

X-Chromosome Control of Genome-Scale Recombination Rates in House Mice

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ABSTRACT Sex differences in recombination are widespread in mammals, but the causes of this pattern are poorly understood. Previously, males from two interfertile subspecies of house mice, *Mus musculus musculus* and *M. m. castaneus*, were shown to exhibit a ~30% difference in their global crossover frequencies. Much of this crossover rate divergence is explained by six autosomal loci and a large-effect locus on the X chromosome. Intriguingly, the allelic effects at this X-linked locus are transgressive, with the allele conferring increased crossover rate being transmitted by the low crossover rate *M. m. castaneus* parent. Despite the pronounced divergence between males, females from these subspecies exhibit similar crossover rates, raising the question of how recombination is genetically controlled in this sex. Here, I analyze publicly available genotype data from early generations of the Collaborative Cross, an eight-way panel of recombinant inbred strains, to estimate crossover frequencies in female mice with sex-chromosome genotypes of diverse subspecific origins. Consistent with the transgressive influence of the X chromosome in males, I show that females inheriting an *M. m. castaneus* X possess higher average crossover rates than females lacking the *M. m. castaneus* X chromosome. The differential inheritance of the X chromosome in males and females provides a simple genetic explanation for sex-limited evolution of this trait. Further, the presence of X-linked and autosomal crossover rate modifiers with antagonistic effects hints at an underlying genetic conflict fueled by selection for distinct crossover rate optima in males and females.

KEYWORDS recombination; house mouse; *Mus musculus*; meiosis; X chromosome; Collaborative Cross; haplotype

THE rate of crossing over in a genome is constrained by several biological considerations. At a minimum, one crossover per chromosome pair is needed to ensure correct segregation at the first meiotic division (Nicklas 1974; Hassold and Hunt 2001). Excessively low crossover rates may lead to the irreversible accumulation of deleterious alleles via Muller's ratchet (Muller 1964). At the other extreme, high crossover rates may be associated with genome instability and increased *de novo* mutation (Arbeithuber *et al.* 2015). Additionally, elevated recombination rates can rapidly dissolve high fitness haplotypes, leading to a reduction in organismal fitness (Charlesworth and Barton 1996). Despite these constraints, there is marked variation for crossover rates in nature. The rate of crossing over differs between

species (Dumont and Payseur 2011a; Smukowski and Noor 2011), among individuals (Broman *et al.* 1998; Thomsen *et al.* 2001; Koehler *et al.* 2002; Kong *et al.* 2004, 2008; Sun *et al.* 2004; Borodin *et al.* 2008; Coop *et al.* 2008; Dumont *et al.* 2009; Wong *et al.* 2010; Fledel-Alon *et al.* 2011), and between the sexes (Broman *et al.* 1998; Coop *et al.* 2008; Dumont *et al.* 2009; Kong *et al.* 2010). Although recent investigations have cast light on the molecular mechanisms controlling the fine-scale distribution of recombination hotspots (Baudat *et al.* 2010; Parvanov *et al.* 2010; Billings *et al.* 2013; Baker *et al.* 2014, 2015; Powers *et al.* 2016), the genetic and evolutionary processes that shape genome-scale crossover rates remain poorly understood.

House mice belonging to the *Mus musculus* species complex provide an especially powerful model system for elucidating the genetic control of genome-scale crossover rate variation. As the premiere biomedical mammalian model system, house mice are equipped with unparalleled genetic and genomic resources to facilitate identification of causal genetic variants. Furthermore, mouse chromosomes are well suited to cytogenetic analysis of MLH1, a mismatch repair protein localizing to the site of most crossover events.

