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Report

Sex, Not Genotype, Determines Recombination Levels in Mice

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Recombination, the precise physical breakage and rejoining of DNA between homologous chromosomes, plays a central role in mediating the orderly segregation of meiotic chromosomes in most eukaryotes. Despite its importance, the factors that control the number and placement of recombination events within a cell remain poorly defined. The rate of recombination exhibits remarkable species specificity, and, within a species, recombination is affected by the physical size of the chromosome, chromosomal location, proximity to other recombination events (i.e., chiasma interference), and, intriguingly, the sex of the transmitting parent. To distinguish between simple genetic and nongenetic explanations of sex-specific recombination differences in mammals, we compared recombination in meiocytes from XY sex-reversed and XO females with that in meiocytes from XX female and XY male mice. The rate and pattern of recombination in XY and XO oocytes were virtually identical to those in normal XX females, indicating that sex, not genotype, is the primary determinant of meiotic recombination patterns in mammals.

Early linkage studies of such evolutionarily diverse species as *Drosophila melanogaster* (Morgan 1912), *Bombyx mori* (Haldane 1922), and mouse (Dunn 1920) provided evidence of sex-specific differences in recombination. Subsequently, it has become clear that, when recombination occurs in both sexes of an organism, one sex usually has a higher overall rate than that of the other. For example, female recombination rates are significantly higher than male rates in human, dog, pig, zebrafish, and most mouse strains (Donis-Keller et al. 1987; Mikawa et al. 1999; Neff et al. 1999; Singer et al. 2002; K. Koehler, A. Lynn, and T. Hassold, unpublished data), whereas the opposite is true for sheep and wallaby (Crawford et al. 1995; Zenger et al. 2002). In virtually all such organisms, however, sex not only affects the rate of recombination but also profoundly influences the placement of exchange events along the length of the chromosome. For example, the overall rate of recombination in the human female is nearly twice that of the male, but males exhibit significantly higher rates of recombination in telomeric regions than females do (Donis-Keller et al. 1987; Broman et al. 1998; Kong et al. 2002; Matise et al. 2003).

What is the basis for these sex-specific patterns? Conceptually, the possibilities can be separated into two broad categories. The differences could be strictly genetic, with germ cells following recombination programs dictated by sex-chromosome genotype—that is, regardless of other factors, XY germ cells behave as males and XX germ cells behave as females. Alternatively, the differences could be attributed to sex-specific factors—that is, the primary determinant of recombination is the environment in which the germ cell finds itself rather than its genotype.

It is known that, in *Drosophila,* the addition of a Y chromosome to an otherwise normal female fly has no effect on recombination levels (Merriam 1967). However, to our knowledge, in vertebrates, the basis of sexual dimorphism in recombination rates has been examined previously only in a species in which environmental triggers can cause functional sex reversal (Yamamoto 1961; Kondo et al. 2001). Specifically, in the medaka, a small freshwater teleost native to Asia, the feeding of hormones to larvae can induce sex reversal, producing XX males and XY females. Genetic maps of normal XY male and XX female medaka show "typical" sex-specific recombination patterns: overall, the female map is longer than the male map, with males showing greater recombination rates in the distal regions of the chromosome

