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Genetic map of Fleckvieh cattle

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Yosef Amsalu Abitew, BSc

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Co-Supervisor:
Dr.ir. P (Pascal) Duenk

Department of Animal Sciences
Animal Breeding and Genomics
Wageningen University and Research

Main Supervisor:
Univ.Prof. Dipl.-Ing. Dr.nat.techn. Johann Sölkner
Division of Livestock Sciences (NUWI), BOKU

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Yosef Amsalu Abitew

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Main supervisor: Johann Sölkner (BOKU)

Co-supervisors: Gábor Mészáros (BOKU)

Pascal Duenk (WUR)

Tom Druet (University of Liège)

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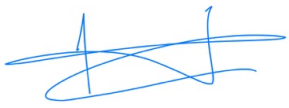
Student's signature 

Abstract

In this MSc. thesis work, we constructed a genomic map of Fleckvieh cattle using ~39K SNPs and 114228 meiosis events from male parents and 42706 meioses of females. Meiotic recombination is a source of genetic variation as it shuffles genetic material between sister chromatids, breaking down linkage disequilibrium in the process. Studying how recombination varies among individuals and breeds is important to understand how it could respond to selection. We used recombination frequencies between neighbouring markers to construct a genetic map for Fleckvieh cattle. We estimated recombination rates from a total of 4,600,610 crossovers. The average genome wide recombination rate was 1.04 centiMorgan per megabase and 0.92 cM/Mb with a length of 26.05 Morgan and 23.16 Morgan in males and females, respectively. Longer chromosomes showed lower recombination rates and vice versa. A higher number of recombinations was observed in males than females, similar to sheep but unlike many mammals. The genetic map showed a more pronounced recombination rate around the end position of each autosome in males whereas a gradual decline was observed around the same position in females. Using genome wide association study, we identified two previously reported candidate genes (REC8 & RNF212) in chromosome 6 and 10. The strongest signal was found on chromosome 19, near SCO1 gene, not associated with recombination rate so far. Genome wide recombination rate is a highly heritable trait with a 50% chip heritability. This is an estimate higher than previous studies from other breeds.

The genetic map of Fleckvieh cattle emerging from this thesis will be useful for many future research endeavours related to genetic diversity as well as genome wide association.

Key words: Cattle, Genetic recombination, SNP, Map, Morgan, Fleckvieh

Zusammenfassung

Erstellung einer genetischen Karte für das Fleckvieh-Rind

In dieser Masterarbeit haben wir eine genomische Karte des Fleckviehs erstellt, wobei wir ~39K SNPs und 114228 Meiose-Ereignisse von männlichen Eltern und 42706 Meiosen von weiblichen Tieren verwendet haben. Die meiotische Rekombination ist eine Quelle genetischer Variation, da sie genetisches Material zwischen Schwesterchromatiden verschiebt und dabei das Kopplungsungleichgewicht aufbricht. Es ist wichtig zu untersuchen, wie die Rekombination zwischen Individuen und Rassen variiert, um zu verstehen, wie eine Population auf Selektion reagieren würde. Wir haben Rekombinationshäufigkeiten zwischen benachbarten Markern verwendet, um eine genetische Karte für Fleckvieh zu erstellen. Wir schätzten die Rekombinationsraten anhand von insgesamt 4.600.610 cross-overs. Die durchschnittliche genomweite Rekombinationsrate betrug 1,04 centiMorgan pro Megabase und 0,92 cM/Mb bei einer Länge von 26,05 Morgan bzw. 23,16 Morgan bei männlichen bzw. weiblichen Tieren. Längere Chromosomen wiesen niedrigere Rekombinationsraten auf und vice versa. Bei den männlichen Tieren wurde eine höhere Anzahl von Rekombinationen beobachtet als bei weiblichen, ähnlich wie bei Schafen, aber im Gegensatz zu vielen anderen Säugetieren. Die genetische Karte zeigte eine ausgeprägtere Rekombinationsrate um die Endposition jedes Autosoms bei Bullen, während bei den Kühen ein allmählicher Rückgang um dieselbe Position beobachtet wurde. Mithilfe einer genomweiten Assoziationsstudie identifizierten wir zwei zuvor publizierte Kandidatengene (REC8 & RNF212) auf Chromosom 6 und 10. Das stärkste Signal wurde auf Chromosom 19 in der Nähe des Gens SCO1 gefunden, das bisher nicht mit der Rekombinationsrate assoziiert war. Die genomweite Rekombinationsrate ist ein stark vererbbares Merkmal mit einer Chip-Heritabilität von 50 %. Diese Schätzung ist höher als jene früherer Studien mit anderen Rassen.

Die in dieser Arbeit erstellte genetische Karte für Fleckvieh wird für viele künftige Forschungsarbeiten im Zusammenhang mit der genetischen Vielfalt und der genomweiten Assoziation von Nutzen sein.

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ከሁሉም አስቀድሜ እናቴ መምህርት የዝባየሽ ቢራራ ከልጂነቴ ጀምሮ እስከ አሁኑዋ ቀን ድረስ ስላደረግሽልኝ ነገር በሙሉ እግዚአብሄር በምድር በሰማይ ውለታሽን ይክፈልልኝ። እኔን ለማሳደግ ስትይ ያየሽውን ፈተና መቸም አልረሳውም። አብረን ያሳለፍናቸውን ጥሩ እና ደስ የማይሉ ጊዜያት ቆጥሮ ያሰብኩት ደረጃ ደርሽ እስከማኮራሽ ድረስ ቸሩ መድሃኒያለም እድሜ እና ጤና ሰጥቶልኝ ሁሌም በደስታ ኑሪልኝ። ይሄን ጽሁፍ ሳዘጋጅ በየቀኑ ስላበረታታሽኝ ከልቤ አመሰግንሻለሁ። ያላንቺ አይሆንም ነበር።

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ቶሚ እና ሉፓ፣ በህይዎቴ ያልጠበኳችሁ አስገራሚ ጓደኞች ናችሁ። አመሰግናለሁ።

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Abbreviations

DSB = Double strand breaks

CO = Crossing-over

cM = centiMorgan

M = Morgan

bp = Base pair

Mb = Mega base pair

Gb = Giga base pair

QTL = Quantitative trait loci

GRR = Genome wide recombination rate

RR = Recombination rate

FAO = Food and Agriculture Organization of the United Nations

LD = Linkage disequilibrium

SNP = Single Nucleotide Polymorphism

GP = Genomic prediction

DNA = Desoxy-ribo Nucleic acid

IBD = Identity-by-descent

EM algorithm = Expectation maximization algorithm

HMM = Hidden Markov model

GRM = Genomic relationship matrix

REML = Restricted maximum likelihood

QQ = Quantile-quantile

GWAS = Genome wide association study

h^2 = Heritability

h^2SNP = Chip-heritability

Introduction

Background

In meiotic prophase I, DNA double-strand breaks (DSBs) lead to the exchange of genetic material between non-sister chromatids. Recombination creates new genetic variation by generating novel combinations of grand-paternal and grand-maternal genetic information, and it helps to remove deleterious mutations that might otherwise accumulate (Piedrafita & Varona, 2019; Tiemann-Boege et al., 2017). Genetic recombination that involves crossing-over (CO) between homologous chromosomes increases genetic diversity and is essential for proper chromosomal segregation at the first meiotic division.

Nowadays, livestock breeding programs frequently incorporate genomic selection and exploit genetic variation to improve production and functional traits. On the one hand, recombination breaks down linkage disequilibrium and creates new genetic combinations which facilitate genetic diversity in eukaryotic genome. On the other hand, recombination is also mutagenic (Arbeithuber et al., 2015), and breaks down favourable allelic combinations previously selected upon (Johnston et al., 2018). Studying recombination rate variation and recombination based genetic mapping is an effective way to understand how they affect the population to respond to selection. A previous study in human suggested that women with higher recombination rates tend to have more offspring (Coop et al., 2008; Kong et al., 2004). Recombination is a heritable trait and responds to selection in the laboratory (Coop et al., 2008; Stapley et al., 2017). Battagin et al., 2016 recently reported that higher recombination rate could intensify the effectiveness of breeding programs, enhance overall selection response and decrease loss of genetic variation.

Recombination frequencies between markers may be used to construct genetic maps which have important applications (Qanbari & Wittenburg, 2020). Genetic and physical maps are two types of maps that help determine the location of genes in a chromosome. Genetic mapping is a technique used to determine the order and relative distance between genetic markers on a chromosome based on their recombination frequencies. Genes inherited from parental generations are identified as genetic markers for a particular trait. Genetic maps are measured in centimorgans (cM). Centimorgan is the distance between markers for which the expected average number of crossovers in a single generation is 0.01 (Rittner & Schneider, 1998). Physical map of genes, on the other hand, is used to indicate the physical distance of two genes using the number of nucleotides as measured by base pair nucleotides (bp). Physical mapping does not consider Mendelian genetic patterns alike genetic maps.

Genetic maps are important in many applications including quantitative trait locus (QTL) mapping, whole genome prediction, haplotype imputation, analysis of recombination processes and selection sweep studies (Druet & Georges, 2015; Singer et al., 2002).

Livestock have an important role in the global food system. Austria's primary cattle breeds are: Fleckvieh/Simmental, Brown Swiss, Holstein Friesian, Pinzgauer, Grauvieh Cattle as well as various special breeds for milk and meat production purposes. Fleckvieh cattle originated from

Austria in the 19th century. It was a crossbred of local breed with the Simmental cattle breed which was imported from Switzerland. The breed is dual-purpose and raised for milk and meat production. The total number of Fleckvieh cattle in Austria amounted to about 1.4 million head in 2020, which accounts for more than 75% of the total cattle population (ZAR, 2020).

In this study, we take advantage of a huge genotyped dataset from almost 300,000 Fleckvieh cattle genotyped with 50K genotypes with a large pedigree dataset to construct a breed specific recombination map.

Hence, a first objective of this study was to construct a genetic map based on the recombination landscape for Fleckvieh cattle and to investigate whether males and females had a similar recombination landscape in all the 29 autosomes. The second objective of this study was to conduct a genome wide association analysis for the phenotype trait genome wide recombination rate (GRR). Additionally, we estimated marker-based heritability of the trait and looked into the variance components.