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Immunofluorescent detection of this protein provides a robust, inexpensive method for quantifying global crossover frequencies in gametocytes isolated from single individuals, including inbred animals (Anderson *et al.* 1999; Koehler *et al.* 2002; Holloway *et al.* 2008). Prior investigations have used MLH1 mapping to establish that males from wild-derived inbred strains representative of the three principal house mouse subspecies—*Mus musculus musculus*, *M. m. domesticus*, and *M. m. castaneus*—exhibit pronounced, heritable differences in their global crossover rates (Koehler *et al.* 2002; Dumont *et al.* 2009; Dumont and Payseur 2011a). At the most extreme, males from one inbred strain of *M. m. musculus* (PWD/PhJ; hereafter PWD) possess a ~30% increase in total crossover number compared with males from a wild-derived inbred strain of *M. m. castaneus* (CAST/EiJ; hereafter CAST) (Dumont and Payseur 2011b). These two interfertile subspecies diverged ~0.5 MYA (Salcedo *et al.* 2007; Geraldès *et al.* 2008), implying very rapid divergence in this meiotic phenotype.

Eight loci contributing to the large difference in global crossover frequency between males from CAST (*M. m. castaneus*) and PWD (*M. m. musculus*) were previously identified in an intersubspecific F₂ QTL mapping experiment (Dumont and Payseur 2011b). Two of these QTL localized to the X chromosome, with one X-linked locus explaining ~35% of the phenotypic variance for crossover rate in the F₂ population. Curiously, alleles at both X-linked loci were inherited in a transgressive fashion, with alleles from the low recombination rate *M. m. castaneus* parent conferring an increase in total MLH1 number. Further evidence for a transgressive influence of the CAST X chromosome has been uncovered in an F₂ intercross with a common laboratory strain (C57BL/6J; Murdoch *et al.* 2010), and is suggested by patterns of crossover rate variation in reciprocal F₁ hybrids between CAST and WSB/EiJ, a wild-derived inbred strain of pure *M. m. domesticus* origin (Dumont and Payseur 2011a; Dumont *et al.* 2011). Thus, the genetic effect of the CAST X chromosome is not a peculiarity limited to one hybrid background or study. Conversely, at each of the six significant autosomal QTL that were identified in this genome-wide scan, the CAST allele was associated with a decrease in crossover rate, with the high-recombination rate allele inherited from the high-crossover rate PWD (*M. m. musculus*) parent. The distribution of allelic effects across autosomes and the sex chromosomes appears nonrandom.

Remarkably, despite the large difference in recombination rate between *M. m. castaneus* and *M. m. musculus* males, females from these two house mouse subspecies have near identical crossover frequencies (Dumont and Payseur 2011b). Differential X-chromosome inheritance between the sexes could lead to differences in the expression of X-linked crossover rate modifiers, potentially accounting for the sex-limited divergence between these two subspecies. However, multiple sex-specific crossover rate modifiers have been identified (Chowdhury *et al.* 2009; Fledel-Alon *et al.* 2011; Kong *et al.* 2014; Ma *et al.* 2015),

raising the question of whether the X-chromosome effect previously described in male house mice also extends to females.

Meiotic recombination unfolds in the fetal ovary, a consideration that makes estimating crossover rates in large numbers of females via MLH1 mapping impractical. Instead, I rely here on an alternative strategy to evaluate evidence for a subspecies-dependent genetic effect of the X chromosome on female recombination. Specifically, I take advantage of genetic diversity present among the eight founding strains of the Collaborative Cross (CC), a recombinant inbred house mouse panel which includes genetic contributions from each of the three principle house mouse subspecies (Churchill *et al.* 2004). By analyzing publicly available genotype data from mice in the early breeding generations of this reference panel, I directly test the effect of X-chromosome genotype on female crossover frequencies inferred from patterns of haplotype transmission. My analysis of crossover rates estimated from genetic data reveals a significant influence of the X chromosome on female recombination and offers a simple inheritance-based explanation for the observed sex dimorphism in crossover rate.

Materials and Methods

Crossover inference in the CC

All data analyzed in this article were obtained from the supplementary files accompanying Liu *et al.* (2014). I briefly describe this data below. Additional details are provided in the original manuscript (Liu *et al.* 2014).

