Variation and genetic control of individual recombination rates in Norwegian Red dairy cattle

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ABSTRACT

Meiotic recombination is an important evolutionary mechanism that breaks up linkages between loci and creates novel haplotypes for selection to act upon. Understanding the genetic control of variation in recombination rates is therefore of great interest in both natural and domestic breeding populations. In this study, we used pedigree information and medium-density (∼50K) genotyped data in a large cattle (*Bos taurus*) breeding population in Norway (Norwegian Red cattle) to investigate recombination rate variation between sexes and individual animals. Sex-specific linkage mapping showed higher rates in males than in females (total genetic length of autosomes = 2,492.9 cM in males and 2,308.9 cM in females). However, distribution of recombination along the genome showed little variation between males and females compared with that in other species. The heritability of autosomal crossover count was low but significant in both sexes (h^2 = 0.04 and 0.09 in males and females, respectively). We identified 2 loci associated with variation in individual crossover counts in female, one close to the candidate gene *CEP55* and one close to both *MLH3* and *NEK9*. All 3 genes have been associated with recombination rates in other cattle breeds. Our study contributes to the understanding of how recombination rates are controlled and how they may vary between closely related breeds as well as between species.

Key words: recombination, genetic shuffling, genetic diversity

INTRODUCTION

During eukaryotic meiosis, homologous chromosomes line up and exchange segments of DNA in a process known as recombination. The process is well conserved across taxa because it has a vital role in assuring homologous chromosome pairing during prophase I, ensuring that the gametes resulting from the meiosis have the correct number of chromosomes (Fledel-Alon et al., 2011; Lenormand et al., 2016). Human studies have shown that a lack of recombination can lead to aneuploidy; that is, the incorrect number of chromosomes in gametes (Sherman et al., 1991; Fledel-Alon et al., 2009). From an evolutionary perspective, recombination is an important mechanism because it breaks the linkages between alleles from genes located on the same chromosomes and creates new haplotypes for selection to act upon (Barton and Charlesworth, 1998; Sved and Hill, 2018). However, recombination may break up beneficial linkages built up over many generations, and there are other costs associated with recombination, such as increased mutation rate associated with double-strand break repair (Arbeithuber et al., 2015). These costs and benefits are thought to explain why the number of crossovers per chromosome seems to have well-conserved upper and lower limits across species (Ritz et al., 2017). Still, there is substantial variation in recombination rates between species, between sexes, and between individuals within breeds and populations of the same species (Lenormand et al., 2016; Stapley et al., 2017). In addition, many species show large variation in recombination rates along the chromosomes, including recombination hotspots and coldspots, with patterns often differing between the sexes (Kong et al., 2008; Halldorsson et al., 2016; Johnston et al., 2016, 2017; Petit et al., 2017; Johnsson et al., 2021).

There has been increasing interest in understanding the variation in individual recombination rates over the last decade. Several studies have been conducted in model species, such as house mice (Dumont et al., 2009; Booker et al., 2017; Wang et al., 2017) and *Drosophila melanogaster* (Hunter et al., 2016; Samuk et al., 2020; Winbush and Singh, 2021); natural populations such as red deer (Johnston et al., 2018) and Soay sheep (Johnston et al., 2016); and domesticated species such as pigs (Johnsson et al., 2021), chickens (Weng et al., 2019), sheep (Petit et al., 2017), and several cattle breeds (Sandor et al., 2012; Ma et al., 2015; Kadri et al., 2016;

Shen et al., 2018). There is a heritable component to individual recombination rates in all species studied (i.e., the proportion of phenotypic variance explained by additive genetic effects), ranging from around 5% in pigs (Johnsson et al., 2021) to 46% in mice (Dumont et al., 2009). Studies on the genetic architecture of individual recombination rates has led to discovery of several meiotic genes associated with individual recombination rate variation. This includes *RNF212* and *Rec8* that have been found in various mammal species (Kong et al., 2008; Sandor et al., 2012; Kadri et al., 2016; Johnston et al., 2018, 2020), and also genes such as *SYCP2*, *HEI10*, *MEIOB*, and several others that are only found in one or a couple of species and populations (Petit et al., 2017; Halldorsson et al., 2019a; Johnsson et al., 2021). Most vertebrate species studied show striking sex differences in the amount and location of recombination in the genome, known as heterochiasmy, but the direction and magnitude can vary markedly across species (Lenormand and Dutheil, 2005; Mank, 2009). However, despite the potential for genome-wide rates to respond rapidly to selection, the direction of heterochiasmy is conserved across relatively distantly related breeds and populations within species; for example, in sheep (Johnston et al., 2016; Petit et al., 2017), cattle (Shen et al., 2018), and pigs (Johnsson et al., 2021). Sex-specific studies of populations and breeds within species remain limited.

Cattle (*Bos taurus*) were domesticated around 10,000 years ago, and their domestication is believed to be one of the cornerstones of the Neolithic revolution (Götherström et al., 2005). Today, dairy and beef breeds are spread around the world, and breeding programs with extensive pedigrees and genotype information exist for a large number of individuals. Recombination rate variation has been studied in several cattle breeding populations, such as Holstein (Ma et al., 2015), Holstein Friesian, Jersey (Sandor et al., 2012), Brown Swiss, Ayrshire (Shen et al., 2018), and beef cattle such as Angus and Limousin (Weng et al., 2014). Only some of the studies have examined sex-specific rates of recombination, but those that do all find higher genome-wide rates in males than in females and some indications for sex-specific control of the trait (Ma et al., 2015; Kadri et al., 2016; Shen et al., 2018). Several genes have been identified as genetic drivers of recombination rate variation in cattle, including *PRDM9* (Ma et al., 2015), which is usually associated with recombination hotspot usage in other species (Paigen and Petkov, 2018).

Recombination is one of the main sources of novel haplotypic variation, which is a prerequisite for selection response and genetic gain in animal and plant breeding. Therefore, it is of great interest to understand how and why recombination rates vary between individuals and sexes, and along the genome. For example, if there is a heritable component to individual recombination rate, it presents an opportunity to select for higher rates, and thereby potentially help quantitative traits respond faster to selection (Battagin et al., 2016). More practically, having detailed information on how recombination rates vary within a breeding population may be important for genomic prediction (Gao et al., 2018) and selection on QTL (Lotterhos, 2019).

Here, we studied the genetic architecture and variation in individual autosomal crossover count (**ACC**) using a large genomic data set of more than 110,000 Norwegian Red cattle (*Bos taurus*), a breed that comprises the majority of all cattle in Norway. Our objectives were to (1) create sex-specific high-density linkage maps to understand variation in recombination landscapes; and (2) determine the heritability of individual ACC and identify potential loci associated with the trait.

MATERIALS AND METHODS

Study Population and Genotype Data

This study was exempt from institutional Animal Care and Use Committee or equivalent ethical approval because no animal procedures were performed to conduct this study and only existing data were used. In this study, we used genotype data and pedigree information from Norwegian Red cattle, which is the most common milk-producing breed in Norway. Genotype data were available from 5 SNP arrays developed for cattle: Affymetrix 54K (customized chip), Illumina NRF v2 (customized chip), Illumina BovineSNP50 v1, Illumina BovineSNP50 v2.0, and Illumina BovineHD. A total of 35,880 common SNP were genotyped for 110,555 individuals across these 5 arrays. The physical positions of the SNP were determined based on the ARS-UCD1.2 reference genome. The data were filtered to remove markers with missing call rates exceeding 0.1 or a Hardy-Weinberg equilibrium exact test P-value $< 10^{-6}$. Only autosomal SNP markers were included. This genotype data set is referred to hereafter as the 35K data set. All individuals were also imputed to a set of 655,309 SNP markers on the Illumina BovineHD chip; this data set included the X chromosome and was used for the GWAS analysis only. This data set is referred to hereafter as the 600K data set.