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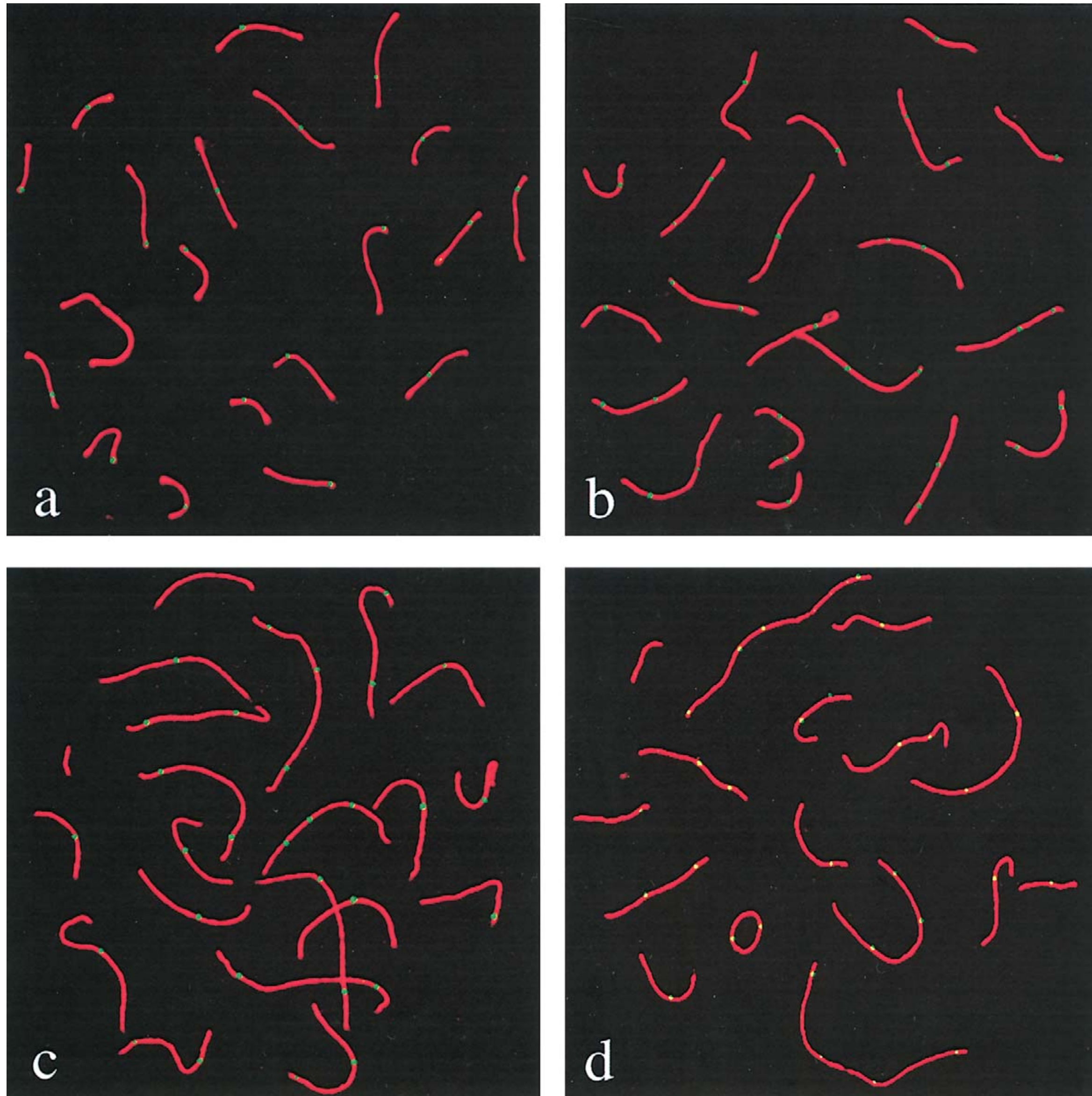

Figure 1 Pachytene-stage cells from an XY^{POS} male (*a*), XX female (*b*), XY^{POS} female (*c*), and XO female (*d*). The SC is identified by an antibody against SCP3 (MIM 604759) (*in red*), and recombinational sites are detected by an antibody against MLH1 (*in green*). The breeding scheme used to generate XY^{POS} and XO female mice has been described elsewhere (Eicher et al. 1982). In brief, it has been demonstrated that sex reversal for XY^{POS} female mice can be complete (i.e., the C57BL/6J XY^{POS} mice are phenotypically female, with two ovaries) or incomplete (i.e., the C57BL/6J XY^{POS} mice are hermaphrodites, with either two ovotestes or an ovary and a contralateral ovotestis). Because some of the C57BL/6J XY^{POS} hermaphrodites have sufficient testicular tissue to be fertile, these mice can be mated to normal C57BL/6J female mice to produce offspring. Only C57BL/6J XY^{POS} mice that appeared completely sex reversed (i.e., they have normally positioned ovaries, with no evidence of testicular tissue) were included as XY^{POS} females in our studies. XO female mice were produced by mating normal C57BL/6J females to C57BL/6J males carrying the Y* chromosome. C57BL/6J males carrying the Y* chromosome have been shown to be prone to meiotic sex-chromosome nondisjunction, and such matings produce ~20% XO female offspring (Hunt 1991). Oocytes were obtained from either E17 or E18 (newborn) female embryos, whereas spermatocytes were obtained from mature males of known fertility. Care and husbandry of all mice were within the guidelines specified by Institutional Animal Care and Use Committee protocols. Surface-spread SC preparation and immunostaining for MLH1 and SCP3 were done as described elsewhere (Koehler et al. 2002). FISH using a mouse X-chromosome paint probe (Vysis) was employed to identify the XX or XY bivalent in all cells, and, for each pachytene-stage cell, two independent observers scored the total number of MLH1 foci per bivalent. The genotypes of putative XY female mice were confirmed by PCR-based genotyping of *Smcx* (MIM 314690) and *Smcy* (MIM 426000) to detect the presence of a Y chromosome, as described elsewhere (Bean et al. 2001).

and females showing greater recombination in the central regions (Kondo et al. 2001). In sex-reversed XY female medaka, recombination patterns are remarkably similar to those in control females (Kondo et al. 2001). Thus, in this species, sex-specific recombination rates are more strongly influenced by factors intrinsic to the sex of the animal than by genetic differences.