The CC is an eight-way panel of recombinant inbred house mice derived from several generations of organized outcrossing followed by >20 generations of sib mating to achieve inbreeding (Churchill *et al.* 2004). The eight CC founder strains include five classical laboratory strains of predominantly *M. m. domesticus* origin (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, and NZO/HILtJ), and one wild-derived inbred strain representative of each of the three principle house mouse subspecies (*M. m. domesticus*, WSB/EiJ; *M. m. musculus*, PWK/PhJ; *M. m. castaneus*, CAST). Although the wild-derived inbred strain of *M. m. musculus* (PWK/PhJ; hereafter PWK) used in the CC differs from that used in the previous QTL study (PWD; Dumont and Payseur 2011b), these two strains are very closely genetically related (Gregorova and Forejt 2000; Kirby *et al.* 2010). Furthermore, loci on the X chromosome and autosomes from PWK mirror the effects of loci on the PWD X chromosome and autosomes on male meiotic crossover rates (Liu *et al.* 2014).

Figure 1 diagrams sex chromosome inheritance in early generations of the CC. The CC founder strains were mated in 237 unique eight-way combinations at generation 0 (G₀) to yield all possible $\left[2 \times \binom{8}{2} = 56\right]$ two-way hybrid genotypes at the G₁ generation. G₁ mice were then intercrossed to generate G₂ animals, each a unique genetic mosaic of four strains. Male and female G₂:F₁ animals—the progeny of

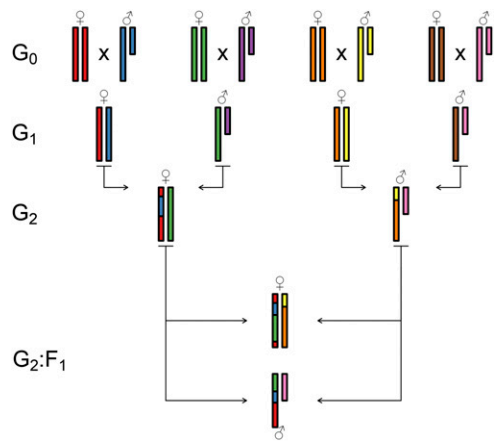


Figure 1 Sex-chromosome inheritance in one representative CC breeding funnel. The eight inbred founder strains were paired in 237 unique combinations at the G_0 generation to randomize parent of origin and sex chromosome inheritance in $G_2:F_1$ progeny. Given a known breeding funnel, crossover events in the G_1 and G_2 generations can be inferred from $G_2:F_1$ haplotypes. Crossovers at the G_1 generation are observed as haplotype switches between strains mated at the G_0 generation. G_2 crossovers manifest in $G_2:F_1$ haplotypes as switches between grandmaternal and grandpaternal alleles. This figure is based on from Liu *et al.* (2014). ♀, female; ♂, male.

G_2 individuals—were densely genotyped on the high-density Mouse Diversity Array (Yang *et al.* 2011) and the strain origin of haplotype blocks along each chromosome was computationally predicted using a pedigree-informed implementation of the Lander–Green algorithm (Lander and Green 1987; Liu *et al.* 2010).

Across the genome of a single $G_2:F_1$ individual, switches from one strain haplotype to that of another strain can be attributed to crossover events that occurred in either the G_1 or G_2 generation. Specifically, crossovers in G_1 meiosis manifest as haplotype transitions between strains mated in the initial G_0 generation. G_2 crossovers are revealed as switches between grandmaternal and grandpaternal alleles (Figure 1). Using this information, Liu *et al.* (2014) previously estimated the total number of crossovers in the G_1 and G_2 progenitors of the CC. Crossover events were further partitioned by parent of origin to permit analysis of sex-specific effects.