Quantification of Individual Crossovers

The pedigree was ordered into 3-generation full-sib families, comprising each unique sire and dam mating combination with their offspring and parents (Figure 1). As recombination rates are estimated for meioses that
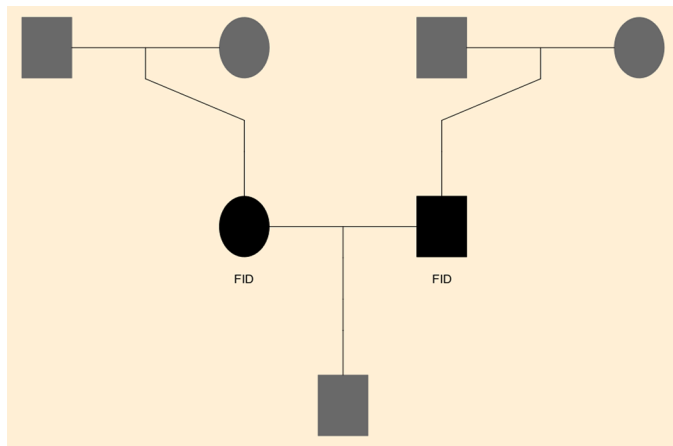
Figure 1. The 3-generation full-sib family structure used to calculate autosomal crossover count (ACC). This structure allows quantification of autosomal crossovers in the gamete transmitted from the focal individuals (FID; black) to the offspring.

occur in the sire and dam, they are referred to hereafter as focal individuals (**FID**). This full-sib family format allowed for phasing of the gametes transmitted from FID to offspring and detection of crossovers occurring during meiosis in the FID. Although an individual can be present in several families; that is, if mated to other individuals or as grandparent or offspring in a different family, this study design meant that each unique meiosis in an FID was only calculated and analyzed once. The total number of full-sib families was 19,603. Due to the breeding structure of cattle, most full-sib families only have one offspring, with a maximum of 5 offspring. In total, ACC were calculated for 603 unique bulls with 19,861 associated offspring and 14,815 unique cows with 19,824 associated offspring.

Linkage Mapping and Estimation of ACC

Sex-specific linkage maps were created with Lepmap3 (Rastas, 2017). Marker order was fixed relative to their physical positions on the ARS-UCD1.2 reference genome, and linkage maps were created for each chromosome separately. It should be noted that all cattle autosomes are acrocentric, with the centromere occurring at the beginning of the genomic sequence for each chromosome. The *filtering2* module was run to filter markers based on segregation distortion, with the argument *dataTolerance* = 0.01 as suggested for multifamily data sets. The *Seperatechromosomes2* module is used to split markers into linkage groups de novo; here, this module was used as a quality control step to exclude any markers that were not assigned to their expected linkage group [logarithm of the odds (LOD) score <5].

The Haldane mapping function option was used to calculate the positions (in cM) of the SNP markers in the *Ordermarkers2* module. The number of markers in the final linkage maps can be found in Table 1. To quantify individual recombination rates, the number of crossovers per autosomal chromatid in each offspring was estimated using the output from the *Ordermarkers2* module. The crossovers were then summed across all autosomes in the offspring and defined as the ACC, which was then assigned to the FID in which the crossover events occurred.

Heritability of Individual Recombination Rates

Genetic variation for the trait ACC was estimated with a repeatability model in DMU version 6 (Madsen et al., 2014) because FID with several offspring either within one family or across families had multiple observations for the trait. We used the REML method with the average information algorithm and fitted the following model:

Table 1. Male and female linkage map lengths[1] for all 29 autosomes

Chromosome	Physical length[2] (Mb)	Male		Female	
		cM	cM/Mb	cM	cM/Mb
1	158.30	128.8	0.81	123.4	0.78
2	136.23	114.6	0.84	111.3	0.82
3	121.01	110.7	0.91	103.6	0.86
4	120.00	107.9	0.90	104.7	0.87
5	120.09	110.2	0.92	101.1	0.84
6	117.81	104.4	0.89	99.3	0.84
7	110.68	106.9	0.97	102.8	0.93
8	113.32	101.4	0.89	96.9	0.86
9	105.45	95.7	0.91	86.9	0.82
10	103.31	102.0	0.99	94.1	0.91
11	106.98	99.2	0.93	92.6	0.87
12	87.22	87.1	1.00	78.2	0.90
13	83.47	93.7	1.12	80.7	0.97
14	82.40	84.5	1.03	76.1	0.92
15	85.01	86.3	1.02	78.3	0.92
16	81.01	86.1	1.06	75.9	0.94
17	73.17	78.3	1.07	67.5	0.92
18	65.82	79.8	1.21	69.8	1.06
19	63.45	89.8	1.42	71.2	1.12
20	71.97	67.7	0.94	64.7	0.90
21	69.86	74.1	1.06	68.3	0.98
22	60.77	73.6	1.21	65.5	1.08
23	52.50	63.1	1.20	57.9	1.10
24	62.32	62.3	1.00	60.6	0.97
25	42.35	58.3	1.38	56.7	1.34
26	51.99	57.5	1.11	56.3	1.08
27	45.61	55.8	1.22	54.7	1.20
28	45.94	55.5	1.21	54.3	1.18
29	51.10	57.6	1.13	55.5	1.09
Total	2,489.14	2,492.9	1.00	2,308.9	0.93

[1]Male and female cM is the estimated total genetic length of the autosomes in centimorgans; cM/Mb is the recombination rate in centimorgans per megabase.

[2]The physical map length is relative to the ARS-UCD1.2 genome.

$$Y = sex + b_1 \times age + b_2 \times het + id1 + id2 + e,$$

where Y is the ACC, sex is the fixed effect of sex, b_1 is the fixed regression of age of the FID when the offspring is born (from ages 1 to 13), b_2 is the regression of ACC on het (defined below) of the FID, e is the residual effect, id1 is the random additive genetic effect of the FID with a covariance matrix proportional to the numerator relationship matrix, id2 is the random effect of the FID permanent environment (i.e., individual identity, capturing environmental effects that are constant across repeated measures on an FID), het is the method-of-moments F coefficient estimates (i.e., observed homozygosity count – expected homozygosity count)/total observations – expected homozygosity count) calculated with the *–het* function in PLINK1.9 (Chang et al., 2015). The narrow-sense heritability (h^2) was defined as the proportion of phenotypic variance explained by the additive genetic effect.

Genome-Wide Associations with Individual Recombination Rates

The MLMA module implemented in the GCTA software package (Yang et al., 2011) was used to look for potential associations between any of the SNP markers from the 600K data set and mean individual ACC. This is a mixed models–based association analysis including the candidate SNP to be tested for association. For SNP markers on the X chromosome, the *–linear* option in PLINK (Chang et al., 2015) was used with the *–xchr* model flag. For the GWAS analysis on males, where several bulls have a large number of observations, an additional GWAS was tested for comparison where the genetic component of the EBV (i.e., estimate + residual) from the variance component estimations in DMU was fitted as a response variable to control for the permanent environmental effect of the bull. The genome-wide significance threshold = 0.05/number of markers per analysis.

