Genetic Control of Mammalian Meiotic Recombination. I. Variation in Exchange Frequencies Among Males From Inbred Mouse Strains

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ABSTRACT

Genetic background effects on the frequency of meiotic recombination have long been suspected in mice but never demonstrated in a systematic manner, especially in inbred strains. We used a recently described immunostaining technique to assess meiotic exchange patterns in male mice. We found that among four different inbred strains—CAST/Ei, A/J, C57BL/6, and SPRET/Ei—the mean number of meiotic exchanges per cell and, thus, the recombination rates in these genetic backgrounds were significantly different. These frequencies ranged from a low of 21.5 exchanges in CAST/Ei to a high of 24.9 in SPRET/Ei. We also found that, as expected, these crossover events were nonrandomly distributed and displayed positive interference. However, we found no evidence for significant differences in the patterns of crossover positioning between strains with different exchange frequencies. From our observations of >10,000 autosomal synaptonemal complexes, we conclude that achiasmate bivalents arise in the male mouse at a frequency of 0.1%. Thus, special mechanisms that segregate achiasmate chromosomes are unlikely to be an important component of mammalian male meiosis.

MEIOTIC exchange, or recombination, is required to ensure the proper disjunction of homologous chromosomes to opposite poles at the first meiotic division. It is well established that this process is under genetic control, with stringent regulation of the number and distribution of exchanges. For example, the distribution of exchange events on chromosomes does not fit a Poisson distribution and thus is nonrandom (Haldane 1931). The most important consequence of this is that, with very few exceptions, every chromosome forms at least one exchange event with its homolog, resulting in the so-called "obligate chiasma" necessary for accurate segregation (Mather 1936, 1937). Further, in most organisms there is a virtual absence of exchange in heterochromatin and a reduction of exchange frequencies in euchromatin that lies near chromosomal elements such as centromeres and telomeres (Mather 1939; Hulten 1974; Schalet and Lefevre 1976; Clarke and Carbon 1980; Wu and Lichten 1994; Mahtani and Willard 1998).

Variation in the frequency of meiotic exchange has been observed among different strains and/or species in several organisms, including flies (Roberts and Roberts 1921; Hawley 1980; Charlesworth and Charlesworth 1985; True *et al.* 1996; Zwick *et al.* 1999), maize (Williams *et al.* 1995), and mice (Reeves *et al.* 1990). Not surprisingly, mutations that affect the number and/or distribution of recombination events have been discovered in these and other organisms (reviewed in Hawley 1988). Recombination rate is also a selectable character in Drosophila (*e.g.*, Roberts and Roberts 1921; Charlesworth and Charlesworth 1985).

There are several different ways to study genetic exchange patterns. Most commonly, genetic linkage methodology is used to analyze the progeny of appropriately marked parents through studying the inheritance of phenotypic markers or molecular polymorphisms. While this has been a useful approach in many organisms, it has limitations. First, the approach relies on analysis of transmitted haploid meiotic products rather than on the cells undergoing meiosis; as a result, only half of all exchanges can be detected (*e.g.*, following a single exchange, only two of the four chromatids will be recombinant) and any recombination-associated selection against gametes will be missed. Second, the technique cannot be used in inbred strains, where all loci are homozygous; thus, in species such as the mouse, it is difficult to investigate genetic variation in recombination rates. Third, conventional linkage analysis requires well-characterized, three-generation (or deeper) kindreds. In humans, this effectively limits the analyses to those meioses available from the CEPH registry (*i.e.*, a few hundred meioses, with a relatively small number of meioses per individual; Dausset *et al.* 1990), complicating efforts to identify intra- or interindividual variation in recombination rates.

Another common method of studying genetic exchange patterns involves cytogenetic analysis of diakinesis-stage gametes, a timepoint at which chiasmata can be directly

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observed in chromosome preparations. Conducting analysis on the entire gamete, prior to the meiotic divisions, means that all exchanges can be detected, a significant advantage over linkage analysis. Unfortunately, this method cannot be applied to all organisms; in some, like yeast and flies, chromosome size or other features of the meiotic process prevent clear visualization of the chiasmata, while in others, like humans, obtaining gametic material of the appropriate stage is very difficult. However, in the mouse, cytogenetic examination of chiasmata at diakinesis has been widely used to study genetic recombination.

A number of investigators over the past few decades have examined chiasmate bivalents at diakinesis in both murine males and females. It is generally agreed that mice have ~22–24 (in males) to 23–30 (in females) chiasmata distributed among their 20 bivalents (Henderson and Edwards 1968; Polani 1972; Luthardt *et al.* 1973; Polani and Jagiello 1976; Speed 1977; Jagiello and Fang 1979; Gorlov *et al.* 1994; Lawrie *et al.* 1995). Thus, it is usually assumed that chiasma frequency is higher in females than in males, although this has been disputed by at least one recent report suggesting that the gender difference vanishes when the sex bivalent is excluded from the analysis (Lawrie *et al.* 1995).

Differences in chiasma frequency between strains have occasionally been noted (Polani and Jagiello 1976; Speed 1977), but in no case has there been a systematic examination of the effects of genetic background on the level of meiotic exchange. Further, although a wide variety of inbred, outbred, and F_1 progeny have been used in chiasma studies, a series of inbred strains has never been studied in the same set of experiments by the same investigators, making true genetic background comparisons impossible.