Liu *et al.* (2014) converted these crossover counts into genetic map lengths expressed in centimorgans. To facilitate direct comparisons with existing crossover estimates for CAST and PWD based on MLH1 foci analysis (Lynn *et al.* 2002; Dumont and Payseur 2011a,b), I focus on estimated total crossover counts. Due to the random assortment of recombinant chromosomes at the first meiotic division, only half of the crossover events that take place during G_2 meiosis are expected to be transmitted to a given $G_2:F_1$ proband. Likewise, one-quarter of crossovers that occur in a given G_1 meiosis will be detected as haplotype switches in the descendant $G_2:F_1$ animal. The expected total number of crossovers at G_1 (G_2) meiosis is therefore four (two) times the observed number.

Statistical analyses

All analyses were performed in the R Environment for Statistical Computing using function calls provided in base packages (R CoreTeam 2016). Crossover counts were compared using nonparametric Mann–Whitney U -tests and confidence intervals were obtained by bootstrap sampling the observed data 1000 times.

Data availability

All data necessary for confirming the conclusions presented in this article are represented fully within the article and its supplemental files.

Results

Armed with the recognition that G_1 CC females receive half of their genome from each of two founder strains (Figure 1), I first tested how strain background, including any effect of the X chromosome, influences global crossover rates in females. Figure 2A displays the mean number of crossovers in G_1 females with half of their genome derived from either CAST or PWK, averaged over the effects of all other seven possible strain contributors. Females with a CAST X chromosome have significantly higher crossover rates than females with one PWK X chromosome (Mann–Whitney U -test, $P = 1.646 \times 10^{-6}$). Even though inbred females from wild-derived inbred strains representing these two subspecies do not differ in their global recombination rates (Dumont and Payseur 2011b), variation in this trait is uncovered in F_1 hybrids bearing genetic contributions from these subspecies. G_1 females inheriting half of their genome from CAST have higher crossover counts than inbred CAST females (Figure 2A and Supplemental Material, Table S1; Table S2), although differences in methodology (*i.e.*, analysis of haplotype transmission vs. MLH1 foci mapping) complicate the interpretation of this variation. Conversely, G_1 females with half of their genome from PWK have lower crossover counts than closely related inbred PWD females. While genetic effects from the X and autosomes cannot be disentangled in these G_1 hybrids, these patterns are broadly consistent with observations in hybrid males from these two subspecies. Namely, the CAST X chromosome appears to confer an increase in crossover frequency when decoupled from the homozygous CAST autosomal genetic background. Conversely, the PWK X chromosome (autosomes) appears to decrease (increase) global crossover frequency.

G_2 females inherit one recombinant X chromosome from their mother and a nonrecombined X chromosome from their father (Figure 1). I took advantage of this unique aspect of sex-chromosome inheritance to test for an effect of subspecies identity of the paternally inherited X chromosome on an autosomal genetic background that has been shuffled by recombination, allowing the effects of the X chromosome and autosomes to be genetically isolated. G_2 females with a nonrecombined CAST X chromosome have, on average, higher