RESULTS AND DISCUSSION

Broad- and Fine-Scale Recombination Rates

The total genetic length of the Norwegian Red autosomes was 2,492.9 cM in males and 2,308.9 cM in females, equating to an overall rate of autosomal recombination of 1 cM/Mb in males and 0.93 cM/Mb in females (Table 1). The total recombination rate per chromosome varied from 0.81 to 1.38 cM/Mb in males and from 0.78 to 1.34 cM/Mb in females in the 29 autosomes (Table 1). Sex differences in recombination rate were mainly driven by elevated male recombination in

subtelomeric regions of some chromosomes (i.e., the last 10–30 Mb), with the largest effects seen in chromosomes 13, 17, and 19, which are 14, 14, and 20% longer in males than in females, respectively (Figure 2). However, there were few sex differences in variation in recombination rate along the remainder of the autosomal chromosomes (Figure 2). Chromosome 15 is the only chromosome where the female rate was elevated in the subtelomeric region, although the male map length was longer overall (Figure 2). The relationship between the physical length (Mb) and genetic length (cM) of the autosomes was close to linear in both males and females (adjusted R^2 = 0.92 and 0.97, respectively; Figure 3). The linkage maps are available in the Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.20976067 .v1; Brekke, 2022).

Individual Recombination Rates

The ACC per gamete was close to normally distributed in both sexes, with means (± SD) of 24.3 (± 4.3) for males and 22.2 (± 4.9) for females (Figure 4). Gametes with <6 or >50 crossovers were excluded, resulting in a total of 19,861 ACC measures for males and 19,824 for females. The heritability for ACC was 9% in males and 4% in females (Table 2). Most of the phenotypic variance was explained by the error term in both sexes (Table 2). Inbreeding significantly decreased ACC in both sexes (−9.8 and −13.7 ACC per unit het in males and females, respectively), equivalent to a difference of 2.35 ACC for males and 6.35 ACC for females between individuals with the minimum and maximum levels of inbreeding in our data. We observed no effect of age on ACC in either sex in our analysis. Results from the analysis of genetic variation in individual crossover count can be found in Table 2.

Genome-Wide Associations with ACC

We found 2 genomic regions significantly associated with ACC in females, one on chromosome 10 and one on chromosome 26 (Figure 5, Table 3). Under the peak on chromosome 10, there are 2 candidate genes, *MHL3* and *NEK9*, which are 20.7 and 45.0 kb away, respectively, from the top SNP in that region (Figure 5); *MLH3* and *NEK9* are reported to be associated with cattle recombination rates in Kadri et al. (2016) and Ma et al. (2015), respectively. The most highly associated SNP on chromosome 26 was 45.6 kb away from the *CEP55* gene, which is associated with individual recombination rates in Holstein (Ma et al., 2015). It should be noted that the top SNP in the first peak on chromosome 10, which is close to the significance threshold, was 85.0 kb from *RNF212B*, a gene that has been previously associ-
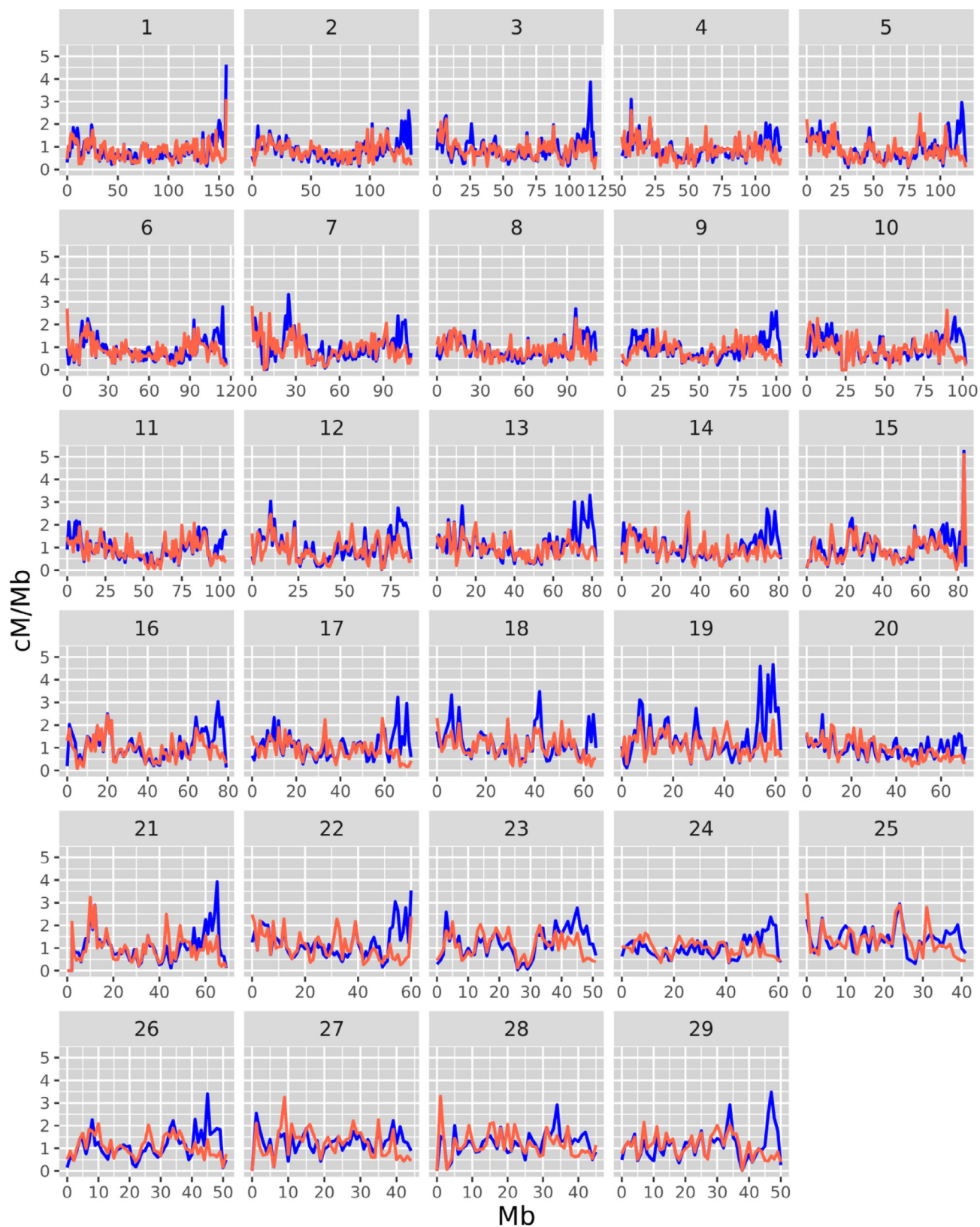
Figure 2. Fine-scale sex-specific recombination rate along the 29 cattle autosomes. The recombination rate (in cM/Mb) within each 1-Mb bin is plotted along the autosomes, with male rates in blue and female rates in red. Cattle autosomes are acrocentric, with the left- and right-hand ends of each map corresponding to the centromeric and subtelomeric regions, respectively.
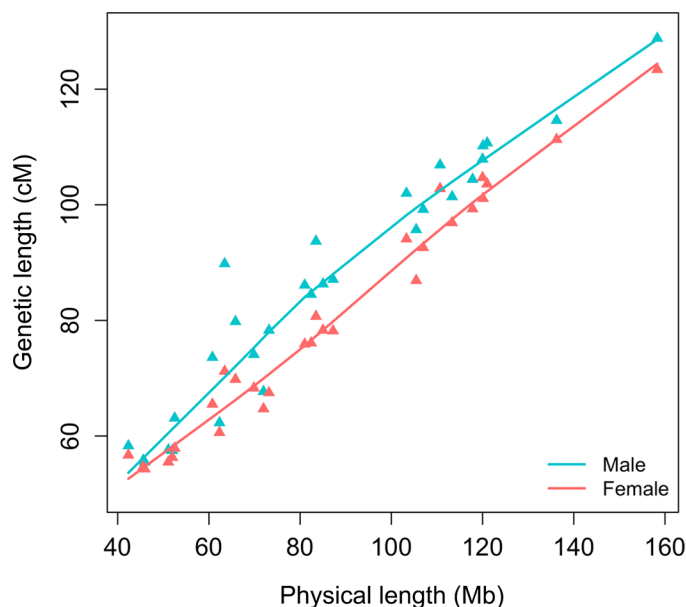
Figure 3. The relationship between the linkage map length (cM) and physical length (Mb) of the 29 autosomes. The relationship is plotted with robust locally weighted regression using the Lowess smoother in R (https://www.r-project.org/).