There is also ambiguity in the literature over the true frequency of nonexchange or achiasmate bivalents in the mouse. Clearly, their occurrence is rare, but a precise figure remains elusive and may even be strain or gender dependent. Several investigators have reported univalents at a rate of up to several percentage points in both male and female mice at diakinesis/metaphase I (Henderson and Edwards 1968; Polani and Jagiello 1976; Jagiello and Fang 1979), but it remains controversial whether the "loose" homolog associations visualized in these preparations can truly be classified as achiasmate. Furthermore, other researchers have reported precocious separation of small mouse chromosomes at postpachytene stages of meiotic prophase, suggesting that "univalents" may in fact be homolog pairs experiencing premature chiasma loss, not genuine nonexchange bivalents (Speed 1982; Speed and Chandley 1983).

To identify possible genetic effects on recombination and to determine the absolute frequency at which achiasmate bivalents arise during murine meiosis, we have applied a recently developed immunostaining assay to directly examine meiotic exchanges in male mice. Specifically, we have analyzed the number and distribution of MLH1 foci in pachytene-stage cells from males of various inbred genetic backgrounds.

Mammalian MLH1, a homolog of the *Escherichia coli* Mut L mismatch repair protein, plays important roles in both somatic mismatch repair and meiotic recombination (Baker *et al.* 1996; Hunter and Borts 1997; Woods *et al.* 1999). MLH1 appears as discrete foci along the synaptonemal complex (SC) at pachytene in various organisms and is thought to be a component of late recombination nodules (RN), a role consistent with meiotic co-localization studies (Plug *et al.* 1998). Furthermore, in both mice and humans, the distribution of MLH1 foci along the SC parallels the pattern of meiotic recombination events (Barlow and Hulten 1998; Anderson *et al.* 1999). The number and location of MLH1 foci are in keeping with previous cytogenetic and molecular studies of meiotic exchange (Hulten 1974; Speed 1977; Jones 1987; Lawrie *et al.* 1995), and the distribution of foci displays positive interference (Barlow and Hulten 1998; Anderson *et al.* 1999). Functional evidence also supports the idea that MLH1 is a component of the late RN and critical for chiasma formation. *Mlh1* null mutant mice of both sexes are sterile and, while meiosis progresses normally until synapsis, the paired chromosomes fall apart into univalents as the SCs disappear (Woods *et al.* 1999), a stage at which homologous chromosome pairs are usually held together by chiasmata. *Mlh1* mutants in yeast are also defective in crossing over (Hunter and Borts 1997).

Analysis of MLH1 foci thus represents a potentially valuable resource for the direct study of meiotic recombination. Since the pachytene-stage SC is physically longer than the typical diakinesis-stage bivalent, this technique also offers greater precision in localizing exchange positions (Anderson *et al.* 1999). Here, we present results from a study applying the analysis of MLH1 foci to detect meiotic recombination events in male mice of four different inbred strains. Our results confirm the utility of MLH1 foci as a marker of meiotic exchanges in mice. Further, we find significant differences in the levels of meiotic exchange among the inbred strains, demonstrating substantial genetic variability in this fundamentally important process.

MATERIALS AND METHODS

Mice: Breeding stock of four inbred strains, C57BL/6, A/J, CAST/Ei, and SPRET/Ei, were housed in Thoren ventilated rack caging in a pathogen-free facility and maintained by brother × sister matings.

Immunostaining: The technique used for making surface-spread synaptonemal complex preparations has been described previously (Peters *et al.* 1997). The immunostaining protocol is similar to that of Anderson *et al.* (1999). Primary antibodies used were rabbit polyclonal antibody against human MLH1 (Calbiochem, San Diego) and goat antibody raised

against rat SCP3 (a component of the lateral elements of the synaptonemal complex, graciously provided by Terry Ashley). Secondary antibodies used were fluorescein-labeled donkey anti-rabbit and rhodamine-labeled donkey anti-goat (Jackson ImmunoResearch, West Grove, PA).

All incubations were performed in a 37° humid chamber. All dilutions were made into 1× ADB [10× stock consisted of 10 ml normal donkey serum (Jackson ImmunoResearch), 3 g BSA (Sigma, St. Louis), 50 μl Triton-X 100, and 90 ml 1× PBS that was then sterile filtered with a 45-μm filter and diluted with PBS].

Antibodies were applied in the following order: MLH1 primary antibody, diluted 1:75 and incubated overnight; column-purified SCP3 primary antibody, diluted 1:50 and incubated for 2 hr; MLH1 secondary antibody, diluted 1:75 and incubated overnight; SCP3 secondary antibody, diluted 1:100 and incubated for 45 min. Slides were then washed with PBS and stained with 4′,6-diamidino-2-phenylindole (DAPI).

Fluorescence microscopy and digital imaging: Slides were examined on a Zeiss Axiophot epifluorescence microscope and imaged with a CCD camera and computer using Vysis Quips PathVysion SmartCapture VP 1.4 software (Digital Scientific).

Scoring: Two independent observers scored blind-coded digital images (at ~×2500 magnification) of each pachytene spermatocyte for the number of autosomal MLH1 foci on every SC; if the observers did not agree on the number of foci present, the cell was discarded. Only autosomal MLH1 foci were analyzed, because the appearance and disappearance of the focus on the XY bivalent and on the autosomes are temporally uncoupled; however, the presence or absence of the XY focus was also noted. Cells were staged according to the criteria described by Moses (1980). Cells with <19 autosomal foci were never observed.

