microwave-decondensed/codenatured FISH technique (42). Cells were microwave decondensed for 5 s in dithiothreitol (DTT) and 30 s in 3,5-diiodosalicylic acid, lithium salt (LIS)/DTT at medium power (550 W). Hybridization buffer (10% dextran sulfate, 2 × standard sodium citrate (SSC), 55% formamide) was pre-warmed to 50°C, added to the
cenM-FISH probes and warmed at 50°C until all probe was dissolved. Probes were applied to the slide, a glass cover slip was sealed in place with rubber cement, the probes and cells were microwave denatured for 80 s at 1100 W and the slide was incubated in a humid chamber at 37°C for approximately 24 h. A posthybridization wash (0.4 × SSC 1% NP-40, 70°C) was carried out, streptavidin–Alexa 647 (Molecular Probes) solution was applied under a plastic cover slip, and the slide was incubated at 37°C for 40 min in a humid chamber. The slide was washed, with constant agita-
tion, for 10 min in 4 × SSC, air dried and mounted in DAPI. Cells previously analyzed by antibody immunostaining were relocated, and six fluorescent images (blue, aqua, green, gold, red and far red) were captured for each cell, using Applied Imaging Cytovision 3.1 software (Applied Imaging Corporation, Santa Clara, CA, USA).

Calculating the inter-CO distances on SCs

After cenM-FISH identification of each chromosome, images of the corresponding SC spreads were analyzed for CO distribution and SC length measurement, using MicroMeasure 3.3 (available from the MicroMeasure website, http://www.colostate.edu/Depts/Biology/MicroMeasure). MicroMeasure is an image analysis application that allows collection of data for a wide variety of chromosome parameters from electronically captured images (43). The inter-CO distances for each chromosome were calculated by measuring the intervals between adjacent COs along the SCs, and were expressed as a percentage of the SC length.

Estimation of the interference parameter \( \nu \)

Gamma distribution is widely used for the analysis of distances between events along a linear axis (12). The interference parameter \( \nu \) is estimated by fitting the observed frequency distribution of inter-CO distances to the gamma distribution by the maximum likelihood method, using matlab 7.0 software (Mathworks Inc., MA, USA). When \( \nu = 1 \), gamma distribution shows exponential distribution, indicating no interference among foci. With \( \nu < 1 \), gamma distribution is exponentially shaped and asymptotic to both the vertical and horizontal axes, indicating negative interference. When \( \nu > 1 \), gamma distribution assumes a mounded, skewed shape and represents positive interference. As \( \nu \) increases, the strength of positive interference is stronger, spacing COs more evenly across the chromosomes.

The goodness-of-fit for a gamma distribution and the significance of the \( \nu \) value were also assessed by the following tests using matlab 7.0 software (Mathworks Inc., MA, USA). First, a Kolmogorov–Smirnov test was performed to see how well the observed frequency distribution of inter-focus distances fits to the gamma distribution. Second, the goodness-of-fit for a gamma distribution was further assessed by maximum likelihood estimates of the shape parameters and computer simulations for 10 000 inter-focus distances from each interference parameter, and then the fitted distribution curves were obtained under the gamma model. Finally, likelihood-ratio tests were used to evaluate whether the gamma distribution or the exponential distribution fits the data significantly better, that is, to determine whether \( \nu \) differs significantly from 1.0.

Statistical analysis

One-way analysis of variance (ANOVA) and subsequent pairwise \( t \)-tests using the pooled variance from the ANOVA were used to examine the differences in interference levels among individuals. The Student’s \( t \)-test was applied to examine the difference in interference levels between normally synapsed and gapped/split SC 9 and between SCs other than SC 9 both in cells with normally synapsed SC 9 and in cells with gapped/split SC 9. The relationships between the two sets of distances from centromere to the nearest COs on the p- and q-arms and between the \( \nu \) for each chromosome were evaluated using Pearson’s correlation analysis.

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