Literature review

The Food and Agriculture Organization of the United Nations (FAO) projects a massive global increase in demand for food of animal origin mainly because of human population growth (FAO, 2011) . To meet this increasing demand for dairy and meat products, cattle breeding industries have been using genomic selection to identify genetically superior animals at an early age. The concept of genomic selection was first proposed in 2001 when (Meuwissen et al., 2001) published their article on predicting total genetic value based on dense marker maps after a simulation study using large numbers of marker genotypes. The study showed a new approach that required a sufficiently high marker density such that every quantitative trait locus (QTL) affecting a related trait would be in linkage disequilibrium (LD) with at least one marker (de Koning, 2016; Meuwissen et al., 2001). The article also demonstrated that genomic selection could improve traits that cannot be measured on the selected candidate, such as milk production on sires. At that time the tools needed for implementation for genomic selection were not yet available. However, after 2008, new genotyping technologies that render the genotyping of numerous single nucleotide polymorphisms (SNPs) cost-effective, genomic prediction (GP) has been implemented in multiple livestock species and genomic selection has been of huge research interest (de Koning, 2016).

The probably most significant event in history of genetic mapping happened when Artur Sturtevant, an undergraduate student in Thomas Hunt Morgan's lab found out that crossing over between two loci was a random event to ensure proper segregation of chromosomes during meiosis, where there is an equal chance of crossing over at any position along chromatids (Brown, 2002; Sturtevant, 1913). Two genes that are more distant from each other will be separated by crossovers more frequently than two genes that are close together. The frequency of genes that are unlinked by recombination corresponds to the distance between them. The number of crossovers is therefore a measurement of the distance between two genes (Brown, 2002). There are specific positions in the genome where crossovers occur at lower and higher frequency than the genome average, called crossover cold spots and hotspots (Bosse et al.,

2012; Z. Weng et al., 2019), somewhat contrary to Sturtevant's original observation and assumption.

Location of crossovers could be affected by epigenetic factors such as DNA methylation and histone modification along with genetic and environmental factors. Previous studies in cattle, human, sheep, and mice showed that variation in recombination among individuals is a heritable trait and may have conserved driving genes (Jeffreys et al., 2000; Kadri et al., 2016; Ma et al., 2015; Petit et al., 2017). Several genes have been identified to be associated with recombination rate variation across many species. Among them, RNF212, HEI10, CPLX1 with PRDM9 being a major specifier of recombination hotspots in sheep (Johnston et al., 2016a), human (Chowdhury et al., 2009) , and mouse (Paigen & Petkov, 2018). Recent studies in cattle recombination also reported an association between variants REC8 and RNF212, and genome wide recombination rate with PRDM9 affecting recombination hotspot usage (Kadri et al., 2016; Sandor et al., 2012).

Genetic maps of livestock and companion animals

For the type of genetic maps considered in this thesis, distance is measured in Morgan and centimorgan (cM), where one cM is the genetic distance between two loci with a recombination frequency of 0.01, or 1% (Lobo & Shaw, 2008). A decently dense marker coverage along chromosomes is needed for detecting and accurately positioning meiotic recombination events. First human genetic maps were based on large numbers of microsatellites (Broman et al., 1998). A comprehensive genetic map of the cattle genome based on 3802 Microsatellites was provided some years later (Ihara et al., 2004), an enhanced linkage map of the sheep genome comprising more than 1000 loci was available even before (Maddox et al., 2001). Genetic mapping, particularly of loci associated with diseases, is also of great interest for breeders of companion animals. For dog, sparse maps were available very early on, a comprehensive map based on >1,500 microsatellites were made available by 2007 (Wong et al., 2010).

The genomic revolution with the advent of high throughput markers, i.e. SNP markers and whole genome sequence, at a cheap cost, provided the opportunity of substantially increasing map resolution and also of constructing genetic maps for sub-species and breeds. In many livestock breeds, genomic selection has been implemented to increase genetic gains and very many animals are being SNP genotyped with commercial arrays at very low cost, typically in the range of € 15-30 per sample. Based on such data, several genetic maps for the most populous cattle breed of the world, Holstein Friesian, were published (Ma et al., 2015; Qanbari & Wittenburg, 2020), with important information about the pattern of recombination along chromosomes as well as on recombination hotspots. Patterns of recombination along the bovine X-chromosome were also investigated (Kadri et al., 2022; Zhang et al., 2020). Recombination pattern in domestic (Petit et al., 2017) as well as wild sheep (Johnston et al., 2016) populations were explored in detail. For horse, the recombination landscape was investigated (Beeson et al., 2019) and the recombination map spans 2.36 Gb and accounts for 2939 cM. Considering the canine map (Campbell et al., 2016), females have a longer map length (2162 cM) than males (1816 cM).

Much work was been devoted to patterns of recombination in specific regions of the genome, particularly MHC (Ballingall et al., 2015; Jaworska et al., 2020; Radwan et al., 2020).

Very recently, recombination events were detected in the sequence of143 single sperm of two bulls (Yang et al., 2022). The authors found that in the absence of evolutionary selection pressure in fertilization and survival, recombination events in sperm are enriched near distal chromosomal ends, revealing that such a pattern is intrinsic to the molecular mechanism of meiosis.

Current methods for haplotype phasing

Construction of genetic maps from genotypes starts with phasing parental haplotypes. Crossover detection and genetic map construction then follows in the next steps based on the frequency of recombination. Haplotypes from SNP array data could be generated through either statistical or laboratory methods (Browning & Browning, 2011). The accuracy of computational phasing depends on the size of the sample in the genotype data and phasing of closely related individuals, such as parent-offspring trios. For related genotyped individuals, identity-by-descent (IBD) information is used to infer haplotypes. In parent-offspring trios, heterozygous positions and missing genotypes are the only positions that the phase is unknown. Linkage programs that assume sites with linkage equilibrium would incorrectly phase haplotypes by inferring IBD in which it is not present (Browning & Browning, 2011). One possible solution for this problem was proposed (Kong et al., 2008). The method is called long-range phasing in families, and is based on combining IBD and IBD-based phasing with population haplotype-frequency models (Browning & Browning, 2011; Kong et al., 2008). Many statistical phasing programs use expectation maximization algorithm (EM algorithm) to phase small numbers of SNPs. EM algorithm is an iterative method to find local maximum likelihood estimates of SNP haplotypes assuming Hardy-Weinberg equilibrium. This algorithm had computational limitations if high number of genetic polymorphisms are present (Excoffier & Slatkin, 1995).Currently, many haplotype phasing algorithms use Coalescent-based methods and hidden Markov models. Approximating recombination with coalescent methods has better performance recognising that new haplotypes are coming from old haplotypes by the processes of recombination (Browning & Browning, 2011; McVean & Cardin, 2005). A hidden Markov model (HMM) is underlying hidden states to infer haplotypes that are not visible directly. Emission probabilities and transition probabilities link unobserved states to observed data and determine changes from one chromosomal position to another, respectively (Browning & Browning, 2011). LINKPHASE3 is a practical, robust, and accurate genotype imputation and phasing algorithm based on hidden Markov model (Druet & Georges, 2015).

Methods for crossover detection

Pedigree Analyses

The pedigree analyses method relies on genotyping parent-offspring trios in humans, or based on large scale crosses between inbred strains of mice in laboratory. This method relies on the transmission of alleles from the parental generation to the next generation (Druet & Georges, 2010; Wirtenberger et al., 2005). The disadvantage of this method is its high cost and its resolutions is restricted by the genotype density and number of meiosis analysed (Ott et al., 2011). The required input data for pedigree-based crossover detection are genotypes and pedigree data. Using a large pedigree more than thousands of individuals will increase accuracy of detecting crossovers (Kohlmeier, 2013).

Gamete based analyses

In single sperm assay a sperm cell is subjected to whole genome amplification and high-throughput genotyping. By directly determining whether the single gamete is recombinant or parental without relying on analysis of pedigree data (ARNHEIM et al., 1991; Flaquer et al., 2008). This method provides a fine scale crossover detection as a result of a large number of meiosis from a single male (Flaquer et al., 2008).

Construction of genetic maps

To construct haplotypes and detect crossovers, based on Mendelian segregation rules, LINKPHASE3 assigns heterozygous SNPs to maternal and paternal homologs if both parents are genotyped. The second step is completing the parental homologs based on linkage information. The last step of haplotype reconstruction is assigning the reconstructed haplotypes to two hidden states (familial and population information) and imputes missing genotypes (Druet & Georges, 2010).

The probability of a given Markov chain can be computed as:

$$\pi_k^1 \theta_{kl}^1 \prod_{m=2}^{M} a_{kk'}^m \theta_{k'l}^m,$$

in which π^1_k is the initial-state probability Θ^m_{kl} is the emission probability α^m_{kk} is the transition probability, α^m_{kk} equals Jm $\pi^{m+1}_{k'}$, where Jm is the probability to have a jump (recombination) between markers m and m+1 and $\pi^{m+1}_{k'}$ is the probability that the Markov chain moves to HS k' when a recombination occurred between markers m and m+1 (Druet & Georges, 2010).

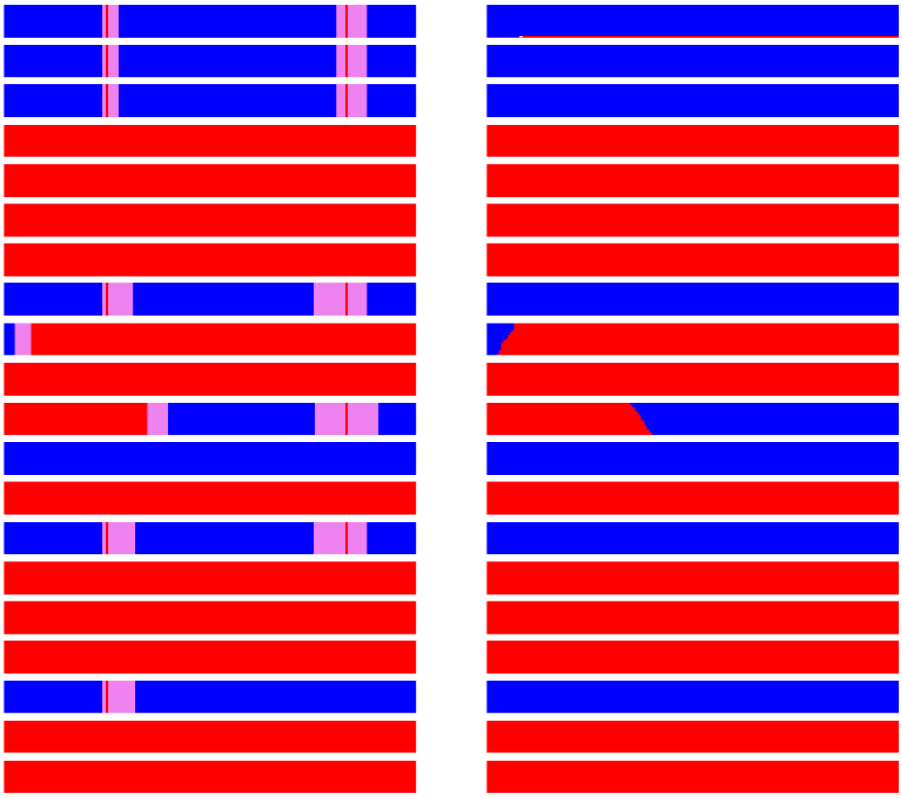

Figure 1. Comparison of inheritance patterns obtained for a small region on BTA1 encompassing 300 SNPs. The colour represents the paternal haplotype inherited (red for grand-paternal, blue for grand maternal origin and violet for unknown) by 20 sibs (one line per animal) in a half-sib family. With LinkPHASE, the inheritance is binary (paternal vs maternal) whereas LINKPHASE3 estimates inheritance probabilities (represented by the height of one colour at a position) which range from 0 to 1 (Druet & Georges, 2015).