In one strain (SPRET/Ei), we observed a small increase in the number of MLH1 foci in late-stage pachytene cells by comparison with earlier pachytene cells. However, no significant stage-related differences were detected in any of the other strains; thus, in analyzing the data, we pooled information from all pachytene stages for each inbred mouse strain.

Test for interindividual variation: Bartlett's test (Bartlett 1937) was used to determine if males within each mouse strain had constant variance. SPRET/Ei was the only strain that did not have constant variance across individuals ($P = 0.0004$), presumably due to increased variance in two of the older mice included in this group. Because of the nonconstant variance across individuals in the SPRET/Ei mice, a standard ANOVA test could not be used as a test for interindividual variation. Instead, a permutation test with an ANOVA-like F-statistic (Manly 1997) was used to determine if there was evidence for interindividual variation in the total number of autosomal MLH1 foci per cell for the four inbred mouse strains. Each strain was tested separately using 10,000 replications.

Measurements: SCs with two MLH1 foci were measured in a subset of cells from the CAST/Ei and SPRET/Ei inbred strains using the freeware computer application MicroMeasure version 3.3 (available on the internet at http://www.colostate.edu/Depts/Biology/MicroMeasure). Relative interfocus distances were calculated as the distance between the two foci expressed as a percentage of the total SC length. For these determinations, the relative centromere-to-MLH1 focus distances (as a percentage of SC length) were first calculated by MicroMeasure, and the absolute value of the difference between these measurements for two foci on the same SC was taken as the relative interfocus distance. MicroMeasure was also used to measure and rank the lengths of all autosomal SCs in the 11 cells containing an SC with zero foci.

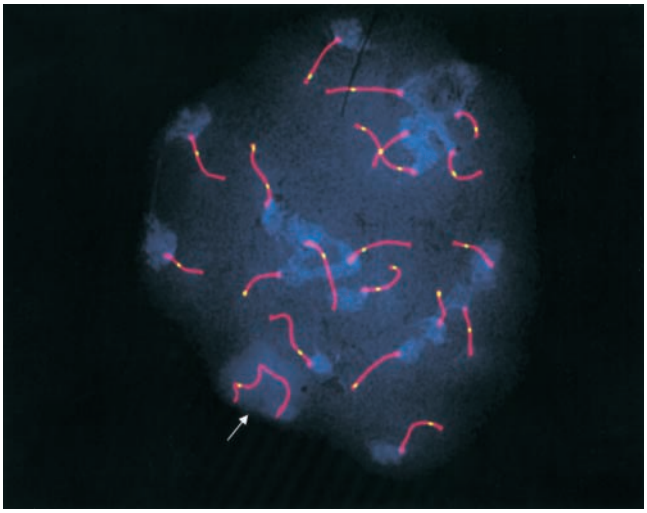

Figure 1.—A typical male pachytene spermatocyte from a CAST/Ei mouse. MLH1 foci are in green, SCP3 (a component of the lateral elements of the synaptonemal complex) is in red, and DNA (DAPI stained) is in blue. There are 23 foci in all: 16 autosomal SCs with 1 MLH1 focus; three autosomes with 2 foci; and the sex bivalent (arrow), which has a single focus in the pseudoautosomal region. All mouse autosomes are acrocentric, and the DAPI-bright "swirls" at one end of each SC represent pericentromeric heterochromatin.

RESULTS

We studied a minimum of five males from each of four inbred strains: C57BL/6, A/J, and the wild inbred strains CAST/Ei and SPRET/Ei. A representative pachytene spermatocyte is pictured in Figure 1 and an overview of the data is presented in Table 1. In all, 652 pachytene-stage spermatocytes were imaged for this study, but only 538 (82.5%) were analyzable. Reasons for discarding cells included high background, unclear cell boundaries, and an inability of independent observers to agree on the number of MLH1 foci present (see materials and methods). On average, 24.5 pachytene cells per animal were imaged for analysis.

The mean number of autosomal MLH1 foci for all 538 cells was 23.8 (range 19–32). Assuming that one focus is the precursor of one chiasma, and that every chiasma is the equivalent of 50 cM, we infer an overall autosomal genetic length of 1190 cM in the male mouse, which is consistent with previous cytogenetic and molecular data (Lawrie *et al.* 1995; Dietrich *et al.* 1996).

Furthermore, the distribution of MLH1 foci was nonrandom and consistent with positive interference, a well-known property of chiasmate meioses. For example, to determine whether two foci on the same SC displayed crossover interference, we measured the autosomal SCs in a subset of cells (15 from two CAST/Ei males and 17 from two SPRET/Ei males) using MicroMeasure, a computer program designed for linear chromosome measurement (Reeves and Tear 2000). In this subset of cells, we identified 146 autosomal SCs with two MLH1