In mammals, functional sex reversal is virtually nonexistent, and deviations in sex-chromosome constitution typically result in sterility. Nevertheless, because meiotic recombination occurs during fetal development in the female, the role of sex-chromosome constitution in setting the recombination patterns can be tested using appropriate mouse models. Specifically, to determine whether the presence of a Y chromosome alters the female recombination pattern, we took advantage of the fact that the transfer of a *Mus domesticus poschiavinus*–derived Y chromosome ("Y^{POS}") onto the C57BL/6J background results in abnormal gonadal development and sex reversal in the majority of C57BL/6J XY^{POS} offspring (Eicher et al. 1982; Albrecht et al. 2000). In addition, to assess the role of X-chromosome dosage, we generated female mice carrying a single X chromosome ("XO" mice) on the C57BL/6J inbred background (see fig. 1 legend).

Fertility is impaired in both XY^{POS} and XO females; XY^{POS} females are sterile, and XO females have a marked reduction in reproductive lifespan. Nevertheless, the prenatal meiotic events of synapsis and recombination proceed normally. Thus, the level and pattern of recombination in these animals can be directly assessed using recently developed immunohistological methods, to examine exchanges in meiocytes (which are visualized as MLH1 [MIM 120436] foci on synaptonemal complexes [SCs] in pachytene-stage spermatocytes or oocytes—e.g., see the work of Anderson et al. [1999], Koehler et al. [2002], and Lynn et al. [2002]).

We examined pachytene-stage cells from four to eight mice from each of four genotype-phenotype combinations: XY^{POS} males, XX females, sex-reversed XY^{POS} females, and XO females. In addition, data from five standard C57BL/6J XY males (see Koehler et al. 2002) were compared with data from XY^{POS} males, to confirm that the Y^{POS} chromosome does not alter recombination patterns in C57BL/6J males. Representative images of pachytene cells from each genotype-phenotype combination are presented in figure 1. The first question we asked was whether the number of autosomal MLH1 foci per cell (i.e., the overall exchange rate) in the XY^{POS} females fit male or female distributions or displayed an intermediate phenotype. The results (table 1) were straightforward: the mean number of exchanges (MLH1 foci) in XY^{POS} females (26.7 ± 3.5) and XO females (27.4 ± 4.4) approximated that of normal females (26.1 ± 3.8) but was highly significantly increased over that of normal males (24.6 ± 2.6) (for XY^{POS} females vs. normal males, $t = 4.7$, $P < .01$; for XO females vs. normal males, $t = 3.6$, $P < .01$). When we examined MLH1 counts on individual chromosomes, we found that the proportions of XY^{POS} female chromosomes with zero, one, two, and three MLH1 foci (0.01, 0.60, 0.37, and 0.02, respectively) were similar to those of XX females (0.01, 0.62, 0.36, and 0.01) but were significantly different from those of XY^{POS} males (0.00, 0.71, 0.28, and 0.01; $\chi^2 = 56.6$; $P < .01$). In addition, the XO females also demonstrated proportions (0.02, 0.56, 0.37, and 0.05) more similar to those of the XX females than to those of XY^{POS} males ($\chi^2 = 53.4$; $P < .01$).

Table 1

Total Number of Autosomal MLH1 Foci

Genotype and Phenotype	No. of Cells	Average No. of Foci	SD	Range
C57Bl/6J XY^{POS} male	116	24.6	2.6	19–32
C57Bl/6J XX female	77	26.1	3.8	19–34
C57Bl/6J XY^{POS} female	75	26.7	3.5	20–35
C57Bl/6J XO female	14	27.4	4.4	22–36

NOTE.—The total numbers of mice were six XX females, eight XY^{POS} females, four XY^{POS} males, and two XO females.