Data and Methods

Genotype and pedigree data

This study used a large pedigree information with a subset of genotype data of 298850 male and female Fleckvieh cattle provided by ZuchtData. Individuals were genotyped with custom SNP chips, all including the contents of the 50K Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego). Data received had already undergone quality control for routine genomic prediction by ZuchtData. The total number of autosomal SNP included in this study was 38999, numbers of SNP per chromosome are given in Table 1. We used the Btau_5.0.1 genome assembly to map the genotype data.

Haplotype reconstruction and crossover detection

All genotyped animals were phased for each chromosome separately. Haplotype reconstruction was done using LINKPHASE3 (Druet & Georges, 2015), a programme that works well for large half-sib families, alike in our dataset. It works by assigning heterozygous SNPs to paternal and maternal homologs of the offspring based on Mendelian segregation rules after

the offspring were genotyped. Then the algorithm reconstructs haplotypes in parents using linkage information (Druet & Georges, 2010). The last step is based on a hidden Markov model (HMM). Two hidden states describe haplotypes transmitted to the offspring as a combination of the two parental homologs (Druet & Georges, 2015). The programme utilizes linkage and half-sib family information and applies the HMM using the Baum-Welch algorithm. These two homologs (emission probabilities) are linked with marker alleles with a certain likelihood (Druet & Georges, 2015). The probability of inheritance of the paternal or the maternal chromosome at each marker position was calculated for each offspring using the forward-backward algorithm of the programme. Markers with a map confidence score less than 0.9 were considered errors and removed from the recombination map. The programme is written in FORTRAN and was compiled with *GFORTRAN* (gcc V4.4.3, 1987).

Recombination rates

LINKPHASE3 uses an EM algorithm to compute recombination rates between all pairs of successive markers. The probability that a recombination l detected between markers m1 and m2 occurs between two markers m and m+1 (m1 <= m <m2) is equal to:

$$p_m^l = \rho(m) / \sum_{m_i = m1, m2} \rho(m_i)$$

where ρ(m) is the current estimate of the recombination rate between m and m+1. After estimating these probabilities for all markers intervals and all identified CO, recombination rates are updated as:

$$\rho(m) = \sum_{l=1,nrec} \frac{1}{ngam} p_m^l$$

With *nrec* being the number of detected CO and *ngam* the number of gametes (or progeny). The process is repeated 100 times (Druet & Georges, 2015).

Recombination events were considered phase changes in the transferred gametes compared to the two inherited reconstructed haplotypes (Z. Q. Weng et al., 2014). Total recombination rate was estimated for each non-overlapping 1 mega-base (Mb) window across all 29 autosomes. The construction of recombination maps was obtained from LINKPHASE3 output *emap,* which were identified from informative markers.

Results of this study are based on 114228 meiosis events from male parents and 42706 meioses of females. The X- chromosome was excluded from all data analysis for quality control purposes.

Table 1 . Number of SNPs per chromosome

Chr	Num. SNPs	Chr	Num. SNPs	Chr	Num. SNPs
1	2596	11	1705	21	1110
2	2096	12	1309	22	976
3	1933	13	1403	23	827
4	1918	14	1394	24	965
5	1671	15	1296	25	764
6	1296	16	1272	26	845
7	1639	17	1231	27	729
8	1845	18	1021	28	752
9	1598	19	1078	29	818
10	1678	20	1234	Total	38999

Genome-wide association analysis and estimating heritability for recombination frequency

The average total number of recombinations per gamete formation across the autosomal genome for parents with 10 or more offspring was used as phenotype for the genome wide association study and heritability. A total of 1566 individuals fulfilled the criterion of having 10 or more offspring in the available data set of genotyped animals. The number of total SNP genotypes were 38999. For the association study, a univariate linear mixed model for SNP marker association test was fitted according to the following model:

$$\mathbf{y = \mu + Sex + x\beta + \varepsilon}$$

Where y was vector of the phenotype, μ was the intercept, **x** was a vector of genotype markers, β was the size of marker effects, and ε was the vector of errors. The sex of the parent (Male, Female), was considered as a covariate in the model (Zhou & Stephens, 2012).

Single-SNP associations based on the genome-wide efficient mixed model association algorithm were performed using GEMMA (Zhou & Stephens, 2012). A genetic relatedness matrix was estimated to account for population structure.

Chip heritability (h^2) of genome wide recombination phenotype was estimated using GCTA v.1.25.3 (Yang et al., 2011). We used GCTA to estimate the genetic relationships (GRM) between individuals from the genotype data. The genetic relationship between individuals j and k was estimated by the following equation:

$$A_{jk} = \frac{1}{N}\sum_{i=1}^{N}\frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1-p_i)}.$$

Where Ajk is the genomic relationship averaged over all positions in the genome, N being the number of SNPs, x (coded as 0,1, or 2) is the number copies of the reference allele, and p is the frequency of the reference allele frequency of SNPs.

The variance components were estimated based on the restricted maximum likelihood (REML) approach as:

$$\mathbf{h^2_{SNP} = \sigma^2_G/(\sigma^2_G + \sigma^2_e)}$$

Where h^2SNP was the proportion of phenotypic variance explained by the genome wide SNPs.

Results and Discussion

Recombination rates

Recombination rates were estimated based on the *emap* output of LINKPHASE3 in 1 Mb windows. We plotted chromosomal positions to the recombination rates along the whole autosomal genome (~2.5Gigabases), see Figure 2. Note that all centromeres in cattle are located at the beginning of autosomes and telomeres being at the end of each chromosome. To evaluate the number of recombination events, we looked into genome wide number of crossovers. The total number of recombination events was 4,600,610 in 3,931,8492 chromosome-wise meioses.

Table 2. Comparison of the physical (Mb) and genetic maps (cM) of female and male Fleckvieh cattle

Chr	Physical map (Mb)	Female genetic map (cM)	Male genetic map (cM)
1	1.58229218	1.56268727	1.70308671
2	1.36697236	1.13656069	1.19319666
3	1.21403393	1.03742449	1.13913538
4	1.20641946	1.03397624	1.09135581
5	1.21175859	1.01834184	1.16414213
6	1.19216563	0.99529121	1.09208122
7	1.12628884	0.99442935	1.06037345
8	1.13367096	0.99752175	1.05685149
9	1.05587941	0.87461408	0.99023832
10	1.04215086	0.92569992	1.06191607

11	1.07246475	0.97049024	1.09530444
12	0.91091598	0.79281135	0.88660276
13	0.84148909	0.82075299	0.94018053
14	0.84616190	0.76526205	0.87183255
15	0.85257312	0.75742774	0.86042237
16	0.81322588	0.74745892	0.89398695
17	0.74998349	0.66094776	0.82113329
18	0.65978584	0.67874763	0.81155033
19	0.64007021	0.71155638	0.89918104
20	0.71793734	0.62744626	0.69156146
21	0.71136925	0.70082381	0.79628632
22	0.61378199	0.61167473	0.76039552
23	0.52091670	0.54656087	0.64357164
24	0.62643699	0.60058304	0.66057668
25	0.42851121	0.49724772	0.57009084
26	0.51680135	0.55889686	0.62630823
27	0.45347958	0.4984807	0.54483068
28	0.46194755	0.50936626	0.53179343
29	0.51502868	0.53014804	0.59440751
Total	25.08451312	23.16323019	26.05239381

Table 3. Average recombination rates of males and females per chromosome, with males having a slightly higher recombination rate

Chr	Female RR (cMMb-1)	Male RR (cMMb-1)
1	0.987609804	1.076341482
2	0.831443797	0.872875484
3	0.854526768	0.938306049
4	0.857061971	0.904623845
5	0.84038343	0.960704665
6	0.834859842	0.916048234
7	0.882925689	0.94147559
8	0.879904121	0.932238301
9	0.828327621	0.937832778
10	0.888259038	1.018965786
11	0.904915747	1.021296448
12	0.870345199	0.973309042
13	0.97535785	1.117281901
14	0.904391996	1.030337752
15	0.888402088	1.009206542
16	0.919128299	1.099309518
17	0.88128308	1.094868488
18	1.02873931	1.230020835
19	1.111684888	1.404816262
20	0.873956855	0.963261585
21	0.985175856	1.119371297
22	0.996566761	1.238869065
23	1.049228927	1.235459796

24	0.958728571	1.054498203
25	1.160407729	1.330398894
26	1.081453947	1.211893564
27	1.099235163	1.201444793
28	1.102649554	1.151198724
29	1.029356346	1.154125067
Total Average	0.923407605	1.038584791

The autosomal recombination rate was higher in bulls (1.04 cM/Mb) than in cows (0.92 cM/Mb), for chromosome-wise recombination rates see Table 3. There was a trend of shorter chromosomes having higher recombination rates. The genome wide autosomal map length was 26.05 Morgan (M) in males and 23.16 M in females, see Table 2. This result is consistent with recent findings in cattle (Kadri et al., 2016; Ma et al., 2015; Qanbari & Wittenburg, 2020) and Soy sheep (Johnston et al., 2016b). With a likelihood-based approach, Qanbari & Wittenburg, reported a total male genetic map length of 25.35 in German Holstein cattle breed (Qanbari & Wittenburg, 2020).