TABLE 1

Mean number of autosomal MLH1 foci per cell

Strain	Male	Age (mo)	No. cells scored	Range	Mean no. autosomal foci	SD
CAST/Ei	1	7.5	24	19–25	21.5	1.6
	2	3	24	19–27	21.8	1.9
	3	3	24	19–24	21.4	1.4
	4	8	17	19–23	21.2	1.4
	5	4	22	19–24	21.3	1.5
Total			111	19–27	21.5	1.6
A/J	1	4	26	20–29	24.4	2.2
	2	4	32	20–29	23.2	2.0
	3	4.5	16	20–27	23.9	2.0
	4	8	25	20–26	22.4	1.6
	5	8	24	21–29	24.5	1.9
Total			123	20–29	23.6	2.1
C57BL/6	1	9	33	20–28	24.0	1.9
	2	6.5	21	21–28	23.6	1.9
	3	8.5	29	21–28	24.6	2.0
	4	3	20	22–28	24.8	2.0
	5	3	19	21–29	24.6	1.8
Total			122	20–29	24.3	2.0
SPRET/Ei	1	10	24	20–28	24.8	2.1
	2	10	37	22–30	24.7	2.0
	3	11	25	22–29	25.4	1.8
	4	12	18	23–29	25.1	1.5
	5	26.5	34	20–31	24.4	3.1
	6	24.5	22	21–29	24.5	2.0
	7	21.5	22	20–32	26.3	3.3
Total			182	20–32	24.9	2.4
Total for all strains			538	19–32	23.8	2.4

foci (43 from CAST/Ei cells and 103 from SPRET/Ei cells), and for each of these we calculated the relative interfocus distance as a percentage of SC length (see MATERIALS AND METHODS). Assuming that foci are placed randomly on the same SC, we would expect that, on average, this interfocus distance would be 33% of the total length of the SC (ANDERSON *et al.* 1999). However, the mean interfocus distance for the CAST/Ei SCs was 62.2%, and 41/43 SCs had interfocus distances >33%; for the SPRET/Ei SCs, the mean interfocus distance was 58.4%, and 100/103 SCs had interfocus distances >33%. Clearly, foci were nonrandomly positioned on SCs in both strains.

Thus, our observations support previous findings for positive interference in the distribution of exchange events within the mouse genome (*e.g.*, LAWRIE *et al.* 1995; BROMAN *et al.* 2002). More importantly, in combination with our observations on the overall number of MLH1 foci per cell, they confirm previous reports that MLH1 marks the sites of genetic exchange (BAKER *et al.* 1996; BARLOW and HULTEN 1998; ANDERSON *et al.* 1999). Therefore, we conclude that MLH1 focus analysis is a valid method for studying meiotic crossover patterns in mammals.

Distributions of autosomal MLH1 foci among individual inbred mice: We analyzed 111 pachytene nuclei from five CAST/Ei males, 123 pachytene nuclei from five A/J males, 122 pachytene nuclei from five C57BL/6 males, and 182 pachytene cells from seven SPRET/Ei males. The range and mean number of autosomal MLH1 foci observed for each male are presented in Table 1 and the distributions for each are illustrated in Figure 2.

A permutation test (see MATERIALS AND METHODS) provided evidence for significant interindividual variation in the total number of MLH1 foci among the A/J mice ($P < 0.0001$). From Figure 2B, it can be seen that the distributions of A/J mice 1, 3, and 5 were essentially identical, but that males 2 and 4 were somewhat different. No significant interindividual variation was observed for any of the other three inbred strains.

Distributions of autosomal MLH1 foci among inbred strains: The mean number of autosomal MLH1 foci per cell at pachytene was 21.5 from CAST/Ei with a range of 19–27, 23.6 in A/J with a range of 20–29, 24.3 in C57BL/6 with a range of 20–29, and 24.9 in SPRET/Ei with a range of 20–32 (Table 1). Distributions for the four strains are given in Figure 3.

We tested for significant differences among strains; due to the significant interindividual variation among the A/J males, interstrain comparisons were performed