ated with individual recombination rates in domestic sheep, Soay sheep, cattle, and red deer (Kadri et al., 2016; Petit et al., 2017; Johnston et al., 2018, 2020). No markers showed significant associations with ACC in males (Figure 6); however, the most highly associated SNP corresponded to a region on chromosome 6 containing *RNF212*, which was previously associated with cattle recombination rate (Kadri et al., 2016). The GWAS on males with EBVs as response variable showed similar results where no markers exceeded the significance threshold; see Supplemental Figure S1 (https://doi.org/10.6084/m9.figshare.20975701.v1; Brekke, 2022).

There were no significant associations on the X chromosome for either sex (Supplemental Figure S2; https://doi.org/10.6084/m9.figshare.20976307; Brekke, 2022).

In this study, we took advantage of the extensive genotyping in the breeding program of Norwegian Red cattle to study individual recombination rates. We found that recombination rates in this breed vary between the sexes and within and between chromosomes. Individual recombination rates are heritable in both sexes, and we found 2 loci significantly associated with the trait in females, both close to genes that have previously been associated with recombination rate variation in vertebrates. In the following section, we discuss the results in more detail and consider the possible causes and implications of variation in recombination rates for a breeding population.

Sexual Dimorphism in Recombination Landscapes

In total, the genetic length of the male autosomes is 8% longer than that of the female autosomes, as reported in other cattle breeds studied, such as Holstein, Holstein Friesian, and Jersey, where male maps were 8 to 9% longer (Ma et al., 2015; Kadri et al., 2016). The genetic length of individual autosomes was consistently higher in males than in females and was driven by the subtelomeric regions. Along the rest of the autosomes, the sex-specific patterns of recombination rate were almost overlapping and sometimes slightly higher in females (Figure 2). Overall, these patterns were similar to those found in previous cattle studies, indicating conservation of recombination patterns and their sex differences across breeds and autosomes. The modest sex difference observed in cattle is in contrast to other mammal species, such as sheep, where male maps are 24% longer (Johnston et al., 2016), or in pigs (Johnsson et al., 2021), red deer (Johnston et al., 2017), and humans (Broman et al., 1998; Halldorsson et al., 2019b), where the female maps are 20 to 27%, 18%, and 38 to 39% longer, respectively.

Association of ACC with CEP55, NEK9, and MLH3 in Females

We found 2 loci that exceeded the significance threshold in females but no significant peaks in males. The sex difference may be due to the difference in the number of FID, but comparisons with other studies indicate that there are likely to be actual sex differences in genetic architecture of the trait. On the region on chromosome 10, the top SNP was at position 86.32 Mb, relatively close to *NEK9* at 86.33–86.37 Mb and *MLH3* at 86.28–86.30 Mb; *NEK9* and *MLH3* were associated with individual recombination rates in both sexes in the studies of Ma et al. (2015) and Kadri et al. (2016). *NEK9* is involved in spindle organization and alignment and segregation of the chromosomes during oocyte meiosis (Yang et al., 2012). *MLH3* is a MutL homolog involved in postreplicative mismatch repair and has been shown to interact with the meiosis-specific protein MSH4, that has a well-documented role in recombination (Santucci-Darmanin et al., 2002). The second peak, on chromosome 26, was near the *CEP55* gene, which is involved in spindle organization (Xu et al., 2015). This association was also specific to females in Ma et al. (2015). It should be noted that there is a peak on chromosome 10 that is close to the significance threshold; this peak was near the *RNF212B* gene that is associated with individual crossover rates in both male and female cattle in Kadri et al. (2016), as well as other ruminants such as red deer females (Johnston

Table 2. Results from the analysis of genetic variance of individual autosomal crossover count (ACC)[1]

Sex	N_{FID}	N_{obs}	Mean ACC (SD)	h^2 (SE)	V_p	V_e	het $_{effect}$
Female	14,815	19,824	22.2 (4.9)	0.04 (0.01)	24.0	21.2	−13.7 (1.25)
Male	603	19,861	24.3 (4.3)	0.09 (0.02)	18.2	16.01	−9.8 (2.8)

[1]N_{FID} = number of focal individuals in the respective model; N_{obs} = total number of ACC measures; V_p and V_e = phenotypic and error variances, respectively; het = slope of the inbreeding coefficient when fit as a fixed effect.

et al., 2020) and both male and female sheep (Petit et al., 2017; Johnston et al., 2018). *RNF212B* is a close paralog to *RNF212*, a gene that is known to affect recombination rates in mammals (Reynolds et al., 2013).

Sex Differences in Recombination in a Broader Context

Most species studied to date show a difference in recombination rate between males and females, in the overall rate or the pattern of recombination along the genome, but it is more common among mammals to have a female-biased heterochiasmy (Johnston et al., 2017; Wang et al., 2017; Halldorsson et al., 2019a; Johnsson et al., 2021). A long-standing theory has been that selection and domestication increase recombination rates (Burt and Bell, 1987); Ma et al. (2015) suggest that higher selection pressure in bulls may explain the higher male recombination rates. However, in domestic pigs, where there is also a higher selection pressure in males, heterochiasmy is female biased (Johnsson et al., 2021; Brekke et al., 2022). Furthermore, the theory of higher recombination rates in domesticated species versus their wild counterparts has been challenged in at least 3 pairs of species (dog vs. wolf, sheep vs. mouflon,

and goat vs. ibex), where no difference in recombination rate was observed (Munoz-Fuentes et al., 2015). Sexual dimorphism may also occur in selection at the gamete level that differs between species; in a study on Soay sheep, the authors hypothesized that the higher male recombination rates may be due to high levels of sperm competition in Soay sheep males as they have a highly promiscuous mating system, although this could not be formally tested (Johnston et al., 2016).