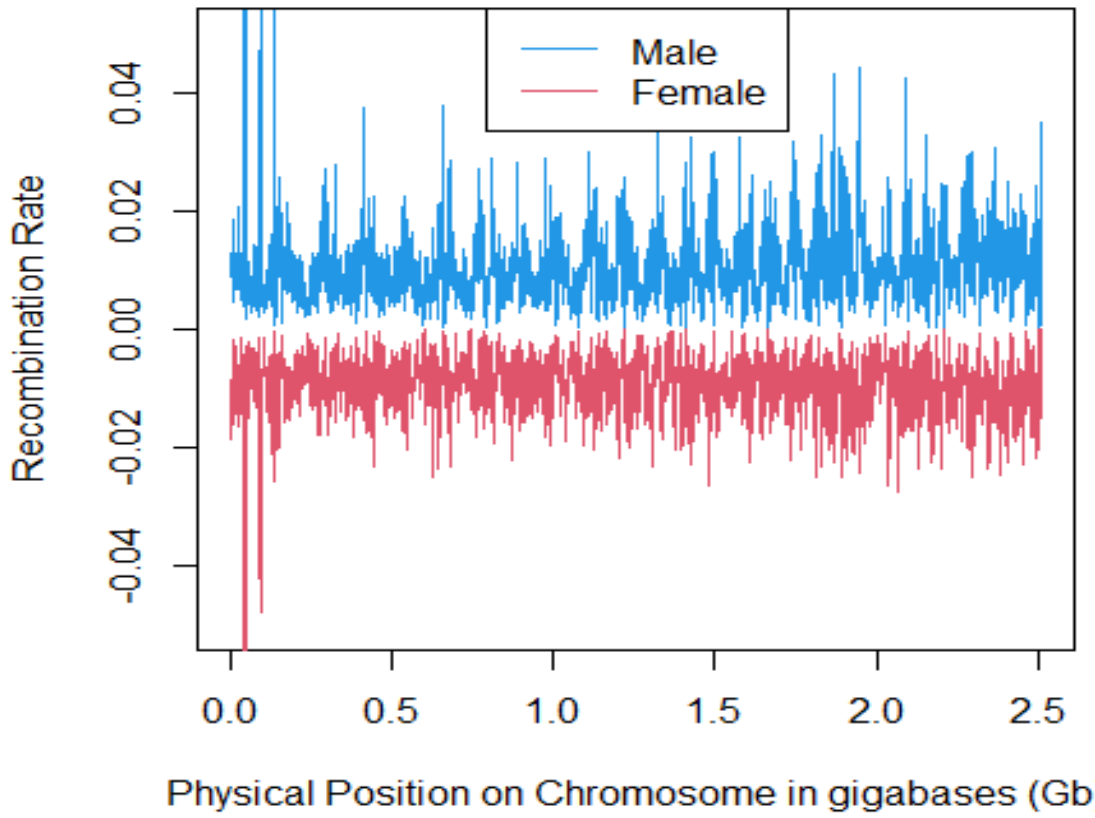

Figure 2. Recombination rates of male and females calculated in 1 Mb windows across all 29 autosomes

We fitted a smooth-spline plot of recombination rates against the relative physical position on all 29 autosomes. The physical positions are scaled by dividing all positions in a 1 Mb window across all chromosomes by the total length of each chromosome. Overall, the recombination map showed that, male recombination rate is higher around the beginning and the end position of the chromosome, while slightly higher female recombination rates are exhibited in the middle.

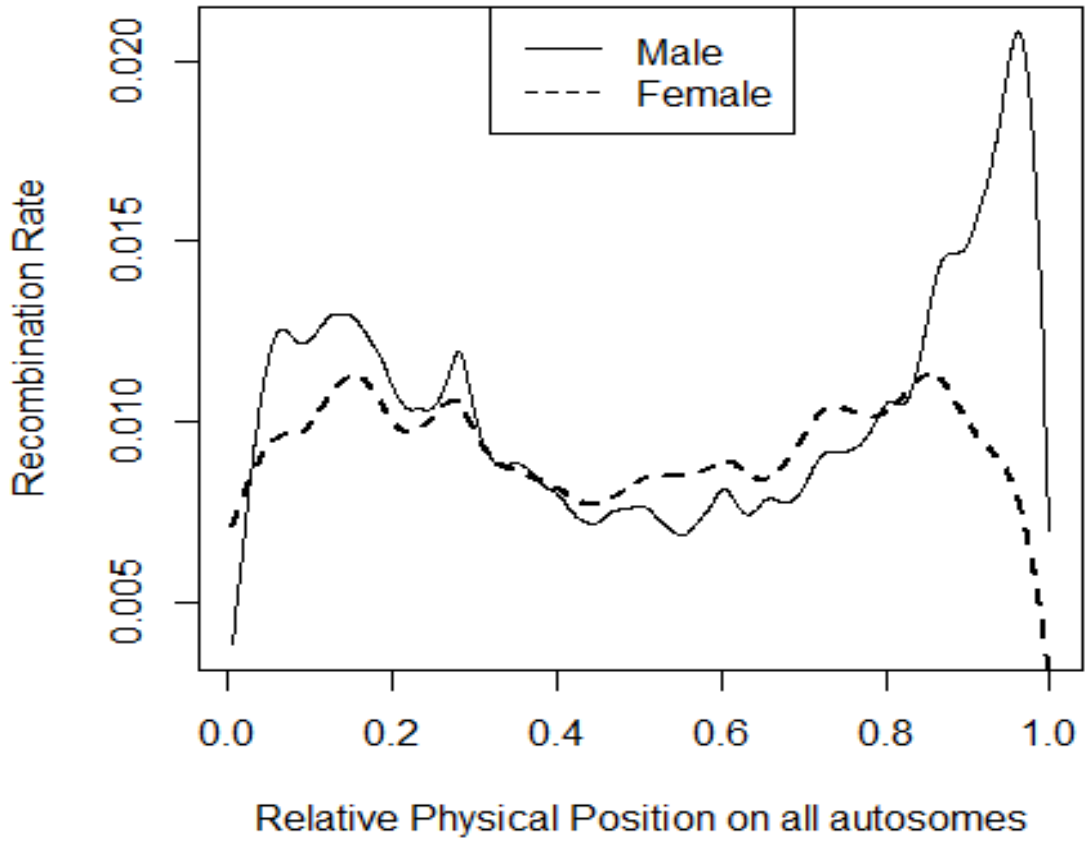

Figure 3. Smooth spline plotting of male and female recombination rates across all autosomes with relative physical distance

Landscape of recombination rates in male and females

From the recombination rates of males and females, we plotted the rate of recombination in 1 Mb windows, see Figure 4 for a sample of two chromosomes and Appendix 1 for all autosomes. A notable pattern to emerge from landscape of recombination in males and females is that the distribution of crossovers is distributed in a non-random way across the genome with higher recombination around the beginning and the end of chromosomes. In line with previous studies in cattle, human, and mice (Broman et al., 1998; Kong et al., 2010; Ma et al., 2015), a more pronounced peak and much higher crossover was also observed around the end of each

chromosome in males than in females. Low recombination rates around the middle of chromosomes were observed consistent with previous findings (Ma et al., 2015).

A recent study in three cattle populations from France, the Netherlands and New Zealand reported a larger crossover in males (23.3 Morgan) than in females (21.4 Morgan) (Kadri et al., 2016). While in most mammals, it is reported that females have a longer genetic map than males (Jensen-Seaman et al., 2004; Johnsson et al., 2021; Tortereau et al., 2012), males exhibit higher recombination rates in cattle and sheep (Johnston et al., 2016b; Ma et al., 2015; Qanbari & Wittenburg, 2020). The highest recombination rate was observed on chromosome 1 while the lowest were on chromosome 27 and chromosome 25 for males and females respectively. The physical map length shows a very high correlation (0.97 and 0.96 in males and females respectively) with the genetic map distance. This result is in line with previous studies (Ma et al., 2015).

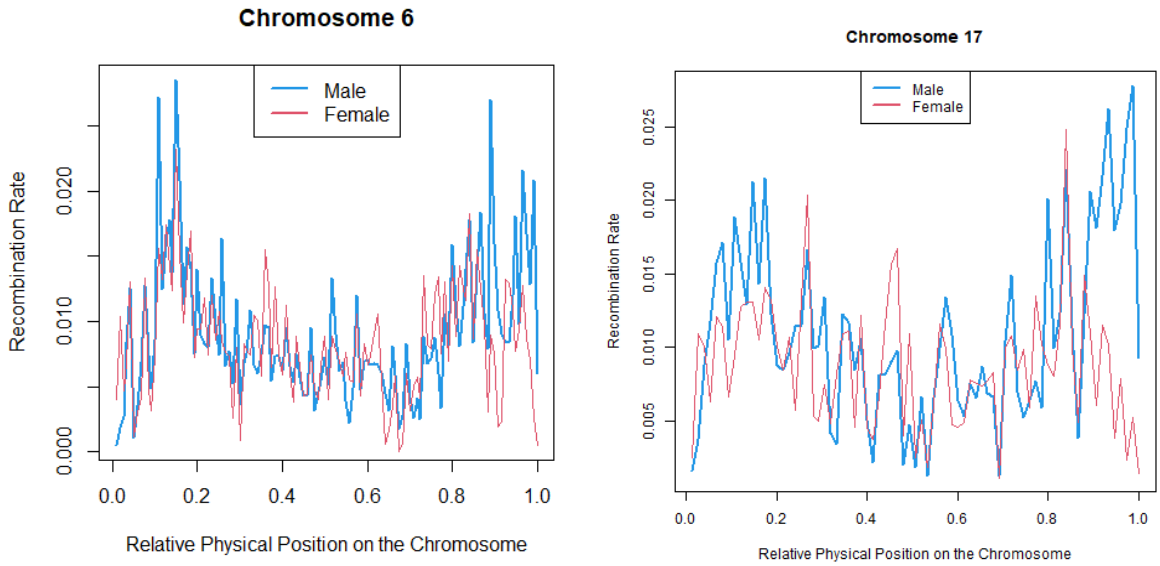

Figure 4. Recombination rate landscapes on chromosome 6 and 17

Landscape of recombination hotspots for male and female recombination

We defined the recombination hotspots in the autosomes recombination rates greater than 2.5 standard deviation from the average genome-wide recombination rate based on (Ma et al., 2015; Qanbari & Wittenburg, 2020). We excluded recombination rates having more than 5 standard deviations from the mean to account for false-positive hotspots. Figure 5 provides chromosomal hotspots of recombination in male and female parents for a sample of chromosomes, see appendix for graphs for each autosomal chromosome.