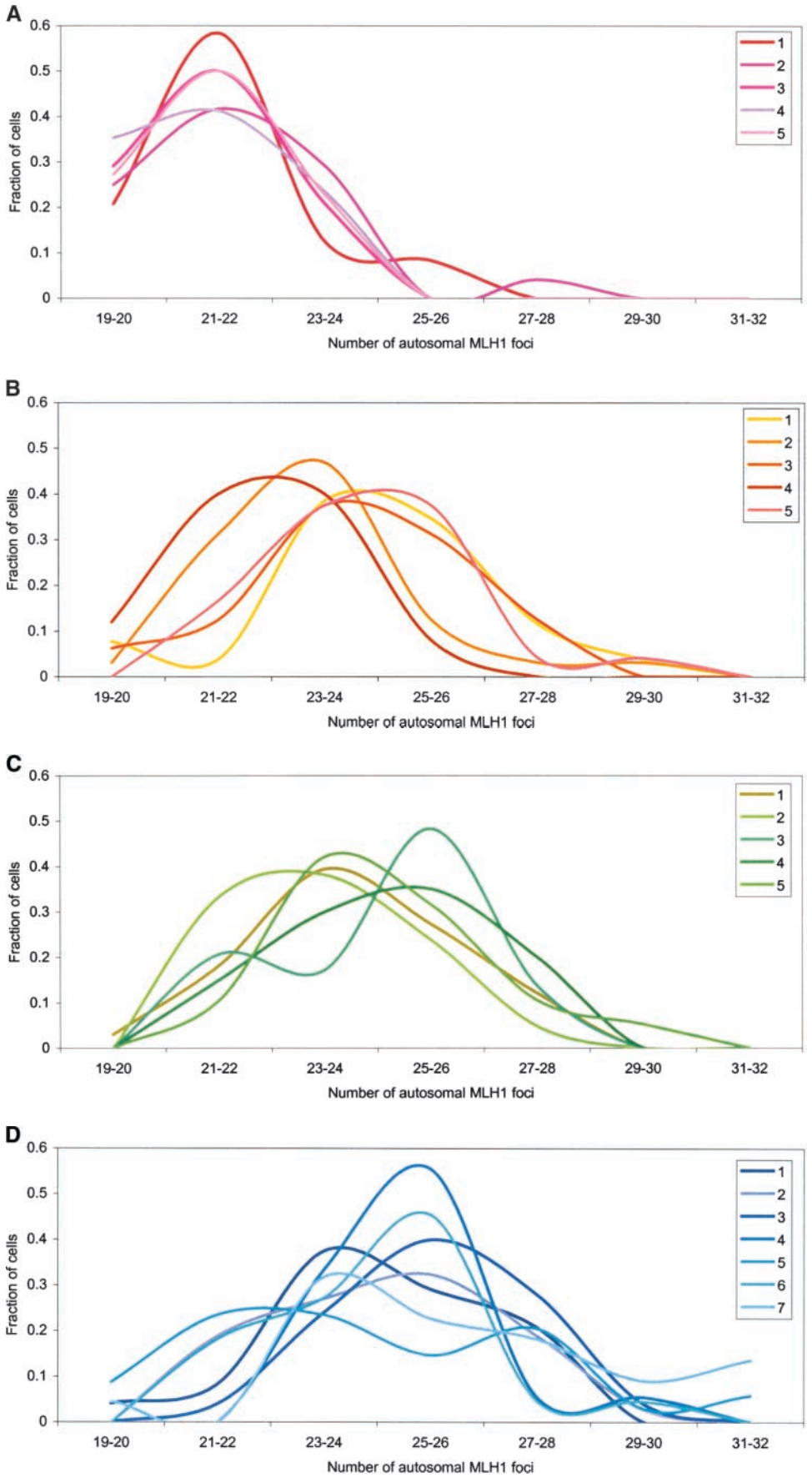

FIGURE 2.—Variation in exchange frequency among individual male mice of four inbred strains. The distribution of autosomal MLH1 foci per cell is presented for each animal studied in the following inbred mouse strains: (A) CAST/Ei; (B) A/J; (C) C57BL/6; (D) SPRET/Ei. The only strain to exhibit significant interindividual variation was A/J ($P < 0.0001$).

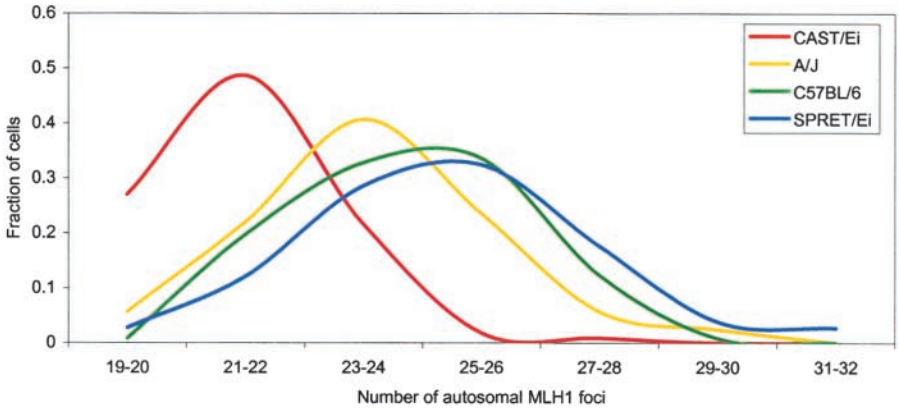

FIGURE 3.—Variation in exchange frequency among four inbred mouse strains. The mean numbers of autosomal exchanges (MLH1 foci) were significantly different among the strains. CAST/Ei had the lowest mean value and the smallest variance, suggesting that it may have a degree of crossover interference higher than that of the other inbred strains.

both with and without this strain. The mean numbers of autosomal MLH1 foci in the strains SPRET/Ei, C57BL/6, and CAST/Ei were highly significantly different from each other ($F = 102.0$; $P = 1.1 \times 10^{-36}$). When A/J males were included in the analysis, the effect was even more significant ($F = 68.5$; $P = 1.7 \times 10^{-37}$).

To determine whether these differences in mean focus number might be due to differing patterns of exchange between strains, we randomly selected five cells from each CAST/Ei and SPRET/Ei male, the two strains with the lowest and highest mean numbers of autosomal MLH1 foci, respectively. For these, the location of each focus was visually classified as centromeric, proximal, medial, distal, or terminal. Centromeric and terminal foci were defined as being within one focus's width of the end of the SC; the rest of the SC was divided into thirds. Single- and double-exchange SCs were considered separately. Our analysis revealed no obvious differences between the two strains, as neither single nor double exchanges displayed significantly different placement patterns (Figure 4). In fact, for both strains the placements were typical of the standard expectations for single- and double-crossover distributions (reviewed in HAWLEY 1988).