Since male-female differences in recombination rates are reflected not only in the total number of recombination events but also in their placement along the chromosome, we evaluated the location of MLH1 foci along the SC in each of the four genotype-phenotype combinations. SC lengths were measured (in microns) using MicroMeasure 3.3 (see MicroMeasure Support Site), and the positions of MLH1 foci in relation to the centromere were recorded. These distributions are presented for SCs with one (fig. 2*a*) or two (fig. 2*b*) MLH1 foci. From these histograms, it is apparent that the placement of exchanges in both XY^{POS} and XO females was similar to that in normal XX females but was markedly different from that in XY^{POS} males. That is, for SCs with a single exchange, the distributions for phenotypic females followed a normal bell-shape distribution, whereas, in the phenotypic males, telomeric locations were overrepresented. Specifically, ~20% of male exchanges but only 4% of female exchanges occurred in the distal 10% of the SC. Similarly, SCs with two exchanges showed a comparable sex-specific effect (fig. 2*b*). Thus, XY^{POS} and XO females behaved like normal females with respect to both the location and the number of exchanges.

There are numerous sex-related differences in mammalian meiosis that could potentially influence recombination patterns—for example, temporal differences in initiation and progression of meiosis (e.g., see Hassold and Hunt 2001), differences attributable to imprinted regions (Robinson and Lalande 1995), and differences in pairing and synapsis of homologs (e.g., see Tease and