Ma et al. (2015) looked at development of recombination rates over time and found a steady decrease in recombination rates in males over the last 40 years, which coincided with a decrease in fertility. A recent study of fertility in Norwegian Red bulls also showed a slight but significant unfavorable genetic trend from 1994 to 2016 (Olsen et al., 2020). One hypothesis could be that, historically, selection has led to higher recombination rates because individuals with unique combinations of alleles are selected (Charlesworth and Barton, 1996), which may indirectly select for alleles associated with higher recombination. However, once bulls have favorable haplotypes, the gametes from low-recombination individuals are those leading to favorable offspring, resulting in selection for lower recombination rates, at least on some chromosomes. A study that compared
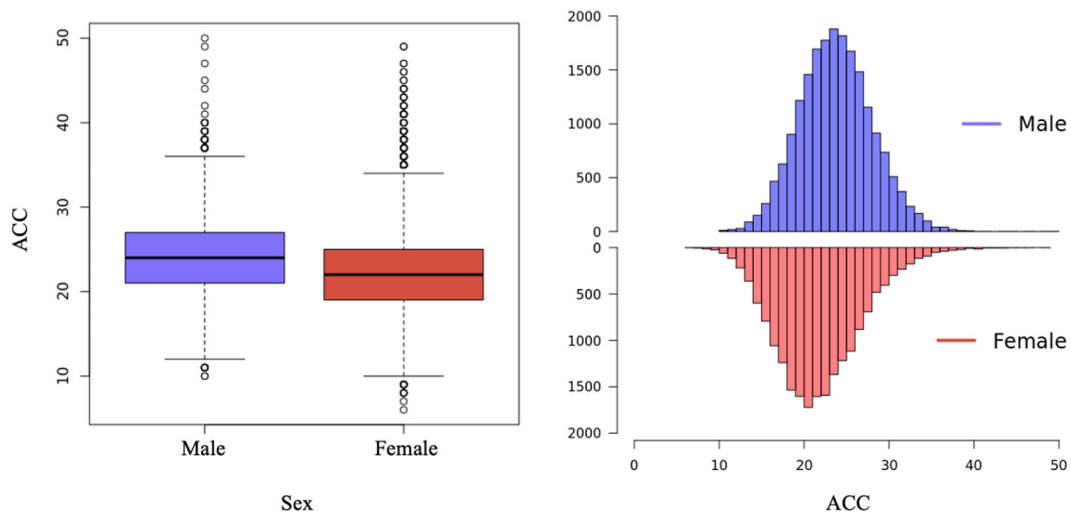

Figure 4. Distribution of autosomal crossover count (ACC) in females (red) and males (blue). The midline in the boxplot (right) is the median and the box is from the 25th percentile to the 75th percentile.

Table 3. Top SNP in genome-wide association with autosomal crossover count (ACC)

Sex	Chromosome	SNP	Position (bp)	MAF[1]	Beta[2] (SE)	P-value	Candidate gene
Female	10	BTA-78285-no-rs	86,322,591	0.27	0.45 (0.07)	7.93E-12	*NEK9*
							MLH3
Female	26	BovineHD2600003818	14,891,061	0.18	0.56 (0.08)	1.34E-13	*CEP55*

[1]Minor allele frequency.

[2]Additive effect size (i.e., the slope) of allele.

recent and historical recombination rates in Norwegian Red bulls found lower recent recombination rates compared with historical rates on chromosomes with important QTL for milk production (Sodeland et al., 2011). This could indicate that the sex difference in recombination rate may have been larger. Overall, biological explanations for the difference in recombination rates between sexes remain to be understood, as most hypotheses have species that contradict the theory, and there is a lack of empirical studies on heterochiasmy.

Genetic Variation in Individual Rates in Both Sexes

Heritability was higher in males than in females, which is consistent with other studies in cattle, but the heritability estimates in our study were lower than those of other studies (Sandor et al., 2012; Kadri et al., 2016). Sandor et al. (2012) only included bulls in their study and estimated heritability to be 0.22, whereas Kadri et al. (2016) found heritability of 0.13 in males

and 0.08 in females, which is closer to our results but still substantially higher. This could be due to breed differences in allele frequencies for loci affecting recombination rates. The standard error was slightly higher in males, probably because the number of FID was much lower in males (603) than in females (14,815), but the standard errors were not very high in either sex (0.01 and 0.02 in females and males, respectively).

The observed effect of inbreeding on recombination rates may be due to long runs of homozygosity affecting the ability to detect crossovers, rather than a true effect of inbreeding on reducing recombination rates. Most of the phenotypic variance was explained by the error terms (Table 3). This may be because we are studying recombination in gametes in live offspring (i.e., successful gametes), and it may not be a random sample from meiosis, but selection may occur at the haploid level in both males and females. Studies in human show signs of selection against nonrecombinant chromatids in meiosis II (Ottolini et al., 2015).
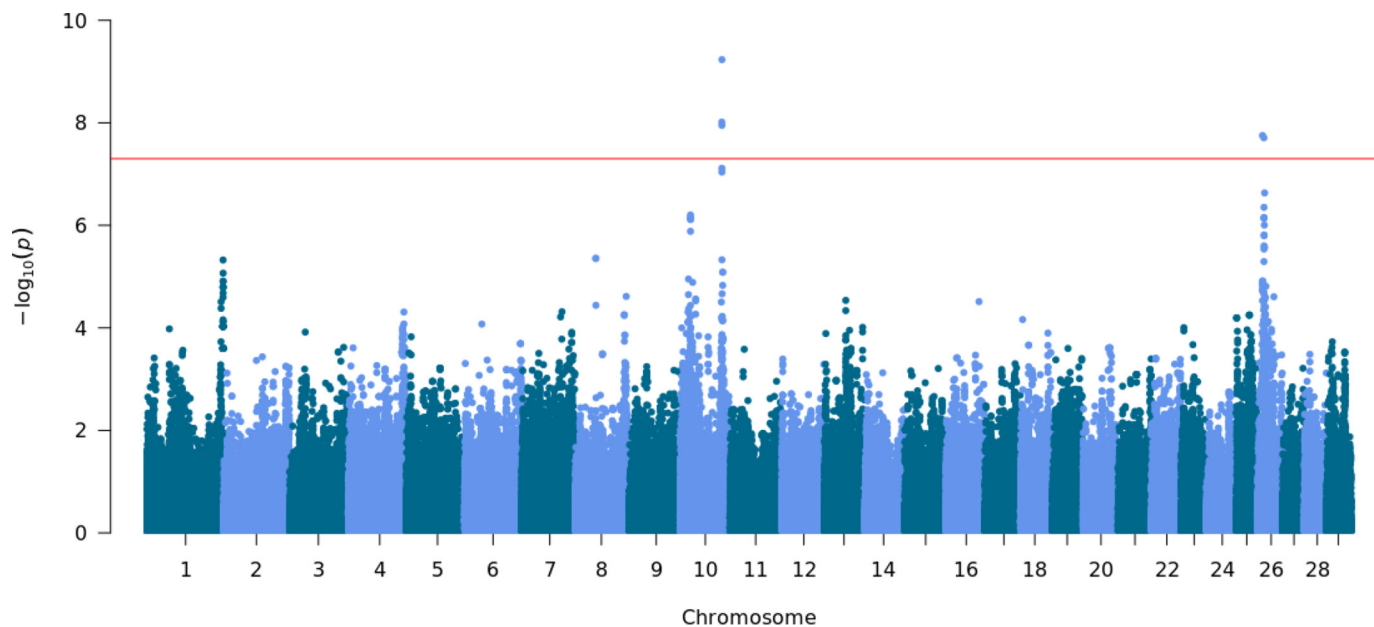