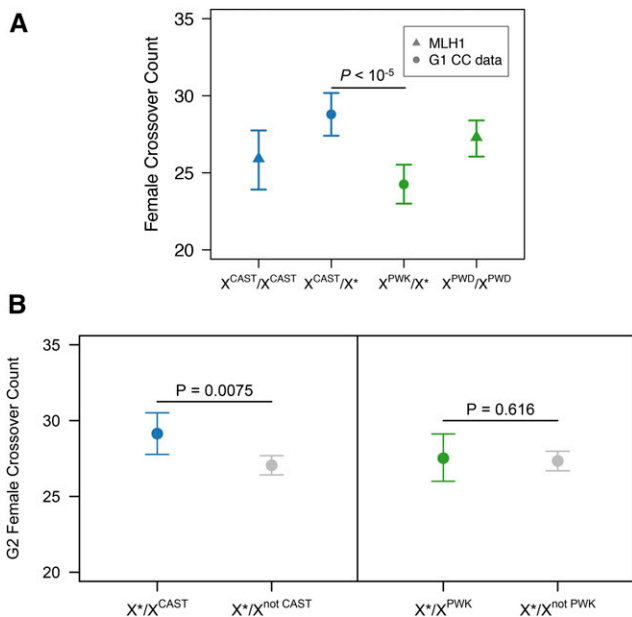


Figure 2 Crossover rate variation in (A) G_1 and (B) G_2 females as a function of X-chromosome genotype. In (A), crossover rates inferred from MLH1 mapping in inbred CAST (X^{CAST}/X^{CAST}) and PWD (X^{PWD}/X^{PWD}) females are also shown for comparison. G_1 females with one CAST X chromosome and a second X chromosome inherited from one of the remaining seven CC founder lines are denoted by the notation X^{CAST}/X^* . Similarly, X^{PWK}/X^* G_1 females inherit one PWK X chromosome and one X chromosome from a different CC founder strain. In (B), mice inheriting a nonrecombined CAST (PWK) X chromosome from their father and a recombinant X chromosome derived from other CC strains are denoted X^*/X^{CAST} (X^*/X^{PWK}). G_2 mice with a nonrecombined, paternally inherited X chromosome from one of the other seven CC strains are designated $X^*/X^{not\ CAST}$ ($X^*/X^{not\ PWK}$). P -values were calculated using nonparametric Mann–Whitney U -tests. Error bars denote 95% confidence intervals computed from 1000 bootstrap replicates of the observed data.

crossover counts than females who do not carry the CAST X chromosome (Mann–Whitney U -test, $P = 0.0075$; Figure 2B). This result indicates that a genetic factor on the CAST X increases crossover rates in female meiosis, matching its previously described influence on male meiotic crossover rate.

In contrast, the frequency of crossing over in G_2 females inheriting a PWK X chromosome is indistinguishable from the crossover frequency in G_2 females who do not inherit a PWK X chromosome (Mann–Whitney U -test, $P = 0.616$; Figure 2B). There are multiple possible interpretations for this negative result. First, power to find a significant difference is low due to the limited number of G_2 females carrying a paternally inherited PWK X chromosome ($n = 50$ meioses). Second, the effect of crossover rate modifiers segregating in the genetic background of G_2 animals could obscure any true biological effect of the PWK X chromosome on crossover rates. Third, if the PWK X chromosome and *M. m. domesticus* X chromosomes have effects of similar magnitude, the PWK X chromosome may have no discernable influence on crossover rates due to the preponderance of *M. m. domesticus* alleles segregating in the CC. Consistent with this possible interpre-

tation, the estimated effect of the PWD X chromosome appears attenuated on a C57BL/6J genetic background relative to its effect size on a CAST background (Dumont and Payseur 2011b; Balcova *et al.* 2016). Finally, recessive X-linked crossover modifiers cannot be detected in the heterozygous backgrounds tested here.

Discussion

By analyzing the distribution of crossover breakpoints as a function of X-chromosome genotype in mice from early generations of the CC, I have demonstrated that a genetic factor residing on the CAST X strongly influences female crossover rates. This result parallels earlier observations of a large X-chromosome influence on male recombination (Murdoch *et al.* 2010; Dumont and Payseur 2011b; Liu *et al.* 2014), and highlights the disproportionate effect of this chromosome on the regulation of crossover rates in house mice. Taken together, these observations suggest a genetic basis for the observed crossover rate dimorphism between males and females. Further, these findings raise the possibility that a conflict-based mechanism drives the rapid evolution of recombination rate in this model system.

Genetic control of heterochiasmy in house mice

Heterochiasmy—or sexual dimorphism for recombination rate—is widespread in the animal kingdom, but the causes of this pervasive pattern remain largely unknown. Differential X-chromosome inheritance in males and females provides a plausible genetic explanation, but this possibility has, to date, been backed by limited empirical support. Although X-chromosome inactivation equilibrates the expression of X-linked genes between heterogametic XY males and homogametic XX females in most mammalian tissues, the inactivated X is reactivated in female primordial germ cells (Sugimoto and Abe 2007). Female meiosis is thus one of the few cellular events controlled by genes expressed from both X chromosomes. The male X chromosome is transcriptionally silenced during much of the meiotic cell cycle, but these inhibitory signals are not established until late zygotene/early pachytene, after crossover repair has initiated (Turner 2007). These considerations demonstrate the potential for variation in the absolute expression of X-linked genes between males and females at the time crossing over takes place, as well as sex differences in the ratio of X-linked to autosome ($X:A$) gene expression.