The effect of age on recombination frequency: To determine whether recombination patterns change with age, we attempted to study several animals per strain from the age categories of 3–4 months and 8–10 months. The only strain for which animals from the 3- to 4-month-old category were unavailable was SPRET/Ei; this strain also includes an additional category of very aged mice (~2 years). We did not observe any significant variation in recombination frequency with age in any strain studied (Figure 5), and the 2-year-old SPRET/Ei males had the same mean autosomal number of MLH1 foci as the 10- to 12-month-old SPRET/Ei animals (24.9). However, the variances of two of the three 2-year-old males' distributions were much larger than those of the younger animals (SPRET/Ei males 5 and 7; see Table 1); because of this, SPRET/Ei was the only strain to fail Bartlett's test for constant variance ($P = 0.0004$).

Analysis of nonexchange SCs: From the 538 cells included in this study, we analyzed a total of 10,222 autosomal synaptonemal complexes. Overall, the frequencies of autosomal SCs with one, two, or three MLH1 foci in our data set were 74.9, 24.7, and 0.3%, respectively; only 11 of the 10,222 SCs (0.1%) were judged to be lacking an MLH1 focus (Table 2). Thus, nonexchange or achiasmate chromosome pairs are extremely rare in male mice.

We then asked whether these nonexchange SCs were the shortest complexes in their respective cells, since as a general rule, shorter chromosomes have fewer crossovers than do longer ones (MATHER 1936; LAWRIE *et al.* 1995). Using MicroMeasure (REEVES and TEAR 2000), we measured all autosomal SCs in each of the 11 cells containing an SC with zero MLH1 foci. Autosomal SCs in each cell were ranked 1–19, from longest to shortest. Of the SCs without foci, two were rank 7, three were rank 14, one was rank 15, two were rank 16, one was rank 18, and two were rank 19. Thus, although many SCs without foci ranked in the bottom third in terms of length, they were by no means the shortest SCs in their respective cells.

DISCUSSION

Genetic background strongly influences the recombination rate in mice: Our observations add to preliminary observations (BARLOW and HULTEN 1998; ANDERSON *et al.* 1999) that MLH1 foci mark the sites of meiotic exchanges in mammals and provide direct evidence that the frequency of meiotic exchange varies with genetic background in mice. In analyses of male meiosis in four inbred strains, we identified strains with "low" (CAST/Ei), "medium" (A/J), and "high" (C57/BL6, SPRET/Ei) levels of recombination; the mean number of exchanges per cell varied by ~15% between the low and high strains. The difference was not attributable to variation in the number of achiasmate bivalents or bivalents with multiple exchanges. Indeed, almost all bivalents contained either one or two exchanges, regardless of strain; thus, the among-strain variation was simply due to differing proportions of one- and two-exchange bivalents.

Our results also resolve ambiguities surrounding pre-

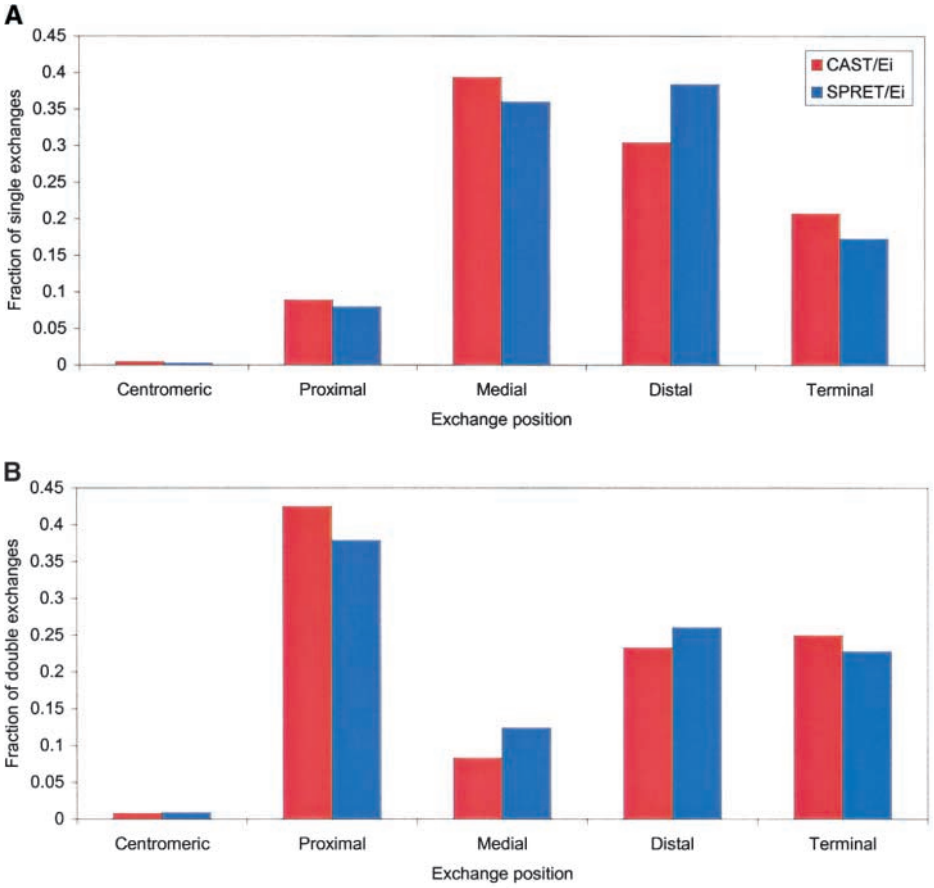

FIGURE 4.—Comparison of the pattern of exchanges between CAST/Ei and SPRET/Ei strains. The physical locations of exchanges (MLH1 foci) along the SC were classified as centromeric, proximal, medial, distal, or terminal (see MATERIALS AND METHODS). No significant differences were noted in the general patterns of exchange placement between these two inbred mouse strains for either (A) single or (B) double exchanges.