Figure 5. Genome-wide associations between individual autosomal crossover count (ACC) in females and SNP markers from the 600K data set. The red line is the significance threshold equivalent to $P < 0.05$ after multiple testing.
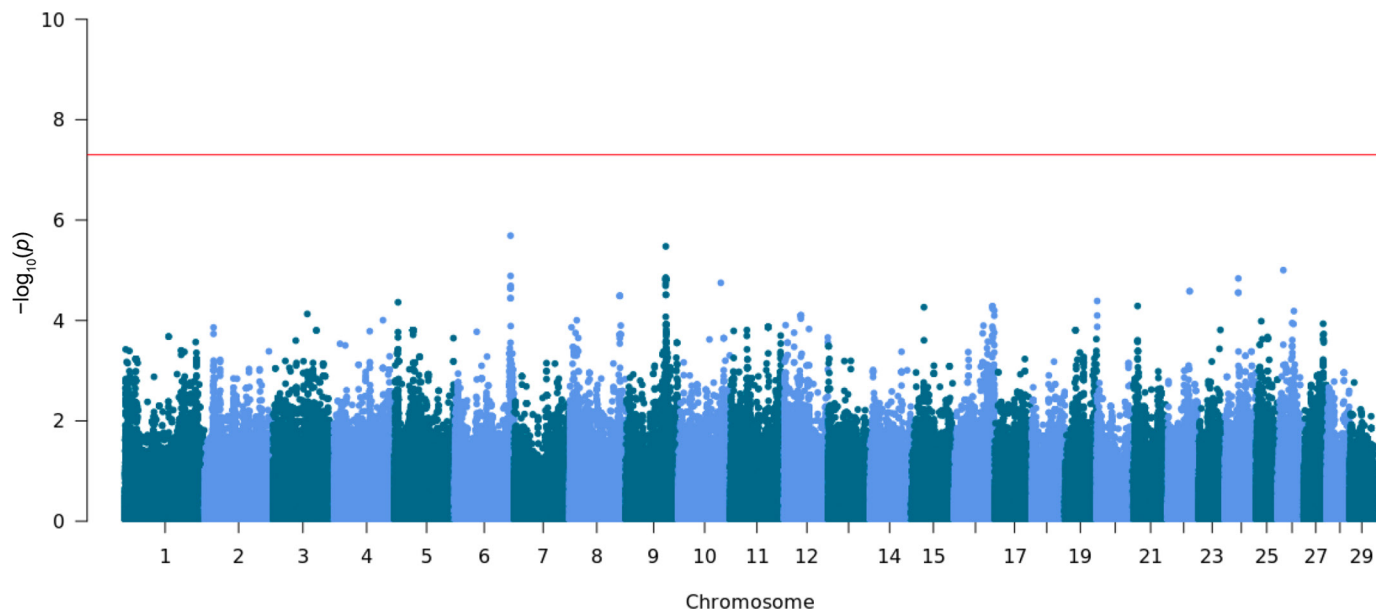
Figure 6. Genome-wide associations between individual autosomal crossover count (ACC) in males and the SNP markers from the 600K data set. The red line is the significance threshold, equivalent to $P < 0.05$ after multiple testing.

Reflections and Implications for Selective Breeding

Recombination is one of the main contributors to within-family genetic variation and is therefore important for breeding. With greater use of genomic information in breeding work and selection based on genomic evaluations, insights into breed-specific patterns of variation in recombination rates may be of great importance. Recombination rate is relatively evenly distributed along the autosome compared with that in many other species and it more closely resembles how recombination rates are typically modeled in phasing and imputation software such as Beagle (Browning and Browning, 2007) and SHAPEIT (O'Connell et al., 2014), which default to a constant recombination rate of 1 cM/Mb. This might suggest that there is not much to gain by using breed- and sex-specific linkage maps in phasing and imputation in cattle. Although there are clear sex differences in the subtelomeric regions, to our knowledge, no phasing or imputation software is currently available that use sex-specific linkage maps. Indeed, a sex-averaged map would not show this pattern at the ends, because most autosomes have opposite patterns in males and females.

Individual recombination rate is a heritable trait in cattle and could therefore be used to select for increased genetic variance. However, studies show that the emphasis on this trait in breeding work would have to be unrealistically high to have an effect on the selection response (Battagin et al., 2016). Also, there may be disadvantages to having high recombination rates,

such as increased mutation rates (Arbeithuber et al., 2015) and breaking of favorable linkages (Charlesworth and Barton, 1996). Given that at least one recombination is needed for proper segregation of chromosomes in meiosis and that aneuploidy is one of the main reasons for pregnancy loss in humans (Sherman et al., 1991; Hassold et al., 1995; Koehler et al., 1996; Fledel-Alon et al., 2009), it may be important for fertility to ensure that recombination rates are not too low. It would be interesting to look specifically at effects of low recombination rates on fertility as well as potential selection between gametes, perhaps by studying recombination in sperm and egg cells instead of in live offspring.

CONCLUSIONS

In this study, we found that both patterns and rates of recombination differed between the sexes in the subtelomeric regions but were relatively evenly distributed and overlapping between sexes in the rest of the autosomes. The genome-wide recombination rates in Norwegian Red cattle are comparable to those of other cattle breeds studied. In agreement with other studies, we found a low but significant heritable component to the genetic variation, but that the trait has a large error variance. It would be of interest to study sperm and egg cells to potentially explain more of the phenotypic variance in recombination rates in cattle. We found genes associated with individual recombination rates that have been previously found in other studies, suggesting that some of the genetic architecture of the

trait is well conserved across cattle breeding populations and other ruminants. Further studies should look at the mechanisms leading to different recombination rates between sexes as well as how individual rates affect fertility. We provide here an example of the genetic basis of recombination rates in a domesticated breed under strong selection, and our findings contribute to the understanding of the underlying mechanisms of individual recombination in cattle as well as in mammals in general.

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REFERENCES

Arbeithuber, B., A. J. Betancourt, T. Ebner, and I. Tiemann-Boege. 2015. Crossovers are associated with mutation and biased gene conversion at recombination hotspots. Proc. Natl. Acad. Sci. USA 112:2109–2114. https://doi.org/10.1073/pnas.1416622112.

Barton, N. H., and B. Charlesworth. 1998. Why sex and recombination? Science 281:1986–1990. https://doi.org/10.1126/science.281.5385.1986.

Battagin, M., G. Gorjanc, A. M. Faux, S. E. Johnston, and J. M. Hickey. 2016. Effect of manipulating recombination rates on response to selection in livestock breeding programs. Genet. Sel. Evol. 48:44. https://doi.org/10.1186/s12711-016-0221-1.

Booker, T. R., R. W. Ness, and P. D. Keightley. 2017. The recombination landscape in wild house mice inferred using population genomic data. Genetics 207:297–309. https://doi.org/10.1534/genetics.117.300063.

Brekke, C. 2022. Autosomal linkage map NRF. figshare. Dataset. https://doi.org/10.6084/m9.figshare.20976067.v1.

Brekke, C., P. Berg, A. B. Gjuvsland, and S. E. Johnston. 2022. Recombination rates in pigs differ between breeds, sexes and individuals, and are associated with the *RNF212, SYCP2, PRDM7, MEI1* and *MSH4* loci. Genet. Sel. Evol. 54:33. https://doi.org/10.1186/s12711-022-00723-9.

Broman, K. W., J. C. Murray, V. C. Sheffield, R. L. White, and J. L. Weber. 1998. Comprehensive human genetic maps: Individual and sex-specific variation in recombination Am. J. Hum. Genet. 63:861–869. https://doi.org/10.1086/302011.

Browning, S. R., and B. L. Browning. 2007. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am. J. Hum. Genet. 81:1084–1097. https://doi.org/10.1086/521987.

Burt, A., and G. Bell. 1987. Mammalian chiasma frequencies as a test of two theories of recombination. Nature 326:803–805. https://doi.org/10.1038/326803a0.

Chang, C. C., C. C. Chow, L. C. A. M. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee. 2015. Second-generation PLINK: Rising to the challenge of larger and richer datasets. Gigascience 4:7. https://doi.org/10.1186/s13742-015-0047-8.

Charlesworth, B., and N. H. Barton. 1996. Recombination load associated with selection for increased recombination. Genet. Res. 67:27–41. https://doi.org/10.1017/S0016672300033450.

Dumont, B. L., K. W. Broman, and B. A. Payseur. 2009. Variation in genomic recombination rates among heterogeneous stock mice. Genetics 182:1345–1349. https://doi.org/10.1534/genetics.109.105114.