This $X:A$ disparity could also underlie male-limited, crossover rate divergence between *M. m. castaneus* and *M. m. musculus*. If crossover rate modifiers follow a simple additive genetic model, the global rate of crossing over will be determined by the ratio of X chromosome to autosome ploidy, with crossover rate modifiers localizing to these distinct genomic compartments exerting polarizing phenotypic effects (Figure 3). In CAST males, the action of autosomal recombination rate suppressors will be weakly counterbalanced by the crossover-promoting effect of the single X chromosome. In contrast, in CAST females,







Autosomes	Sex Chrs	X:A	CO Rate
CAST 	 ♂	1:2	Low
	 ♀	2:2	Moderate
MUSC 	 ♂	1:2	High
	 ♀	2:2	Moderate

Figure 3 The ratio of X chromosome to autosomal ploidy number may explain subspecies and sex differences in crossover rate in house mice. Chromosomes are depicted as arrows, with the arrow point denoting the directional effect of resident crossover rate modifiers. Chromosomes from *M. m. castaneus* (CAST) are shown in blue, with chromosomes from *M. m. musculus* (MUSC) shown in green. The ratio of X chromosome to autosome ploidy (X:A) is 1:2 in hemizygous XY males, but 2:2 in XX females. In females with parity of the X:A ratio, the influence of modifiers on the X and autosomes are counterbalanced, yielding moderate crossover (CO) rates. In contrast, in males, the X:A ratio is less than one and crossover rates are dominated by the contributions from autosomal modifiers. ♀, female; ♂, male.

the influence of recombination-rate modifiers on the autosomes will be further offset by the twofold higher expression of crossover-promoting factors on the X chromosome. In *M. m. musculus*, the effects of autosomal and X-linked crossover rate modifiers are reversed compared to *M. m. castaneus*. Males harbor a single copy of an X-linked crossover suppressor, in contrast to the two copies expressed in females, resulting in higher crossover rates in *M. m. musculus* males relative to females.

This simple genetic model lays out a set of testable predictions. First, female mice with an autosomal genetic background from CAST and a pair of X chromosomes from *M. m. musculus* are predicted to have exceptionally low crossover rates. Conversely, female mice with an autosomal genome complement from PWD and a set of CAST X chromosomes should have very high crossover rates (Figure 3). These predictions can be tested directly by deriving reciprocal X-chromosome congenic lines from these strains and measuring crossover rates in animals with these genetic backgrounds. Second, crossover rates in sex-reversed XX males should be similar to those of genetically matched XX females. Similarly, crossover rates in XO or sex-reversed XY females should be more similar to those of XY males than to diploid XX females. These hypotheses can be readily evaluated using genome-editing technologies like CRISPR/Cas9 to generate sex-reversed mice, or via genetic crosses with commercial stocks characterized by high rates of *de novo* sex-chromosome aneuploidy (Eicher *et al.* 1991; Hunt 1991) or elevated frequencies of sex-reversed offspring (Eicher *et al.* 1982).

Despite the large genetic influence of the X chromosome on crossover rates in house mice, few sex-linked recombination modifiers have been identified in other species (Hunter and Singh 2015; Ma *et al.* 2015). The genetic architecture of genome-scale recombination rate is arguably most well

characterized in humans, but to date no X-linked modifiers have been identified in this species. Thus, differential inheritance of sex-linked modifiers is not a universal cause of heterochiasmy, and the underlying biological mechanisms of sex differences in recombination rate likely vary among species.

The genetic architecture of crossing over in house mice: a role for genetic conflict?