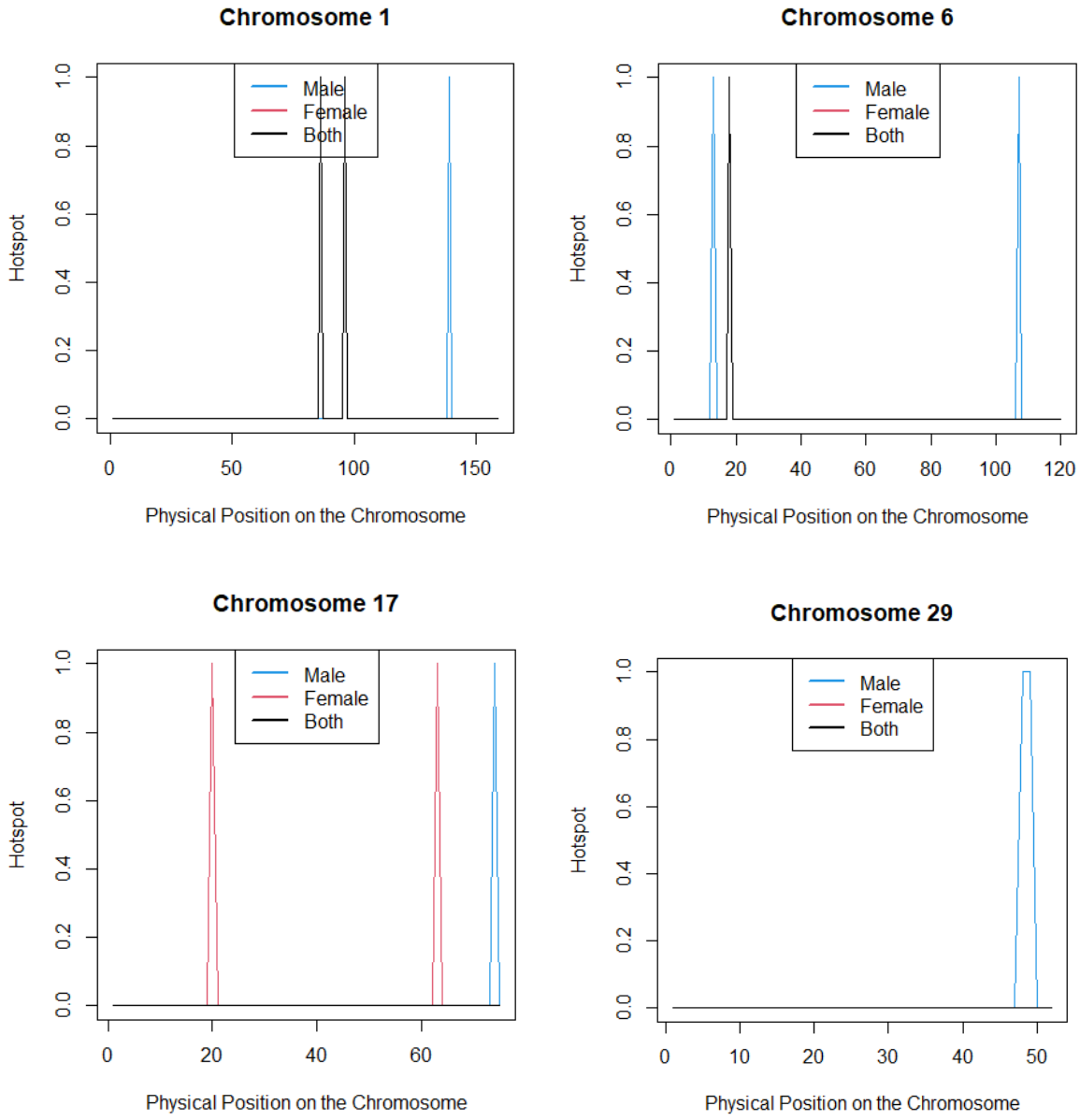

Figure 5. Recombination hotspots in Chromosome 1,6,17 & 29, with recombination rate exciding 2.5 standard deviations from the average recombination rate in the genome.

Genome-wide association and heritability study for recombination frequency

We conducted a genome wide association study of genome wide recombination rates for 1566 sires from our genotype dataset, based on the availability of 10 and more genotyped offspring. For each sire, the average number crossovers across all meiosis divided by numbers of offspring were used as a phenotypic trait. We performed the association analysis using a linear mixed model (LMM) with 38999 total SNPs. We used a genome wide significant level of $-\log10(p) = 5.86$, applying Bonferroni correction. We plotted a Quantile-quantile (QQ) plot of the observed p-values versus the quantile distribution of expected p-values to determine if there are likely a large number of false positive results in the GWAS (Figure 6).

The results from the GWAS (Figure 7, Table 4) show two significant signals on chromosomes 9 and 19 and one suggestive signal on Chromosome 6. The strongest candidate genes, also

based on previous studies, are RNF212 (Chr 6) and REC8 (Chr 9). The strongest signals came from chromosome 19. The candidate gene associated with these SNPs was SCO1 (Synthesis of Cytochrome C Oxidase 1), a gene not reported to be involved in recombination rate so far. However, contrary to the findings of (Ma et al., 2015; Qanbari & Wittenburg, 2020), we did not find SNPs associated with the PRDM9 gene, which has been reported to influence recombination rate and hotspot usage. Kadri et al., 2016 reported six variants associated with recombination in cattle based on 14,401 animals. Five likely coding genes, namely HFM1,MSH4, RNF212, MLH3, and MSH5 and a non-coding variant in RNF212B, a paralog for RNF212, might be involved in recombination rate in cattle (Kadri et al., 2016). In sheep, an association study also identified six candidate QTL for global recombination rate (Johnston et al., 2016a). The candidate genes reported are REC8, GAK, PCGF3, CLPX1, RNF22 and its paralog RNF212B. (Ma et al., 2015) reported thirteen associated loci from a genome wide association study. Nine loci on female recombination rate and the remaining four on male recombination rate with three shared loci between the two sexes. The candidate genes reported are, CPLX1, REC114, PABPN1, FMN1 and NEK9, in chromosomes 6 and 10. Consistent to our result, they couldn't find any significant association with the PRDM9 gene in the genome wide recombination for males.

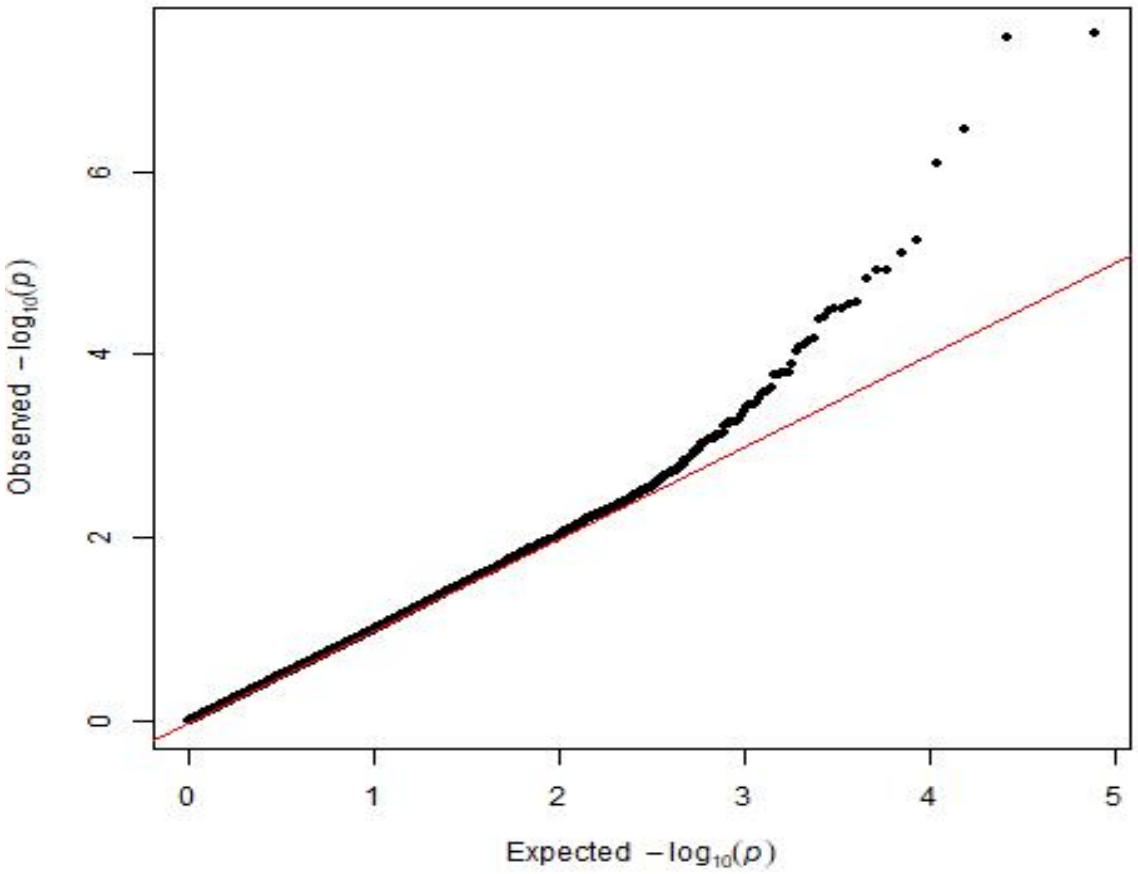

Figure 6. Quantile-quantile (QQ) plot of the observed p-values versus the quantile distribution of expected p-values for the GWAS study