vious meiotic studies of male mice by demonstrating that genetic background is a major determinant of overall recombination rate. This indicates that at least part of the difficulty in precisely defining exchange frequencies has been the use of a variety of inbred, outbred, randombred, and F_1 animals in the experiments (HENDERSON and EDWARDS 1968; POLANI 1972; LUTHARDT *et al.* 1973; POLANI and JAGIELLO 1976; SPEED 1977; JAGIELLO and FANG 1979; LAWRIE *et al.* 1995).

Comparison with previous cytogenetic studies of exchange frequency in the male mouse: It is difficult to compare our data with those of previous chiasma-based studies in mice, especially because the only commonality is a single inbred strain in one report—that of SPEED (1977), who also examined C57BL/6 males. He observed a mean number of 22.1 recombination events (excluding the obligate XY focus) per cell, compared to 24.3 in our study. The discrepancy could reflect substrain differences, genetic drift over the intervening 25 years, or artifactual differences. However, we favor the suggestion of ANDERSON *et al.* (1999) that the increased length of the synaptonemal complex over the diakinesis bivalent makes the MLH1 focus analysis method of estimating exchange frequency more accurate than that of chiasma counts.

Indeed, previous chiasma count studies have varied greatly in their success in accurately counting and lo-

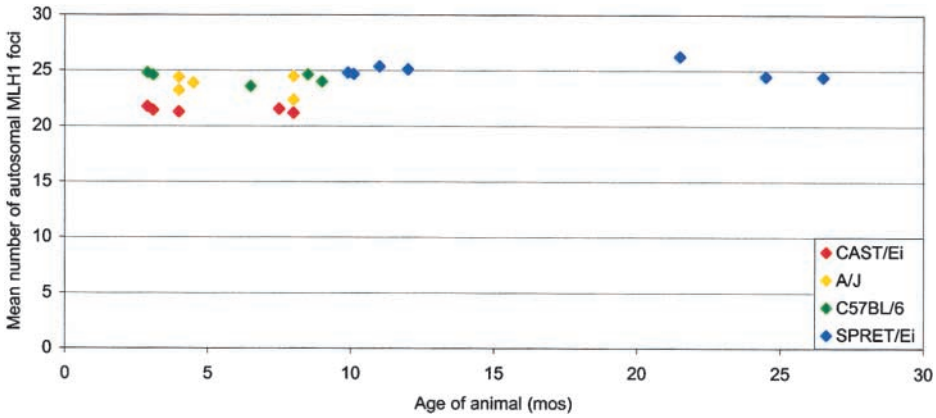

FIGURE 5.—The relationship between age and mean number of exchanges (MLH1 foci) for four inbred strains. No obvious age effect was observed.

TABLE 2

Frequency of autosomal SCs with zero to three MLH1 foci

Strain	Male	Age (mo.)	No. SCs scored	Frequency of SCs with 0 foci	1 focus	2 foci	3 foci
CAST/Ei	1	7.5	456	0	395	61	0
	2	3	456	1	388	67	0
	3	3	456	1	397	57	1
	4	8	323	1	284	38	0
	5	4	418	0	368	50	0
Total			2,109	3	1832	273	1
				0.001%	0.869%	0.129%	<0.001%
A/J	1	4	494	1	353	139	1
	2	4	608	0	474	133	1
	3	4.5	304	0	226	78	0
	4	8	475	3	386	85	1
	5	8	456	1	323	132	0
Total			2,337	5	1762	567	3
				0.002%	0.754%	0.243%	0.001%
C57BL/6	1	9	627	0	465	159	3
	2	6.5	399	1	301	97	0
	3	8.5	551	1	387	162	1
	4	3	380	0	267	110	3
	5	3	361	0	256	104	1
Total			2,318	2	1676	632	8
				<0.001%	0.723%	0.273%	0.003%
SPRET/Ei	1	10	456	0	317	139	0
	2	10	703	1	497	200	5
	3	11	475	0	318	155	2
	4	12	342	0	233	108	1
	5	26.5	646	0	466	176	4
	6	24.5	418	0	299	118	1
	7	21.5	418	0	262	152	4
Total			3,458	1	2392	1048	17
				<0.001	0.691	0.303	0.005
Total for all strains			10,222	11	7662	2520	29
				0.001%	0.75%	0.247%	0.003%

calizing chiasmata; even the best preparations often contain bivalents whose crossover status is at best ambiguous. Some investigators have simply not been successful in visualizing chiasmata (Speed and Chandley 1983); some have attempted to estimate the number of chiasmata solely on the shape of the bivalent (Slizynski 1960); others have reported number but not position; and those that have reported location have usually physically divided the bivalent into only a few intervals. In contrast, the use of MLH1 foci to localize exchange events has several distinct advantages, including the comparative ease of preparation, the availability of a large number of pachytene-stage cells, and the increased resolution afforded by analysis of the SC. Clearly, these analyses must be cautiously interpreted, since we cannot exclude the possibility that some pachytene-stage cells will be eliminated before reaching diakinesis/metaphase I. This concern notwithstanding, we predict that examination of MLH1 foci at pachytene in other genetic backgrounds and in hybrid crosses of mice will constitute a more reliable approach to analyzing meiotic recombination rates than those previously published.