The genetic control of male and female crossover rate by X-linked and autosomal loci with opposing phenotypic effects raises the intriguing, albeit speculative, possibility that crossover rate variation in house mice is shaped by an arms race between loci on the X chromosome and loci on the autosomes. In particular, if optimal crossover rates differ between the sexes (Trivers 1988; Lenormand 2003), crossover rate modifiers may accumulate on the X chromosome, reducing the fitness of the opposite sex (Rice 1984). Such loci will impose selection for compensatory modifiers elsewhere in the genome to mitigate the deleterious effects incurred by the other sex (Rice 1984). This scenario could fuel ongoing antagonistic genetic conflict between X-linked and autosomal crossover rate modifiers. Over time, this process could contribute to rapid crossover rate divergence between *M. m. castaneus* and *M. m. musculus*.

The genetic conflict hypothesis outlined above establishes several key predictions. First, male and female house mice should possess sex-specific crossover rate optima. Numerous theories have been advanced to explain sex differences in recombination (Trivers 1988; Lenormand 2003; Lynn *et al.* 2005; Petkov *et al.* 2007; Kong *et al.* 2008; Chowdhury *et al.* 2009; Brandvain and Coop 2012). However, it remains unknown whether observed crossover rate differences between male and female house mice arise from sex-biased selection pressures on recombination. On average, female house mice have higher crossover rates than males (Reeves *et al.* 1990; Shifman *et al.* 2006; Cox *et al.* 2009), but there is overlap between the sex-specific crossover rate distributions (Dumont *et al.* 2009; Liu *et al.* 2014). A similar pattern is observed in human genome-scale crossover rate data (Coop *et al.* 2008). Determining whether females (males) at the extremes of the female (male) crossover rate distribution are less fit than individuals with crossover rates closer to the sex-specific mean may help address the question of whether selection drives sex differences in recombination rate.

A second prediction of the genetic conflict model is that the X chromosome should influence recombination rates in both sexes. The X chromosome exerts a significant effect on recombination in PWD and CAST males (Dumont and Payseur 2011b; Balcova *et al.* 2016) and CAST females (above), but the PWK X chromosome has no detectable influence on crossover rates in the small sample of genetically heterogeneous G₂ CC females surveyed here. This lack of evidence does not conclusively rule out the presence of an X-linked modifier of female crossover rates on the *M. m. musculus* X, and instead motivates further experimental investigation. Indeed, the

PWD X chromosome significantly decreases female recombination rate on a C57BL/6J genetic background (Balcova *et al.* 2016).

Third, if genetic conflict has shaped the architecture of genome-scale crossover rate, autosomal crossover rate modifiers should have evolved as increases in recombination rate along the PWD lineage and/or decreases in recombination rate along the CAST lineage. Genetic studies of crossover rate variation in *M. m. domesticus* and *M. m. castaneus* hybrids (Murdoch *et al.* 2010) and *M. m. domesticus* and *M. m. musculus* hybrids (Balcova *et al.* 2016) have uncovered autosomal crossover rate alleles from *M. m. domesticus* with phenotypic effects in both directions. Given the basal evolutionary position of *M. m. domesticus* within the house mouse subspecies complex (White *et al.* 2009), this trend suggests directional accumulation or fixation of recombination rate increasing (decreasing) alleles on the *M. m. musculus* (*M. m. castaneus*) autosomes, as predicted. Based on the moderate number of crossover-modifying loci identified, this suggestive pattern cannot be distinguished from chance alone. Under a conflict-based evolutionary model, recombination-rate modifiers should be subject to directional selection. Fine mapping the QTL identified in CAST and PWD, and applying population genetic tests of lineage-specific evolution to the underlying genomic sequences will shed light on the evolutionary forces contributing to the genetic architecture of crossover rate variation between these subspecies.

A final prediction of the genetic conflict hypothesis is that autosomal and X-linked modifiers with opposing phenotypic effects should be a common feature of crossover rate control in genetically diverse *M. m. musculus* and *M. m. castaneus* animals. To date, only one wild-derived inbred strain (*i.e.*, one haplotype) representative of each of these two subspecies has been tested. Future studies that interrogate the genetic basis of crossover rate variation in other wild-derived inbred strains or outbred populations are required to determine whether the patterns observed in PWD and CAST are broadly representative of *M. m. musculus* and *M. m. castaneus*.