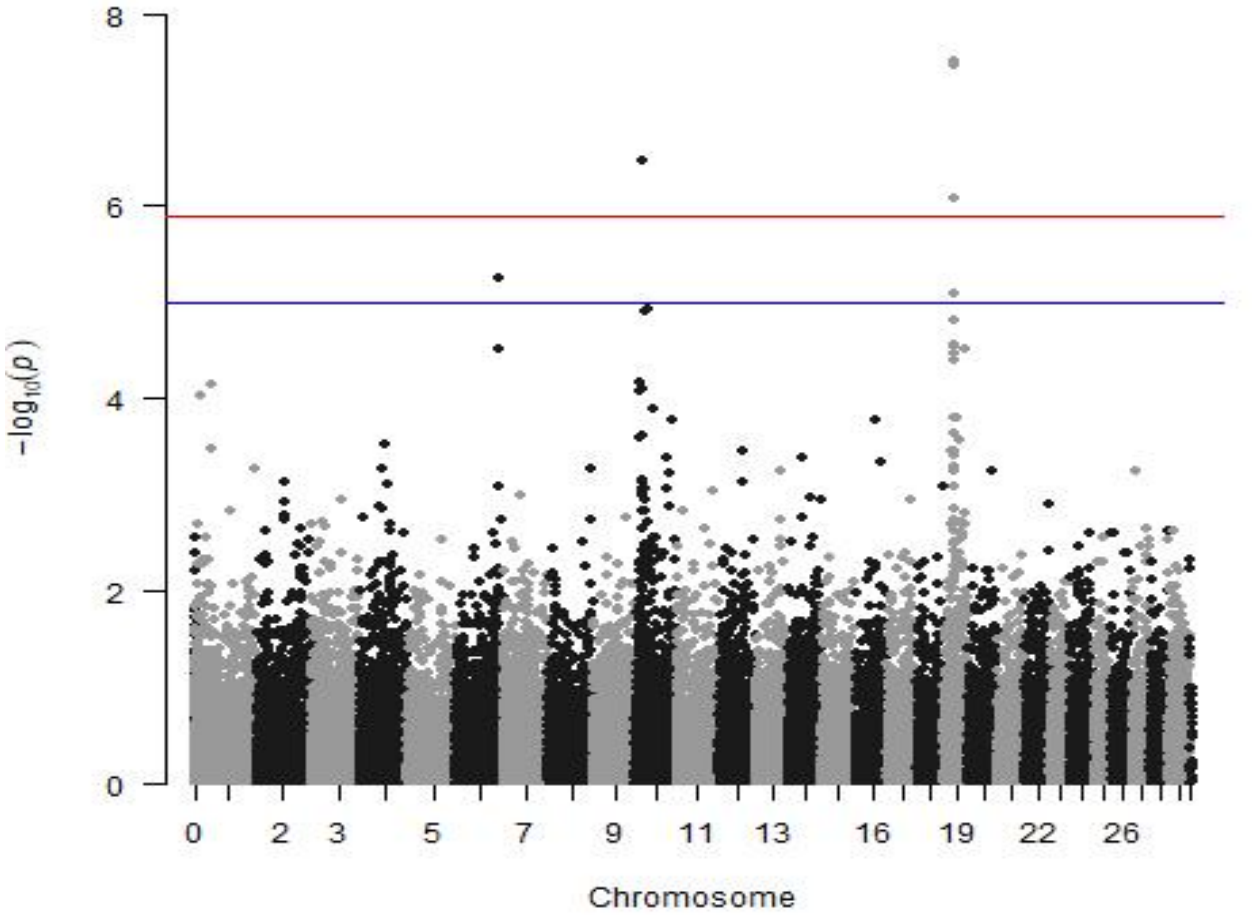

Figure 7. Significant SNPs for the trait genome wide number of recombination (blue line = indicative threshold–log10(p) = 5; red line = Bonferroni threshold–log10(p) = 5.86)

Table 4. Significant SNPs, their position and candidate genes

Chromosome	Physical position (Mb)	p-value	Candidate gene
19	29655745	3.196331e-08	SCO1
19	29729113	3.491151e-08	
10	21225382	3.419654e-07	REC8
19	28095023	8.205766e-07	
6	108998175	5.771408e-06	RNF212
19	27529791	7.960123e-06	
10	33042660	1.1932e-05	
10	26700563	1.222072e-05	REC8

19	27491890	1.525393e-05	
19	24692211	2.758729e-05	
19	25365606	2.909552e-05	
19	53208781	3.109584e-05	
6	108971589	3.114721e-05	RNF212

Variance component and heritability estimates of the trait genome wide recombination is reported in Table 5. The estimate of h^2SNP was 0.515 is based on a sample of 1566 individuals. This result is higher than in previous reports, showing that chip heritability of genome wide recombination to be around 0.23 to 0.26 in Angus and Limousin cattle breeds (Z. Q. Weng et al., 2014). A much lower heritability of global recombination rate (GRR) was reported to be 0.13 in a three cattle population study (Kadri et al., 2016). This estimate from GCTA could not be interpreted as the variance explained by all the SNPs as it could coincide with shared environment effects.

Table 5. Variance components of the trait genome wide recombination-

Source	Variance	SE
V(g)	4.165701	0.482039
V(e)	3.928654	0.319161
V(p)	8.094356	0.336550
V(g)/Vp = h^2SNP	0.514643	0.045712

V(g): genetic variance; V(e): residual variance; V(p): phenotypic variance; V(g)/V(p): heritability based on SNP effects

Conclusion and Recommendations

In conclusion, based on our large pedigree data of Fleckvieh cattle, we identified 4600610 recombination events from 3931849 chromosme-wise meioses. Based on recombination frequency from autosomal 38999 SNPs, we constructed a genetic map for male and female Fleckvieh cattle for the first time. The length of the constructed genetic map is 26.05 Morgans (M) in males and 23.16 M in females. Using 1566 sires and 38999 autosomal SNPs, our genome wide association analysis confirmed two loci already reported to be associated with recombination rate, REC8, and RNF212, as well as a novel candidate gene SCO1. GWAS could be more informative if there were many dam-offspring trios to further look into sex difference in genome wide recombination rate. In previous studies the gene PRDM9 was reported to influence the positions of recombination hotspots. We did not find a strong signal of this gene in our association study for genome wide recombination rate. Performing an association study on the trait recombination hotspot might be a practical way to find better

signals for this particular gene. We confirmed previously reported results on males having higher recombination rate (1.04 cM/Mb) than females (0.92 cM/Mb), alike most other mammals. In most of the autosomes, the recombination map for males showed a higher peak at the end of the chromosome.

We recommend to use the new map build assembly of Bos taurus (ARS-UCD 1.3 (GCF_002263795.2)) for future reference.

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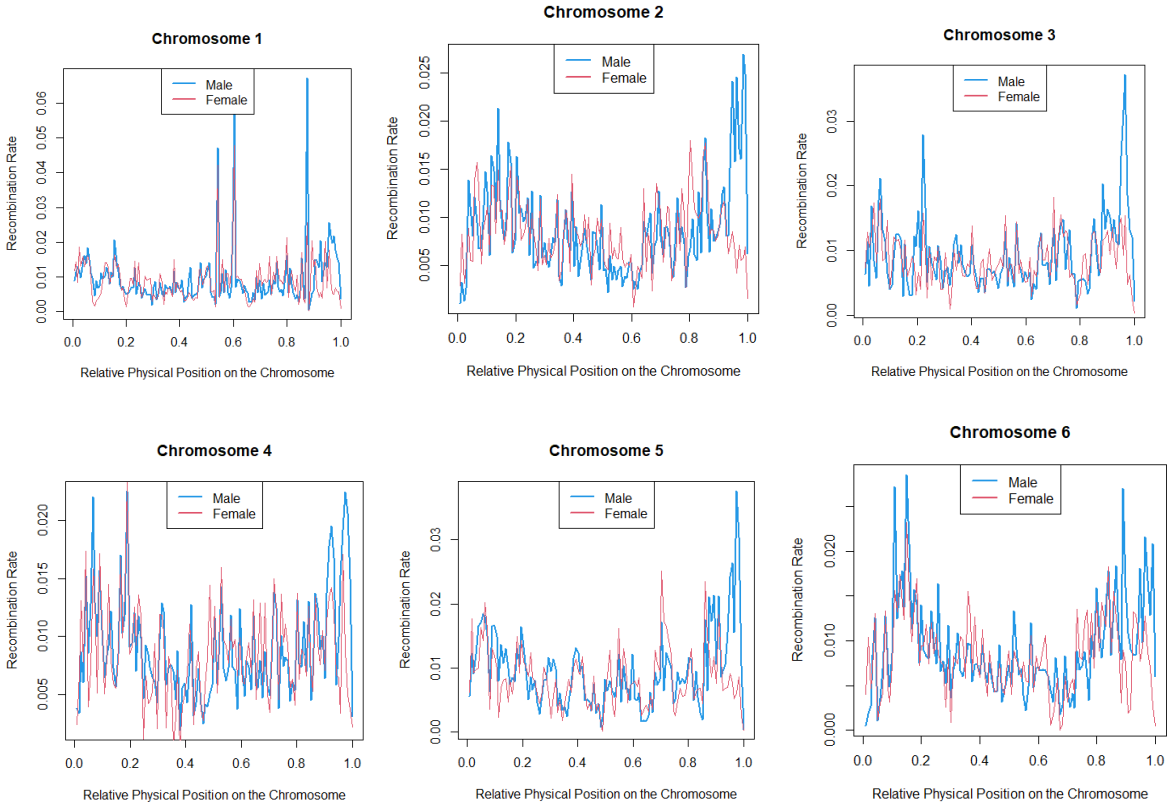
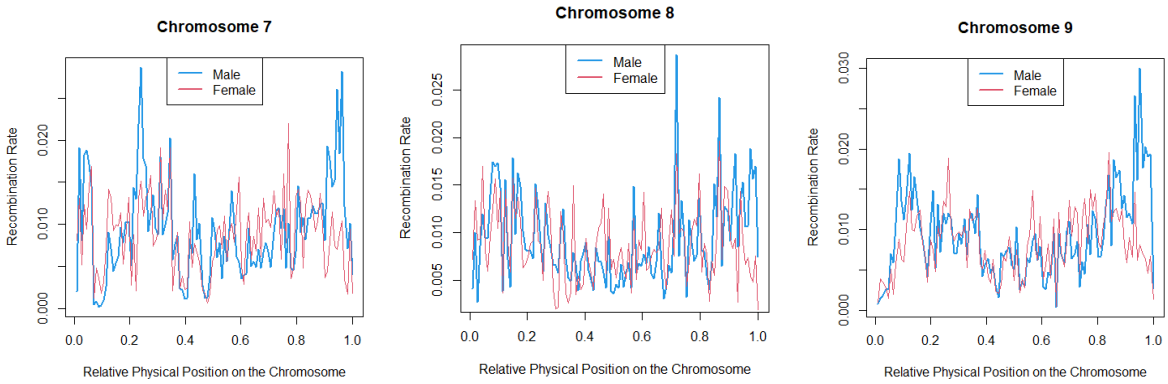
Appendices