Fledel-Alon, A., E. M. Leffler, Y. Guan, M. Stephens, G. Coop, and M. Przeworski. 2011. Variation in human recombination rates and its genetic determinants. PLoS One 6:e20321. https://doi.org/10.1371/journal.pone.0020321.

Fledel-Alon, A., D. J. Wilson, K. Broman, X. Wen, C. Ober, G. Coop, and M. Przeworski. 2009. Broad-scale recombination patterns underlying proper disjunction in humans. PLoS Genet. 5:e1000658. https://doi.org/10.1371/journal.pgen.1000658.

Gao, N., J. Teng, S. Ye, X. Yuan, S. Huang, H. Zhang, X. Zhang, J. Li, and Z. Zhang. 2018. Genomic prediction of complex phenotypes using genic similarity based relatedness matrix. Front. Genet. 9:364. https://doi.org/10.3389/fgene.2018.00364.

Götherström, A., C. Anderung, L. Hellborg, R. Elburg, C. Smith, D. G. Bradley, and H. Ellegren. 2005. Cattle domestication in the Near East was followed by hybridization with aurochs bulls in Europe. Proc. Biol. Sci. 272:2345–2351. https://doi.org/10.1098/rspb.2005.3243.

Halldorsson, B., M. T. Hardarson, B. Kehr, U. Styrkarsdottir, A. Gylfason, G. Thorleifsson, F. Zink, A. Jonasdottir, A. Jonasdottir, P. Sulem, G. Masson, U. Thorsteinsdottir, A. Helgason, A. Kong, D. F. Gudbjartsson, and K. Stefansson. 2016. The rate of meiotic gene conversion varies by sex and age. Nat. Genet. 48:1377–1384. https://doi.org/10.1038/ng.3669.

Halldorsson, B., G. Palsson, O. A. Stefansson, H. Jonsson, M. T. Hardarson, H. P. Eggertsson, B. Gunnarsson, A. Oddsson, G. H. Halldorsson, F. Zink, S. A. Gudjonsson, M. L. Frigge, G. Thorleifsson, A. Sigurdsson, S. N. Stacey, P. Sulem, G. Masson, A. Helgason, D. F. Gudbjartsson, U. Thorsteinsdottir, and K. Stefansson. 2019a. Human genetics: Characterizing mutagenic effects of recombination through a sequence-level genetic map. Science 363:eaau1043. https://doi.org/10.1126/science.aau1043.

Halldorsson, B., G. Palsson, O. A. Stefansson, H. Jonsson, M. T. Hardarson, H. P. Eggertsson, B. Gunnarsson, A. Oddsson, G. H. Halldorsson, F. Zink, S. A. Gudjonsson, M. L. Frigge, G. Thorleifsson, A. Sigurdsson, S. N. Stacey, P. Sulem, G. Masson, A. Helgason, D. F. Gudbjartsson, U. Thorsteinsdottir, and K. Stefansson. 2019b. Human genetics: Characterizing mutagenic effects of recombination through a sequence-level genetic map. Science 363:eaau1043. https://doi.org/10.1126/science.aau1043.

Hassold, T., M. Merrill, K. Adkins, S. Freeman, and S. Sherman. 1995. Recombination and maternal age-dependent nondisjunction: Molecular studies of trisomy 16. Am. J. Hum. Genet. 57:867–874.

Hunter, C. M., W. Huang, T. F. C. Mackay, and N. D. Singh. 2016. The genetic architecture of natural variation in recombination rate in *Drosophila melanogaster*. PLoS Genet. 12:e1005951. https://doi.org/10.1371/journal.pgen.1005951.

Johnsson, M., A. Whalen, R. Ros-Freixedes, G. Gorjanc, C. Y. Chen, W. O. Herring, D. J. de Koning, and J. M. Hickey. 2021. Genetic variation in recombination rate in the pig. Genet. Sel. Evol. 53:54. https://doi.org/10.1186/s12711-021-00643-0.

Johnston, S., M. Stoffel, and J. Pemberton. 2020. Variants at RNF212 and RNF212B are associated with recombination rate variation in Soay sheep (*Ovis aries*). https://doi.org/10.1101/2020.07.26.217802.

Johnston, S. E., C. Bérénos, J. Slate, and J. M. Pemberton. 2016. Conserved genetic architecture underlying individual recombination rate variation in a wild population of soay sheep (*Ovis aries*). Genetics 203:583–598. https://doi.org/10.1534/genetics.115.185553.

Johnston, S. E., J. Huisman, P. A. Ellis, and J. M. Pemberton. 2017. A high-density linkage map reveals sexual dimorphism in recom-

bination landscapes in red deer (*Cervus elaphus*). G3 (Bethesda) 7:2859–2870. https://doi.org/10.1534/g3.117.044198.

Johnston, S. E., J. Huisman, and J. M. Pemberton. 2018. A genomic region containing REC8 and RNF212B is associated with individual recombination rate variation in a wild population of red deer (*Cervus elaphus*). G3 (Bethesda) 8:2265–2276. https://doi.org/10.1534/g3.118.200063.

Kadri, N. K., C. Harland, P. Faux, N. Cambisano, L. Karim, W. Coppieters, S. Fritz, E. Mullaart, D. Baurain, D. Boichard, R. Spelman, C. Charlier, M. Georges, and T. Druet. 2016. Coding and noncoding variants in HFM1, MLH3, MSH4, MSH5, RNF212, and RNF212B affect recombination rate in cattle. Genome Res. 26:1323–1332. https://doi.org/10.1101/gr.204214.116.

Koehler, K. E., R. S. Hawley, S. Sherman, and T. Hassold. 1996. Recombination and Nondisjunction in Humans and Flies. Oxford University Press.

Kong, A., G. Thorleifsson, H. Stefansson, G. Masson, A. Helgason, D. F. Gudbjartsson, G. M. Jonsdottir, S. A. Gudjonsson, S. Sverrisson, T. Thorlacius, A. Jonasdottir, G. A. Hardarson, S. T. Palsson, M. L. Frigge, J. R. Gulcher, U. Thorsteinsdottir, and K. Stefansson. 2008. Sequence variants in the RNF212 gene associate with genome-wide recombination rate. Science 319:1398–1401. https://doi.org/10.1126/science.1152422.

Lenormand, T., and J. Dutheil. 2005. Recombination difference between sexes: A role for haploid selection. PLoS Biol. 3:e63.

Lenormand, T., J. Engelstädter, S. E. Johnston, E. Wijnker, and C. R. Haag. 2016. Evolutionary mysteries in meiosis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 371:20160001. https://doi.org/10.1098/rstb.2016.0001.

Lotterhos, K. E. 2019. The effect of neutral recombination variation on genome scans for selection. G3 (Bethesda) 9:1851–1867. https://doi.org/10.1534/g3.119.400088.

Ma, L., J. R. O'Connell, P. M. VanRaden, B. Shen, A. Padhi, C. Sun, D. M. Bickhart, J. B. Cole, D. J. Null, G. E. Liu, Y. Da, and G. R. Wiggans. 2015. Recombination and genetic control from a large pedigree analysis. PLoS Genet. 11:e1005387. https://doi.org/10.1371/journal.pgen.1005387.

Madsen, P., J. Jensen, R. Labouriau, O. F. Christensen, and G. Sahana. 2014. DMU-A package for analyzing multivariate mixed models in quantitative genetics and genomics. Pages 18–22 in Proceedings of the 10th World Congress of Genetics Applied to Livestock Production.

Mank, J. E. 2009. The evolution of heterochiasmy: The role of sexual selection and sperm competition in determining sex-specific recombination rates in eutherian mammals. Genet. Res. 91:355–363. https://doi.org/10.1017/S0016672309990255.