Genetic mechanisms of global crossover rate evolution

The analysis presented here provides evidence that the subspecies origin of the X chromosome influences female crossover rates in house mice, but it lacks the genetic resolution needed to precisely determine where this modifying locus resides on the X. Furthermore, it remains uncertain whether the ascertained effect is due to the action of a single locus or the cumulative action of multiple X-linked loci. It is also unclear whether the X-linked loci influencing female and male crossover rates are coincident.

Recently, Balcova *et al.* (2016) measured crossover rate variation in a series of PWD and C57BL/6J X-chromosome congenic strains. Although their investigation focused on animals with genetic contributions from *M. m. musculus* and a common laboratory strain of predominantly *M. m. domesticus* ancestry, their findings provide two key refinements to the patterns of X-linked crossover rate control in *M. m. musculus*

and *M. m. castaneus*. First, Balcova *et al.* (2016) identified a crossover rate modifier active in male meiosis that localizes to a 4.7-Mb interval on the X chromosome (mm10 coordinates: chrX:64.88–chrX:69.58 Mb). This region falls under the peak of the male crossover rate QTL previously mapped in a CAST and PWD F₂ intercross population (Dumont and Payseur 2011b), suggesting that these loci are one and the same. The casual gene within this region is unknown, but the narrowed locus harbors multiple promising candidates, including testis- and ovary-expressed genes, a cluster of uncharacterized microRNAs, and several genes of unknown function. Patterns of diversity across this genomic locus are also consistent with the presence of structural mutations differentiating subspecies (Keane *et al.* 2011; Adams *et al.* 2015).

Balcova *et al.* (2016) also characterized an X-linked modifier of female crossover rates in the PWD and C57BL/6J X-chromosome congenic strains. This modifier maps distally to the male crossover rate locus (chrX: 69.58–98.15 Mb; Balcova *et al.* 2016). The female crossover rate modifier residing on the CAST X may be similarly distinct from the male X-linked recombination rate QTL in this strain. My ongoing efforts to characterize the genetic architecture of female crossover rates via QTL mapping of MLH1 foci in an intersubspecific *M. m. castaneus* and *M. m. musculus* mapping population aim to directly evaluate this possibility.

Identifying the genes underlying the X-linked effects on recombination rate variation in house mice represents a key first step toward elucidating the molecular mechanisms of genome-scale crossover rate control and evolution in this experimental system. Intriguingly, the narrowed 4.7-Mb interval harboring the X-linked male crossover rate modifier in PWD and C57BL/6J also contains a male hybrid sterility locus known to interact with *Prdm9* (Mihola *et al.* 2009), an important regulator of fine-scale recombination hotspots (Baudat *et al.* 2010; Parvanov *et al.* 2010). *Prdm9*-based, hotspot-determining mechanisms are broadly conserved between humans and mice (Ségurel *et al.* 2011), but there is little evidence that loci influencing natural variation in global crossover rates are shared between these species. Notably, none of the genes repeatedly associated with genome-scale recombination rate variation in humans map to chromosomal regions harboring QTL for crossover rate variation in mice (Chowdhury *et al.* 2009; Murdoch *et al.* 2010; Dumont and Payseur 2011b; Fledel-Alon *et al.* 2011; Kong *et al.* 2014; Balcova *et al.* 2016). Additionally, no genes within the refined 4.7-Mb interval on mouse chromosome X have been previously associated with genome-scale recombination rate variation in other taxa. Thus, the mechanisms determining natural variation in global crossover rates appear to have evolved via modification at distinct loci along the independent evolutionary lineages leading to humans and mice, an insight that underscores the genetic complexity of this fundamental cellular process.

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X-Chromosome Control of Genome-Scale Recombination Rates in House Mice

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