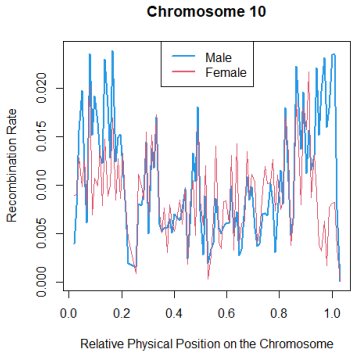
FIGURE S1. RECOMBINATION RATE VERSUS RELATIVE PHYSICAL LOCATIONS BY AUTOSOMES

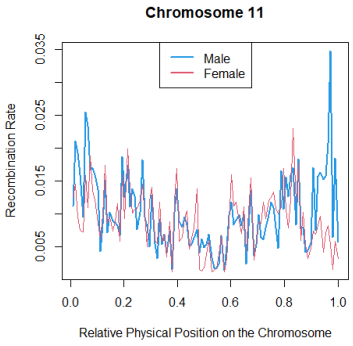


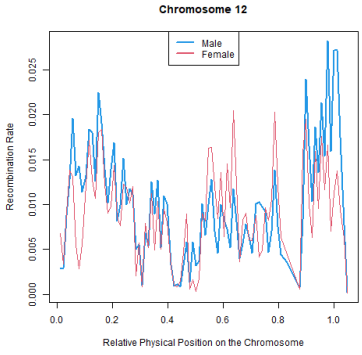

Chromosome 7
Male
Female
Recombination Rate
0.000
0.010
0.020
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

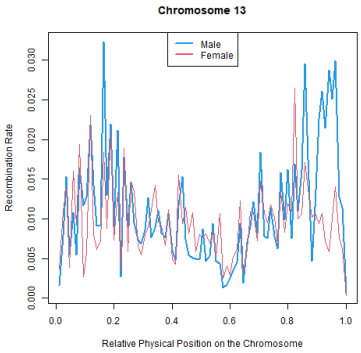
Chromosome 8
Male
Female
Recombination Rate
0.005
0.010
0.015
0.020
0.025
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

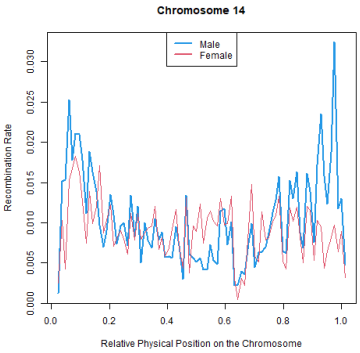
Chromosome 9
Male
Female
Recombination Rate
0.000
0.010
0.020
0.030
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

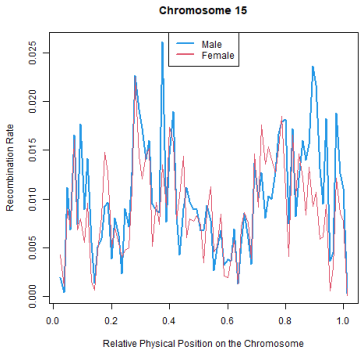

Chromosome 10
Male
Female
Recombination Rate
0.000
0.005
0.010
0.015
0.020
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

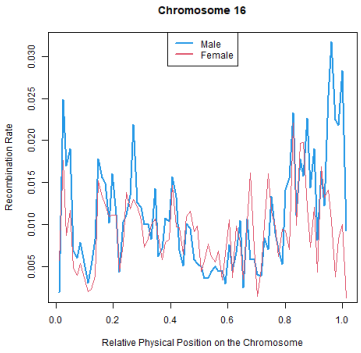

Chromosome 11
Male
Female
Recombination Rate
0.005
0.015
0.025
0.035
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

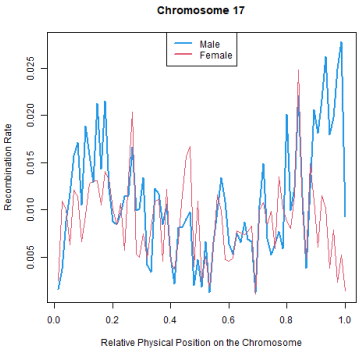

Chromosome 12
Male
Female
Recombination Rate
0.000
0.005
0.010
0.015
0.020
0.025
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

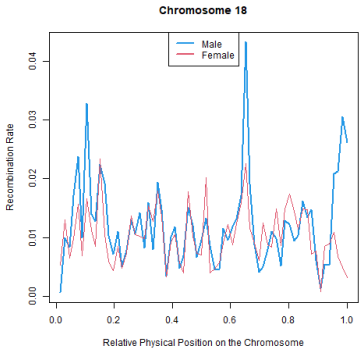

Chromosome 13
Male
Female
Recombination Rate
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

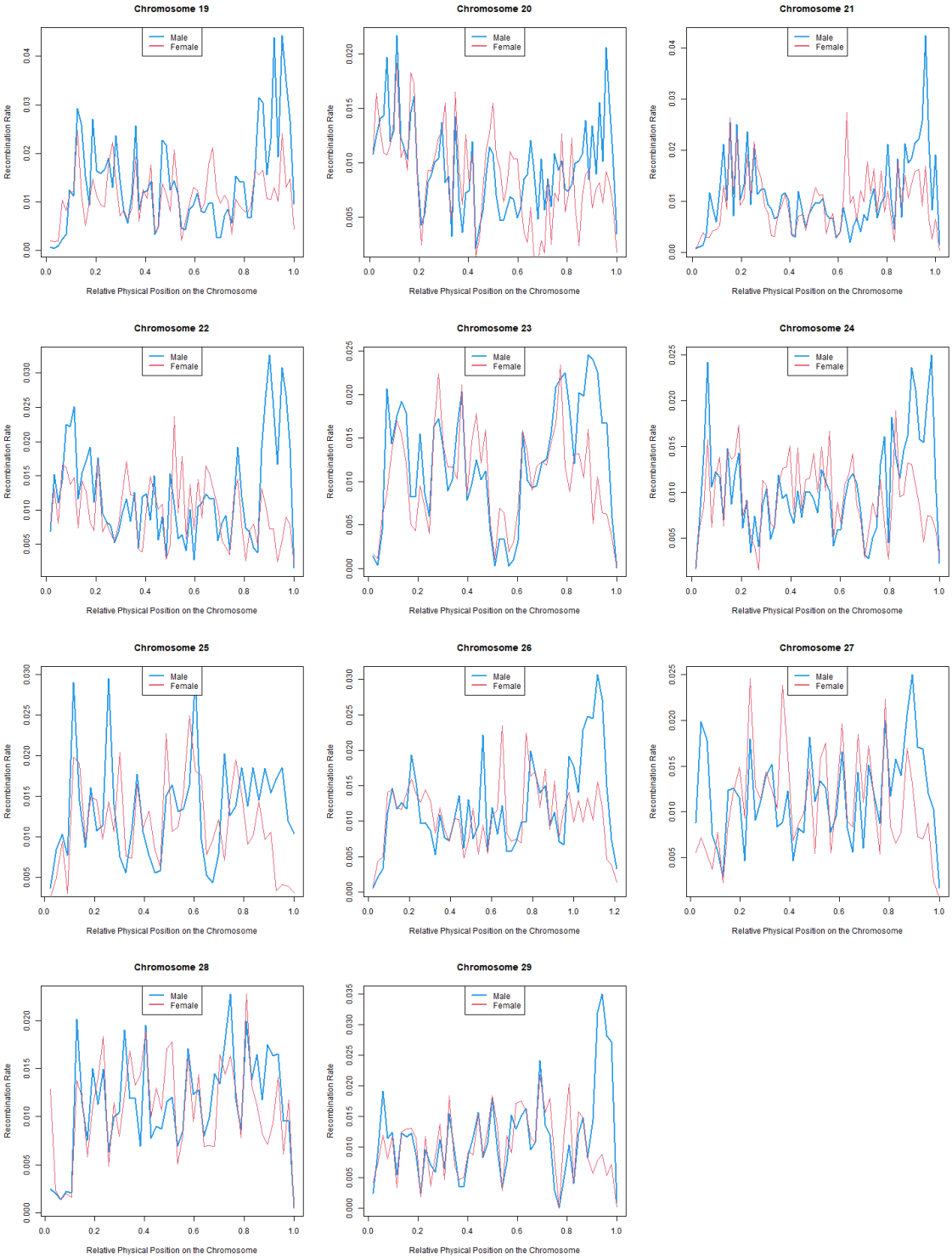

Chromosome 14
Male
Female
Recombination Rate
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome


Chromosome 15
Male
Female
Recombination Rate
0.000
0.005
0.010
0.015
0.020
0.025
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome


Chromosome 16
Male
Female
Recombination Rate
0.005
0.010
0.015
0.020
0.025
0.030
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome


Chromosome 17
Male
Female
Recombination Rate
0.005
0.010
0.015
0.020
0.025
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome


Chromosome 18
Male
Female
Recombination Rate
0.00
0.01
0.02
0.03
0.04
0.0
0.2
0.4
0.6
0.8
1.0
Relative Physical Position on the Chromosome