Munoz-Fuentes, V., V. M. Marcet-Ortega, G. Alkorta-Aranburu, C. L. Forsberg, J. M. Morrell, E. Manzano-Piedras, A. Soderberg, K. Daniel, A. Villalba, A. Toth, A. di Rienzo, I. Roig, and C. Vila. 2015. Strong artificial selection in domestic mammals did not result in an increased recombination rate. Mol. Biol. Evol. 32:510–523. https://doi.org/10.1093/molbev/msu322.

O'Connell, J., D. Gurdasani, O. Delaneau, N. Pirastu, S. Ulivi, M. Cocca, M. Traglia, J. Huang, J. E. Huffman, I. Rudan, R. McQuillan, R. M. Fraser, H. Campbell, O. Polasek, G. Asiki, K. Ekoru, C. Hayward, A. F. Wright, P. Vitart, P. Navarro, J. F. Zagury, J. F. Wilson, D. Toniolo, P. Gasparini, N. Soranzo, M. S. Sandhu, and J. Marchini. 2014. A general approach for haplotype phasing across the full spectrum of relatedness. PLoS Genet. 10:e1004234. https://doi.org/10.1371/journal.pgen.1004234.

Olsen, H. B., B. Heringstad, and G. Klemetsdal. 2020. Genetic analysis of semen characteristic traits in young Norwegian Red bulls. J. Dairy Sci. 103:545–555. https://doi.org/10.3168/jds.2019-17291.

Ottolini, C. S., L. J. Newnham, A. Capalbo, S. A. Natesan, H. A. Joshi, D. Cimadomo, D. K. Griffin, K. Sage, M. C. Summers, A. R. Thornhill, E. Housworth, A. D. Herbert, L. Rienzi, F. M. Ubaldi, A. H. Handyside, and E. R. Hoffmann. 2015. Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. Nat. Genet. 47:727–735. https://doi.org/10.1038/ng.3306.

Paigen, K., and P. M. Petkov. 2018. PRDM9 and its role in genetic recombination. Trends Genet. 34:291–300. https://doi.org/10.1016/j.tig.2017.12.017.

Petit, M., J. M. Astruc, J. Sarry, L. Drouilhet, S. Fabre, C. R. Moreno, and B. Servin. 2017. Variation in recombination rate and its genetic determinism in sheep populations. Genetics 207:767–784. https://doi.org/10.1534/genetics.117.300123.

Rastas, P. 2017. Lep-MAP3: Robust linkage mapping even for low-coverage whole genome sequencing data. Bioinformatics 33:3726–3732. https://doi.org/10.1093/bioinformatics/btx494.

Reynolds, A., H. Qiao, Y. Yang, J. K. Chen, N. Jackson, K. Biswas, J. K. Holloway, F. Baudat, B. de Massy, J. Wang, C. Höög, P. E. Cohen, and N. Hunter. 2013. RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. Nat. Genet. 45:269–278. https://doi.org/10.1038/ng.2541.

Ritz, K. R., M. A. F. Noor, and N. D. Singh. 2017. Variation in recombination rate: Adaptive or not? Trends Genet. 33:364–374. https://doi.org/10.1016/j.tig.2017.03.003.

Samuk, K., B. Manzano-Winkler, K. R. Ritz, and M. A. F. Noor. 2020. Natural selection shapes variation in genome-wide recombination rate in *Drosophila pseudoobscura*. Curr. Biol. 30:1517–1528.e6. https://doi.org/10.1016/j.cub.2020.03.053.

Sandor, C., W. Li, W. Coppieters, T. Druet, C. Charlier, and M. Georges. 2012. Genetic variants in REC8, RNF212, and PRDM9 influence male recombination in cattle. PLoS Genet. 8:e1002854. https://doi.org/10.1371/journal.pgen.1002854.

Santucci-Darmanin, S., S. Neyton, F. Oise Lespinasse, A. Sauniè, P. Gaudray, and V. Ronique Paquis-Flucklinger. 2002. The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. Hum. Mol. Genet. 11:1697–1706. https://doi.org/10.1093/hmg/11.15.1697.

Shen, B., J. Jiang, E. Seroussi, G. E. Liu, and L. Ma. 2018. Characterization of recombination features and the genetic basis in multiple cattle breeds. BMC Genomics 19:304. https://doi.org/10.1186/s12864-018-4705-y.

Sherman, S. L., N. Takaesu, S. B. Freeman, M. Grantham, C. Phillips, R. D. Blackston, P. A. Jacobs, A. E. Cockwell, V. Freeman, I. Uchida, M. Mikkelsen, D. M. Kurnit, M. Buraczynska, B. J. B. Keats, and T. J. Hassold. 1991. Trisomy 21: Association between reduced recombination and nondisjunction. Am. J. Hum. Genet. 49:608–620.

Sodeland, M., M. Kent, B. J. Hayes, H. Grove, and S. Lien. 2011. Recent and historical recombination in the admixed Norwegian Red cattle breed. BMC Genomics 12:33. https://doi.org/10.1186/1471-2164-12-33.

Stapley, J., P. G. D. Feulner, S. E. Johnston, A. W. Santure, and C. M. Smadja. 2017. Variation in recombination frequency and distribution across eukaryotes: Patterns and processes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 3722016045520160455. https://doi.org/10.1098/rstb.2016.0455.

Sved, J. A., and W. G. Hill. 2018. One hundred years of linkage disequilibrium. Genetics 209:629–636.

Wang, R. J., M. M. Gray, M. D. Parmenter, K. W. Broman, and B. A. Payseur. 2017. Recombination rate variation in mice from an isolated island. Mol. Ecol. 26:457–470. https://doi.org/10.1111/mec.13932.

Weng, Z., A. Wolc, H. Su, R. L. Fernando, J. C. M. Dekkers, J. Arango, P. Settar, J. E. Fulton, N. P. O'Sullivan, and D. J. Garrick. 2019. Identification of recombination hotspots and quantitative trait loci for recombination rate in layer chickens. J. Anim. Sci. Biotechnol. 10:20. https://doi.org/10.1186/s40104-019-0332-y.

Weng, Z. Q., M. Saatchi, R. D. Schnabel, J. F. Taylor, and D. J. Garrick. 2014. Recombination locations and rates in beef cattle assessed from parent-offspring pairs. Genet. Sel. Evol. 46:34. https://doi.org/10.1186/1297-9686-46-34.

Winbush, A., and N. D. Singh. 2021. Genomics of recombination rate variation in temperature-evolved *Drosophila melanogaster* populations. Genome Biol. Evol. 13:evaa252. https://doi.org/10.1093/gbe/evaa252.

Xu, Z. Y., X. S. Ma, S. T. Qi, Z. B. Wang, L. Guo, H. Schatten, Q. Y. Sun, and Y. P. Sun. 2015. Cep55 regulates spindle organization and cell cycle progression in meiotic oocyte. Sci. Rep. 5:16978. https://doi.org/10.1038/srep16978.

Yang, J., S. H. Lee, M. E. Goddard, and P. M. Visscher. 2011. GCTA: A tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88:76–82. https://doi.org/10.1016/j.ajhg.2010.11.011.

Yang, S. W., C. Gao, L. Chen, Y. L. Song, J. L. Zhu, S. T. Qi, Z. Z. Jiang, Z. W. Wang, F. Lin, H. Huang, F. Q. Xing, and Q. Y. Sun. 2012. Nek9 regulates spindle organization and cell cycle progression during mouse oocyte meiosis and its location in early embryo mitosis. Cell Cycle 11:4366–4377. https://doi.org/10.4161/cc.22690.

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