

## **Identification of unfavourable homozygous haplotypes associated with with milk and fertility traits in Holsteins.**

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### **Introduction**

Genetic markers have been used to estimate an individual's inbreeding more effectively than traditional pedigree measures, which fail to adequately capture the element of Mendelian sampling and are subject to recording errors. Regions of homozygous loci in a genome, or runs of homozygosity (ROH), are a result of the transmission of identical haplotypes from related parents to the offspring, and are used as a measure of genomic inbreeding.

Identification of ROH allow for the examination of the impact of homozygosity on recorded phenotypes on a genome-wide level, but also at specific regions of the genome. The presence of ROH at site-specific locations of the genome have been associated with fertility in dairy cattle breeds, while some regions showed no detrimental effects (Pryce et al., 2014; Kim et al., 2015). The objective of this study was to identify regions of homozygosity in the genome associated with production and fertility traits in the Canadian Holstein population.

### **Material and Methods**

Genotypes were available for 6,849 females. Cows were genotyped with 50K chip or with lower density and imputed to 50K using FImpute software (Sargolzaei *et al.*, 2014a). Single Nucleotide Polymorphism (SNP) information for 45,187 SNP markers were available before editing. SNPs were excluded based on the following criteria: Minor allele frequency (MAF) less than 5%, SNP call rate lower than 95%, animal call rate lower than 95%, Hardy Weinberg equilibrium (HWE) with p-value smaller than 0.00001 and heterozygosity higher than expected by  $> 0.15$  points. Genotyping quality control was performed using snp1101 (Sargolzaei, 2014b). After genotyping quality control, and removing females without phenotypes, 40,874 SNPs and 3,289 cows were kept for further analysis.

Phenotypic data were extracted from the Canadian Dairy Network database for all the Holstein cows that had their first calving after January 1st, 2000. The different traits retained were three milk production traits (milk, fat and protein yield on a 305-days basis) and 13 fertility and calving traits referring either to the first calving (heifer fertility) or the second calving (and the fertility during the first lactation).

Raw phenotypes were corrected using the GLM procedure of SAS (SAS Institute, Cary NC, 2011) for the 3,289 cows and their contemporary group based on the year and season of calving and the herd. The considered fixed effects depended on the traits and were based on those used for the national genetic evaluation. The total number of animals in the corrective model was 8,917.

To identify unfavorable homozygous haplotypes associated with milk and fertility traits we used the algorithm outlined in Howard et al. (2017). The algorithm is comprised of

three steps to identify unfavorable haplotypes: in step 1, the genome is scanned based on marker windows of decreasing size and ROH genotypes associated with unfavorable phenotype below given threshold are identified; in step 2, the unfavorable haplotype is formally tested for significance with the use of a linear mixed model; and in step 3, nested windows are removed. The default window size setting (50, 45, 40, 35, 30, 25, 20, 15 SNPs) was used.

## Results and Discussion

A total of 16 different traits were used for this study. Trait descriptions, average haplotype effects, and most extreme unfavorable haplotype effects are presented in Table 1. Trait clusters and the total number of unfavorable haplotypes identified are presented in Table 2.

**Table 1:** Unfavourable haplotype effects with standard deviations and extreme haplotype effects for production and fertility traits