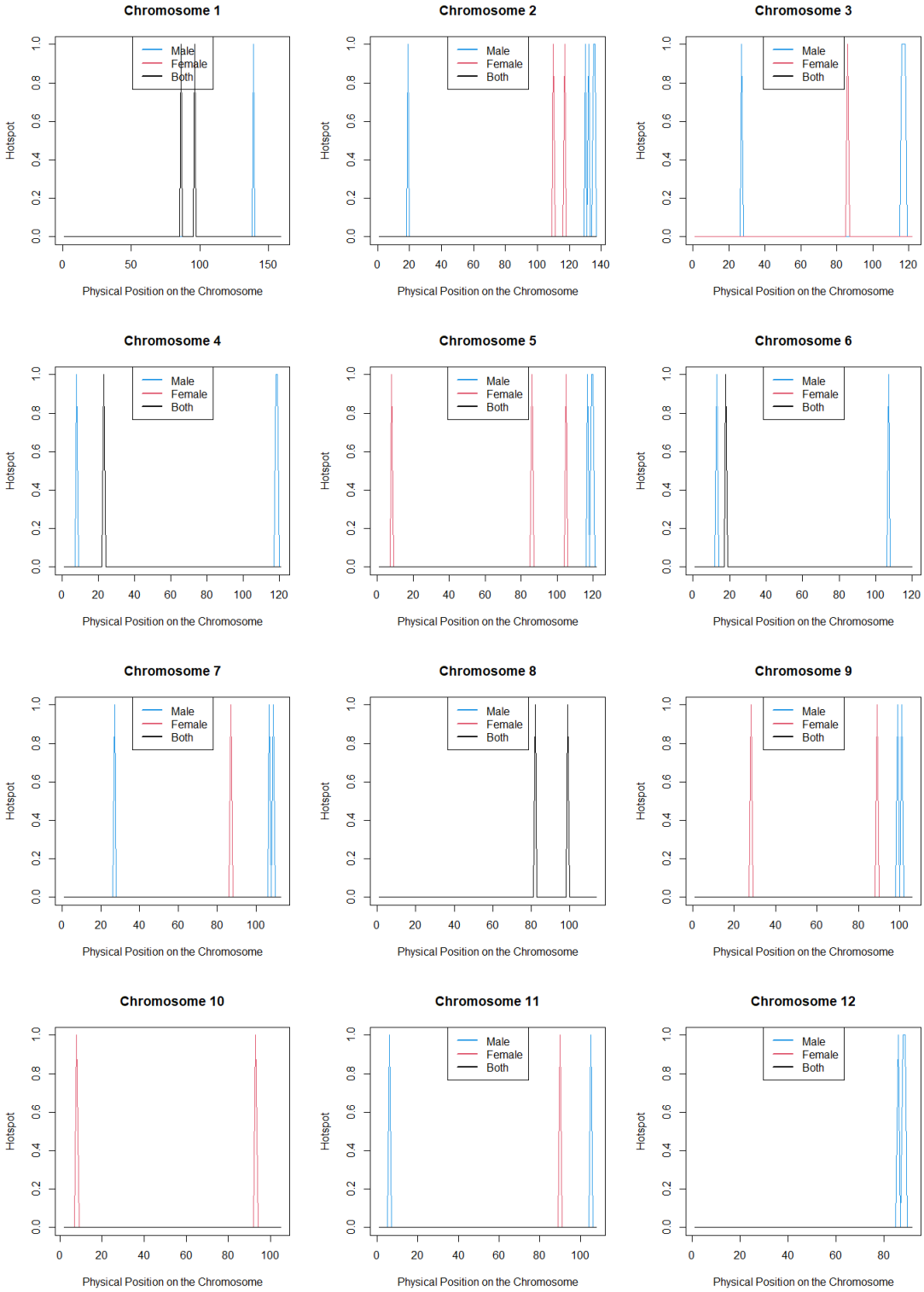


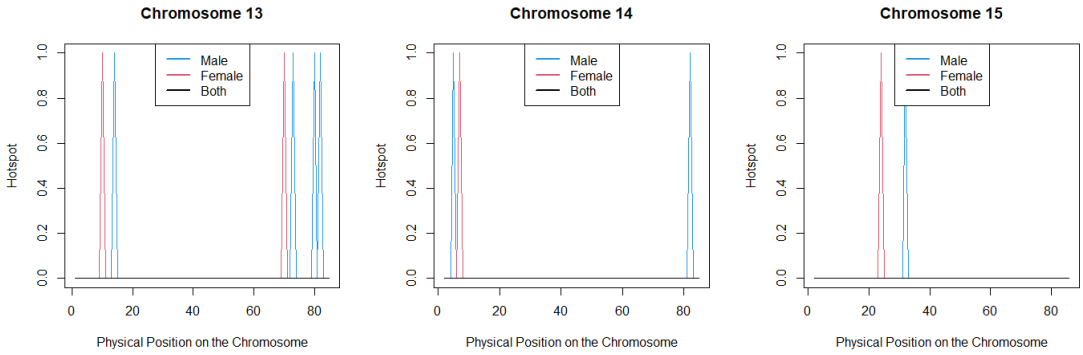
2. **Command to estimate chip heritability**

gcta64 --reml --grm gcta_grm --pheno phenotype.txt --mpheno 1 --out her_test

3. **Data Availability**

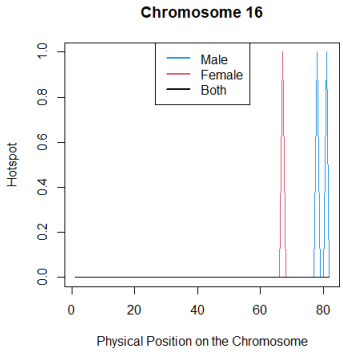
All the data used in this study, Rscripts, commands, and outputs are available upon a formal request to Professor Johann Sölkner (johann.soelkner@boku.ac.at)

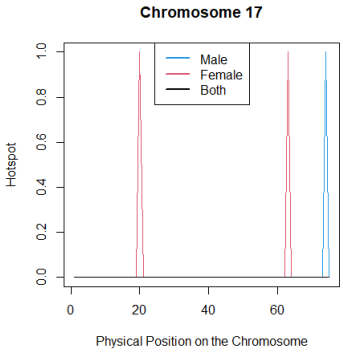


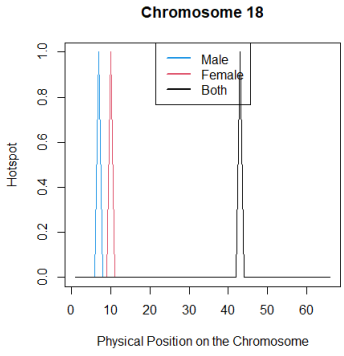

Chromosome 13
Male
Female
Both
Hotspot
Physical Position on the Chromosome

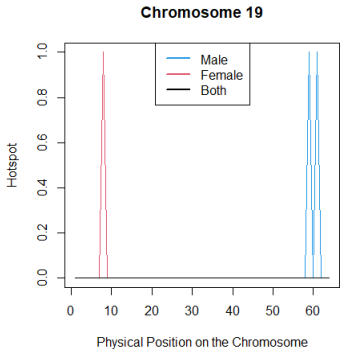
Chromosome 14
Male
Female
Both
Hotspot
Physical Position on the Chromosome

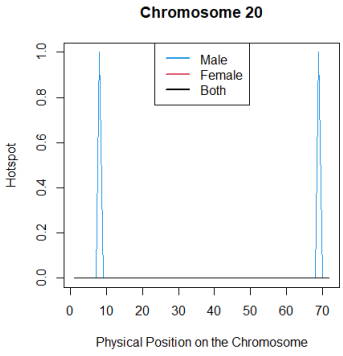
Chromosome 15
Male
Female
Both
Hotspot
Physical Position on the Chromosome

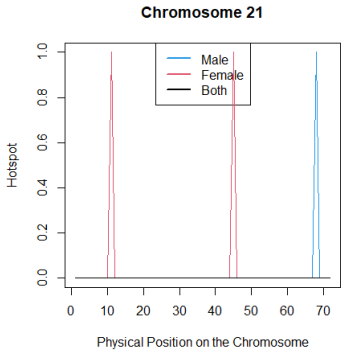

Chromosome 16
Male
Female
Both
Hotspot
Physical Position on the Chromosome

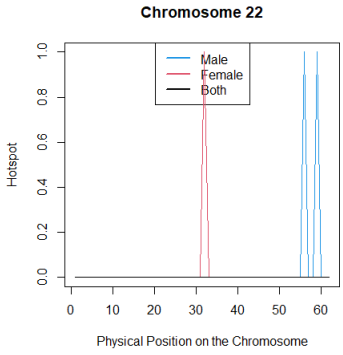

Chromosome 17
Male
Female
Both
Hotspot
Physical Position on the Chromosome

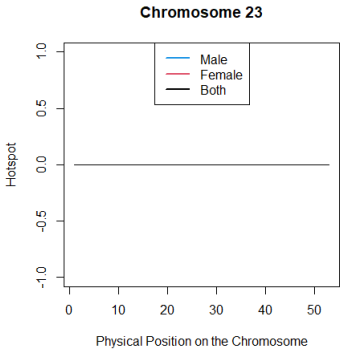

Chromosome 18
Male
Female
Both
Hotspot
Physical Position on the Chromosome

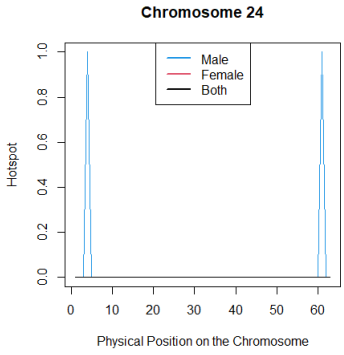

Chromosome 19
Male
Female
Both
Hotspot
Physical Position on the Chromosome

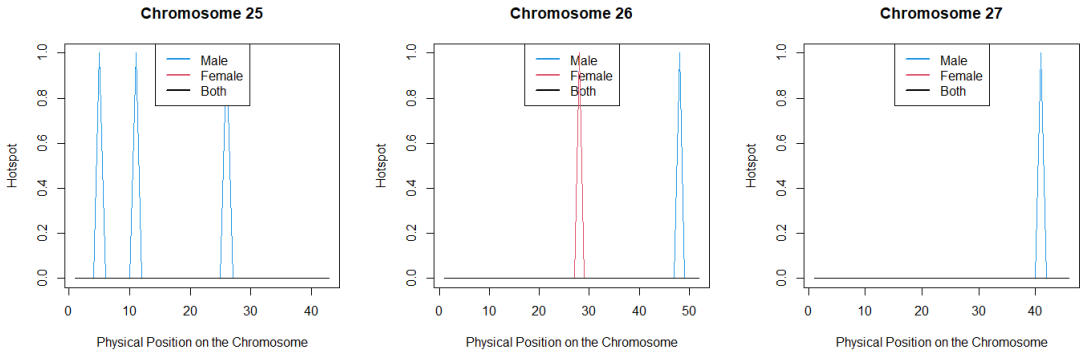

Chromosome 20
Male
Female
Both
Hotspot
Physical Position on the Chromosome

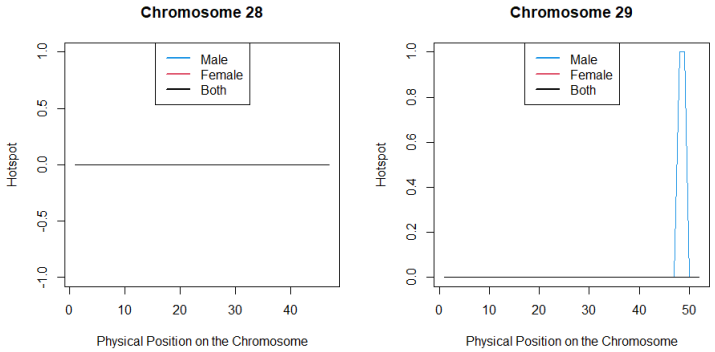

Chromosome 21
Male
Female
Both
Hotspot
Physical Position on the Chromosome


Chromosome 22
Male
Female
Both
Hotspot
Physical Position on the Chromosome


Chromosome 23
Male
Female
Both
Hotspot
Physical Position on the Chromosome


Chromosome 24
Male
Female
Both
Hotspot
Physical Position on the Chromosome


Chromosome 25
Male
Female
Both
Hotspot
Physical Position on the Chromosome
Chromosome 26
Male
Female
Both
Hotspot
Physical Position on the Chromosome
Chromosome 27
Male
Female
Both
Hotspot
Physical Position on the Chromosome


Chromosome 28
Male
Female
Both
Hotspot
Physical Position on the Chromosome
Chromosome 29
Male
Female
Both
Hotspot
Physical Position on the Chromosome