Only one previous study has used MLH1 foci in mouse pachytene nuclei to perform a detailed analysis of meiotic recombination (Anderson *et al.* 1999). These investigators reported a mean of 21.7 ± 2.3 MLH1 foci per autosomal complement in C57BL/6 males, substantially lower than our value of 24.3 ± 2.0. In large part, the difference between the two studies reflects variation in the proportion of SCs scored as having no MLH1 foci, *i.e.*, from ~4% in the study of Anderson *et al.* (1999) to 0.1% in the present study. There is no obvious explanation for this discrepancy, but technical differences such as different imaging systems, or differences in sample preparation or staining, may have influenced the results (see materials and methods; Anderson *et al.* 1999). Clearly, additional analyses will be needed to resolve this issue.

Crossover interference and exchange distribution in the male mouse: Crossover interference has two important consequences (Muller 1916): first, virtually all SCs receive at least one exchange event (Mather 1936) and, second, on SCs with multiple exchanges, the foci are consistently spaced farther apart than would be expected if the distribution were random. The exchange distributions observed in our study fell into the typical pattern exhibited in most organisms (Hawley 1988). In addition, the lower the mean number of autosomal MLH1 foci per cell, the more SCs with single foci and the fewer SCs with multiple foci were observed (Table 2). Conversely, inbred strains with higher mean numbers of autosomal MLH1 foci (*e.g.*, SPRET/Ei) had more multiple-focus SCs and fewer single-focus SCs.

In an attempt to further characterize differences underlying the observed genetic variation in meiotic exchange frequency, we examined both the pattern of exchange placement and the relative interfocus distance (between two foci on the same SC) in strains with the lowest and highest mean numbers of autosomal MLH1 foci per cell, CAST/Ei and SPRET/Ei, respectively. We could not find any significant differences between these strains. Nonetheless, there is a general expectation that the lower the exchange frequency, the higher the degree of crossover interference (nonrandomness). CAST/Ei clearly had both the least random crossover distribution and the lowest exchange frequency of the four inbred strains studied (Figure 3).

The effects of age on genetic exchange: The possibility that age might influence mammalian recombination rates has been a contentious subject, with several groups suggesting an age-related decline in recombination in oocytes from older mice (Luthardt *et al.* 1973; Polani and Jagiello 1976; Speed 1977), hamsters (Sugawara and Mikamo 1983), or humans (Tanzi *et al.* 1992). However, there is little evidence for paternal age-related effects on recombination in mammals (*e.g.*, Broman *et al.* 1998) and, in our study as well, we found no significant effect of age on overall recombination rate (Figure 5).

However, the results for three SPRET/Ei males of advanced age (~2 years) were intriguing. While the mean exchange frequency per cell (24.9) was identical to that of younger SPRET/Ei animals, the range of values for two of the three animals was remarkable (Table 1). We observed several cells with unusually high recombination frequencies (30–32 MLH1 foci), as well as a few cells containing two-focus SCs where the two MLH1 foci were quite close together, a situation that was never observed among younger males. Thus, it may be that, with age, spermatocytes lose their ability to implement tight genetic control over exchange. However, additional analyses of a more extensive series of aged animals will be necessary to confirm or refute these initial observations.

Recombination failure in the male mouse: On the basis of the analysis of >10,000 individual autosomal synaptonemal complexes, we estimate that ~0.1% of all autosomal bivalents are achiasmate, with shorter chromosomes more likely to be involved than larger ones. This value seems to be shared by males of different genetic backgrounds, as the frequency of SCs with zero foci was similar among the four inbred strains we analyzed (Table 2). Thus, strains with low levels of recombination (*e.g.*, CAST/Ei) appear to be no more likely to have achiasmate bivalents than do strains with high levels of recombination (*e.g.*, C57BL/6 and SPRET/Ei).

Our results also imply that, regardless of strain, ~1 in 50 spermatocytes will contain an achiasmate autosome. The biological consequences of this situation are not clear since, in an otherwise chromosomally normal male, it is not known whether the presence of a single unpaired autosome will interfere with the completion of meiosis (*e.g.*, Eaker *et al.* 2001). However, under the simple assumption that such cells produce functional gametes and that the achiasmate bivalents segregate randomly, our results suggest that ~1% of spermatocytes may be aneuploid. This is consistent with previous observations of the frequency of nondisjunction in the male mouse (Bean *et al.* 2001; Marchetti *et al.* 2001; T. Hassold, unpublished observations).

The origin, frequency, and fate of achiasmate bivalents in the female mouse, as in other organisms, may be markedly different than those in the male mouse (Hawley *et al.* 1993; LeMaire-Adkins *et al.* 1997; Koehler and Hassold 1998). Thus, it is difficult to generalize from our observations. Nevertheless, our results suggest that—at least for the male mouse—special mechanisms that segregate achiasmate chromosomes are unlikely to be an important feature of the meiotic process.

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