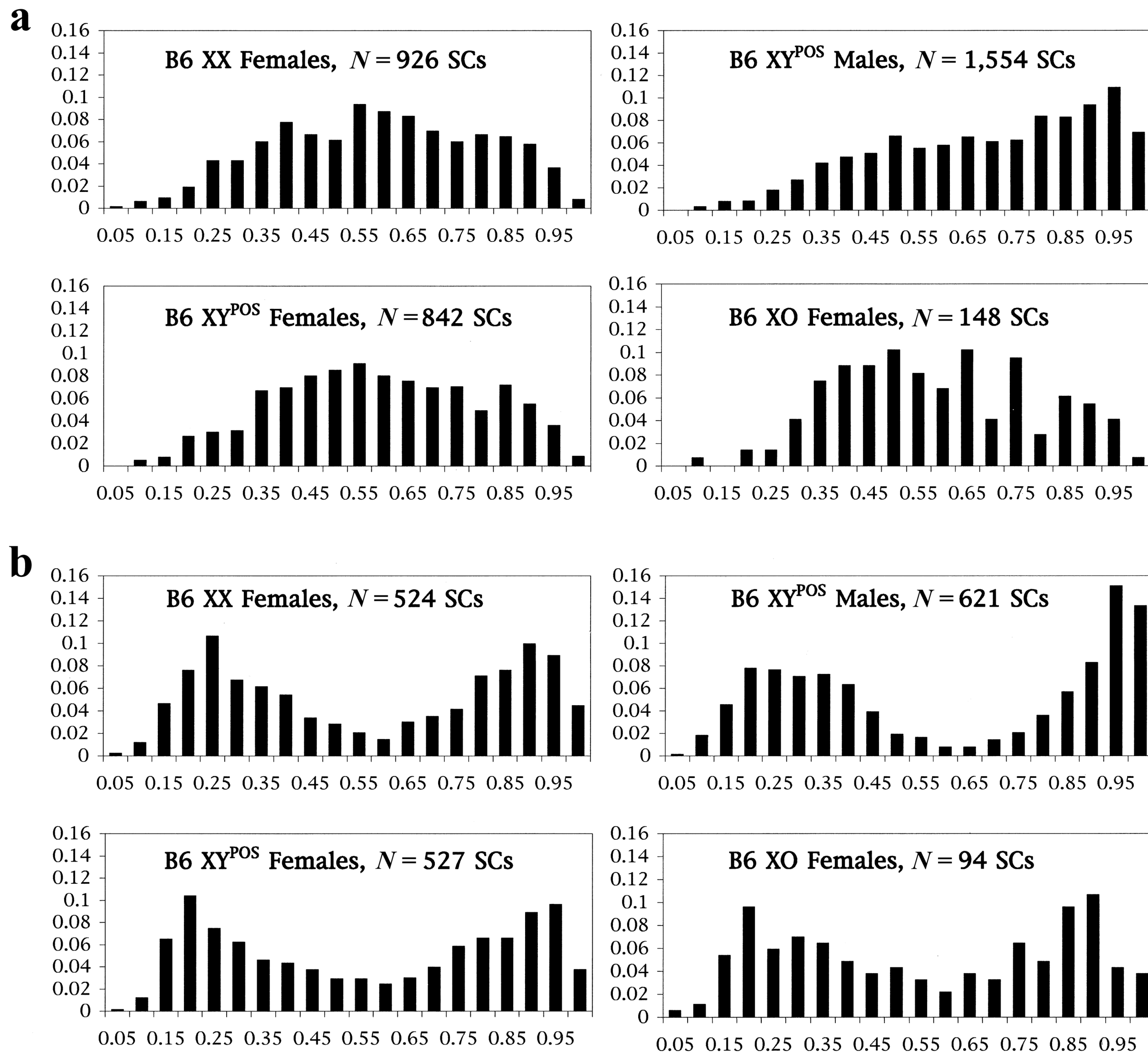

Figure 2 Positions of MLH1 foci on SCs with a single exchange (*a*) or two exchanges (*b*)

Hulten 2004). The most obvious of these are the temporal differences. It is well established that meiosis takes much longer to complete in the mammalian female than in the male (e.g., see Hassold and Hunt 2001), and there is also a common perception that each stage of meiosis lasts longer in the female than in the male. Presumably, then, the female might have higher rates of recombination simply because of an increased window of opportunity. However, the basic premise underlying this reasoning is inaccurate. Female gametogenesis takes longer to complete because of two cell-cycle arrests, the first of which is extremely protracted—it is initiated in the fetal ovary and lasts until the time of ovulation, which may be months, years, or decades later, depending on the species. Importantly, this first arrest phase occurs after the meiocyte has committed to a specific number of recombination events. Thus, there is no obvious, a priori reason to link the length of time of meiosis with recombination rates. Further, experimental data provide little evidence of such a link. That is, if female recombination rates were correlated to the length of time of meiosis, we would expect an increase in the recombination rate (and a decrease in the positive interference) with an increase in the age of the female. In fact, studies have reported either no age-related increases (Broman et al. 1998; Kong et al. 2002) or modest increases (Kong et al. 2004) that are insufficient to account for the differences between males and females. Thus, we think it is unlikely that the protracted nature of female meiosis contributes to male-female differences in recombination.

We also think it is unlikely that imprinting plays a role. Studies of the human have presented evidence of

sex-specific recombination rates within imprinted regions (Robinson and Lalande 1995). However, imprinting has been demonstrated for relatively few regions of mammalian genomes (Jiang et al. 2004), and it is unlikely to provide an adequate explanation for the global patterns of sex-specific recombination that have been observed.

Instead, we suggest that the sex-specific differences in the pairing and synapsis of homologs "set up" spermatocytes and oocytes to have different exchange patterns. The rationale behind this suggestion is straightforward. First, there is ample evidence that the synaptic pathway operates differently in females than in males. For example, recent studies of knockout mice, including those with mutations in loci encoding synapsis proteins, have revealed clear sex-specific differences in phenotypic outcome (see Hunt and Hassold 2002); thus, males and females respond differently to stresses on interhomolog interactions. Further, evidence from electron microscopic and immunofluorescence studies of leptotene- and zygotene-stage gametes indicates that synapsis occurs differently in male and female mammalian gametes. Specifically, in the male, synapsis appears to proceed from a limited number of synaptic initiation sites, the vast majority of which are located near the chromosome ends (Rasmussen and Holm 1978; Pfeifer et al. 2001; Brown et al. 2005 [in this issue]). In contrast, in the female, these sites are more numerous and are frequently interstitial in location (Bojko 1983; Pfeifer et al. 2003). Thus, there are sex-specific differences in the way that homologs are brought together.

Second, these differences in synapsis almost certainly result in differences in recombination. Recent studies of humans and mice indicate a direct correlation between the length of the SC and the overall number of meiotic exchanges (Lynn et al. 2002)—that is, on average, longer SCs house more meiotic exchanges. Since both human and mouse females have longer SCs than do their male counterparts, it is not surprising that the overall number of exchanges is higher in females than in males.

Thus, we suggest that sex-specific differences in recombination are attributable to differences in the way in which the SC is built in males and females. Indeed, it may simply be that synaptic initiation sites are "translated" into crossovers in both sexes and that females—having more such sites—have more crossovers. This is clearly an easy hypothesis to test, since it should be possible to combine immunofluorescence and FISH methodologies to map initiation sites and exchanges on individual chromosomes and to determine whether, indeed, the two colocalize.

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Web Resources

The URLs for data presented herein are as follows:

MicroMeasure Support Site, http://www.colostate.edu/Depts/Biology/MicroMeasure

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MLH1, SCP3, *Smcx,* and *Smcy*)

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