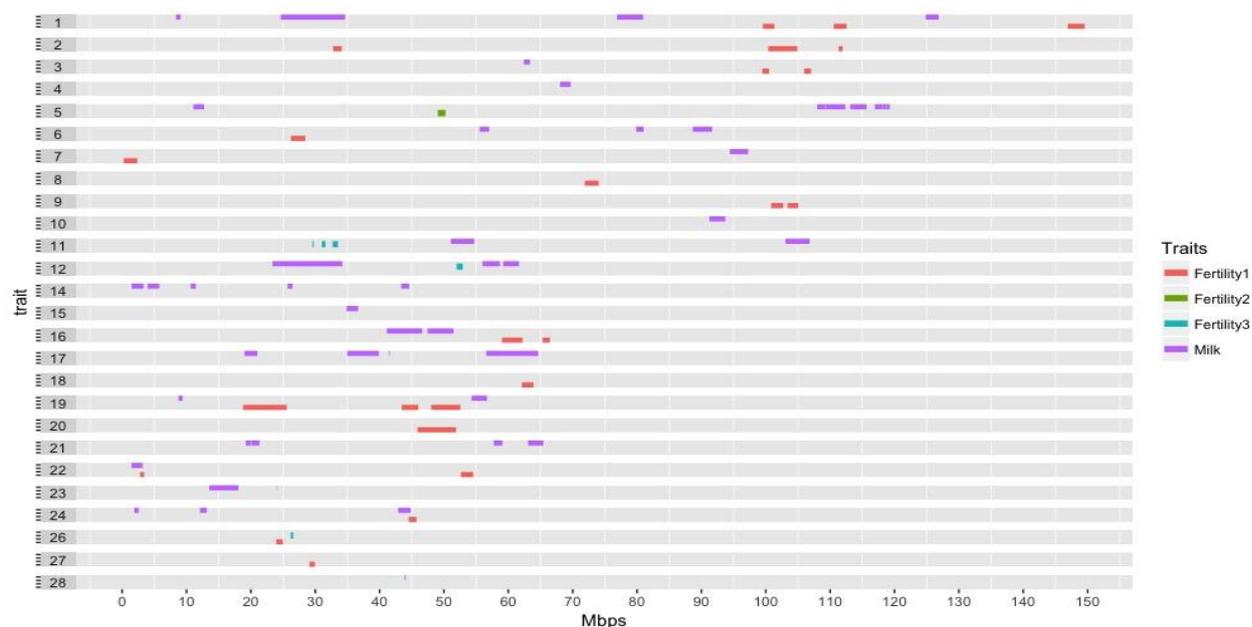
Trait (1st parity unless otherwise stated)	Average unfavourable haplotype effect	Most extreme unfavourable haplotype effect	
		Value	BTA
milk yield (kg)	-295.05 (105.23)	-684.05	3
fat yield (kg)	-10.35 (4.25)	-28.83	5
protein yield (kg)	-8.34 (3.47)	-22.34	21
age at calving in months (heifer)	0.45 (0.14)	0.98	12
age at calving in months	0.56 (0.16)	1.08	15
age at first service in months (heifer)	8.12 (2.30)	17.03	19
calf size 1-4 (heifer)	0.15 (0.05)	0.36	5
calf size 1-4	0.18 (0.05)	0.33	26
gestation length in days (heifer)	1.88 (0.49)	3.47	15
gestation length in months in days	2.07 (0.56)	3.79	3
calving to first service in days	4.86 (1.72)	10.5	14
days open	9.59 (3.06)	25.93	28
first service to calving in days (heifer)	8.83 (2.34)	14.48	14
first service to calving in days	8.82 (2.91)	22.92	11
number of services (heifer)	0.33 (0.08)	0.57	3
number of services	0.29 (0.09)	0.64	1

**Table 2:** Summary of the total number of haplotypes that displayed unfavorable effects within

*trait groups and across multiple trait groups*

Type of cluster	Total number of haplotypes within trait group	Number of haplotypes across multiple traits
Milk, Fat Yield, Protein Yield (first parity)	388	48
Age at first insemination (heifer), age at current/next calving in months (heifer and first parity)	365	26
Gestation length (heifer and first parity), calf size, 1 to 4 (heifer and first parity)	382	6
number of services (heifer and first parity), first service to conception in days (heifer and first parity), calving to first insemination (first parity), days open (first parity)	633	1

Inbreeding has been implicated in reduced performance for certain traits, particularly fitness traits. This noted inbreeding depression has been linked with reduced production and reproductive performance in dairy cows (González-Recio et al., 2007; Pryce et al., 2014). While many haplotypes were found to have favorable effects on production and unfavorable effects on fertility in our study, only two overlapping haplotype clusters could be observed. These haplotypes were found on chromosomes 22 and 24, with both overlaps affecting the clusters of both milk production and age at first insemination (heifer), age at current/next calving in months (heifer), and age at current/next calving in months (first parity) (Figure 1).



**Figure 1:** *Unfavorable haplotypes for fertility and production trait clusters found across multiple trait groups.*

*Milk (milk, fat and protein); Fertility1 traits (age at first insemination (heifer), age at current/next calving in months (heifer), age at current/next calving in months (first parity); Fertility2 traits (gestation length (heifer), calf size, 1 to 4 (heifer), gestation length (first parity), calf size, 1 to 4 (first parity); Fertility3 traits (number of services (heifer), first service to conception in days (heifer), calving to first insemination (first parity), days open (first parity), number of services (first parity), first service to conception in days (first parity)).*

Haplotypes found to negatively affect both milk production and fertility trait clusters can be considered particularly interesting, as they may pinpoint areas in which ROH have detrimental effects on phenotypes of economic value. Such haplotypes, as well as those affecting other trait clusters, should be further analyzed to help better understand genomic areas which are particularly sensitive to homozygosity.

## Conclusions

Genomic information and identification of ROH allow for the examination of the impact of homozygosity on recorded phenotypes on a genome-wide level, but also at specific regions of the genome. The objective of this study was to identify regions of homozygosity in the genome associated with production and fertility traits in the Canadian Holstein population. Multiple haplotypes were identified that had a consistent unfavorable effect both within and across trait groups affecting fertility and production traits. Further investigation of these haplotypes and their effects on fertility and production traits could be useful in better understanding the detrimental effect of homozygosity in dairy populations.

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