The use of runs of homozygosity for intra-chromosomal estimation of inbreeding depression on female fertility in Finnish Ayrshire cattle

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Summary

Inbreeding increases homozygosity, which affects fertility by increasing the frequency of harmful recessive alleles. Impaired fertility reduces profitability of dairy cattle production by decreasing the lifetime milk production of the cow and increasing the costs related to inseminations and veterinary treatments. Genetic diversity is known to vary across the genome, which is why detecting the regions with lower genetic diversity could indicate inbreeding depression. The objective of this study was to estimate the effect of increased homozygosity for number of inseminations required to successfully conceive (AIS) in the Finnish Ayrshire population using runs of homozygosity (ROH) and haplotype analysis. Genomic information from 13 712 Finnish Ayrshire females with fertility phenotypes was analysed. Genotypes were obtained with the Illumina BovineLD v.2 BeadChip low-density panel and imputed to 50K density. The phenotypic records were pre-adjusted for the most important fixed effects prior to the estimation of inbreeding depression. Regional ROH-based inbreeding coefficients (F_{ROH}) were used as covariates in the mixed model equation. First, the chromosomal F_{ROH} was determined as the number SNPs in ROHs divided by the total number of SNPs in the chromosome. Based on the analyses, a 10% increase in F_{ROH} on chromosome 2 or 18 was associated with 0.04 or 0.03 more inseminations for heifers, respectively (P-values < 0.01). Similarly, F_{ROH} estimated on chromosome 15 was associated with 0.05 more inseminations for the second parity cows (P-value < 0.01). Next, an intra-chromosomal sliding window approach was applied to locate more precisely the chromosomal regions showing inbreeding depression. Here F_{ROH} was calculated as the number of SNPs in ROHs belonging to the window divided by the total number of SNPs in the window. Regions associated with inbreeding depression on AIS were found near the beginning of chromosome 2 and towards the ends of chromosomes 15 and 18. The found regions were then examined for homozygous haplotypes associated with increased AIS. The analysis revealed common homozygous haplotypes that were associated with 0.09 or 0.10 more inseminations on chromosomes 2 and 18 for heifers, respectively. Similarly a common haplotype on chromosome 15 was associated with 0.15 more inseminations for second parity cows.

Keywords: inbreeding, inbreeding depression, fertility, runs of homozygosity, haplotype

Introduction

In a closed livestock population all animals will eventually become related and thus inbreeding is unavoidable. This results in inbreeding depression which is defined as the impairment of phenotypic values caused by inbreeding within a population (Falconer &Mackay, 1996). Multiple studies have reported impaired fertility due to inbreeding. For

example, Bjelland et al. (2013) found that days open increased from 1.06 to 1.76 days per 1% increase in the inbreeding coefficient and Pryce et al. (2014) reported a 1% increase in the inbreeding coefficient lengthened the calving interval by 0.18 days. As fertility is an economically important trait, impaired fertility reduces profitability by decreasing the lifetime milk production of the cow and increasing the costs related to inseminations and veterinary treatments.

Traditionally estimates of inbreeding and inbreeding depression have been based on pedigree data, but these estimates are error-prone due to shallow or incomplete pedigree. The availability of single nucleotide polymorphism (SNP) data could overcome this problem by enabling the estimation of inbreeding at the genome level. Runs of homozygosity, which are continuous stretches of homozygous genotypes inherited from a common ancestor, have been concluded in many studies to provide an effective and consistent measure of the genomic inbreeding (e.g., Bjelland et al., 2013; Martikainen et al., 2017). However, as the genetic diversity varies across the genome, an intra-chromosomal approach (Kleinman-Ruiz et al., 2016) could reveal specific chromosomal regions that are strongly affected by inbreeding depression.

The aim of this study was to identify chromosomal regions associated with inbreeding depression on female fertility in the Finnish Ayrshire population.

Material and methods

The genotypes, pedigree data, breed proportions, raw phenotypes, solutions for fixed effects, and estimates of (co)variance components were obtained from NAV, Nordic Cattle Genetic Evaluation (Aarhus, Denmark) and from Faba, The Finnish Animal Breeding Association (Vantaa, Finland). All cows were born between 2002 and 2014.

Phenotypic data

The phenotypic records for the number of artificial inseminations (AIS) were available for 13 712 animals and they were considered separately for heifers (lactation 0) and for cows with one to three lactations (1-3). The phenotypic records were adjusted for fixed effects (herd-year, insemination year-month, calving year-month, and age at insemination) using the solutions of the full Nordic evaluation model.

Genomic data

Cows were genotyped with the Illumina BovineLD v.2 BeadChip low-density panel (Illumina Inc., 2015), which contains 7 931 SNPs, and then imputed to 50K density by the Fimpute software (Sargolzaei et al., 2014). After applying filtering criteria (MAF<0.05 and HWE p-value<0.0001), the dataset included 39 144 SNPs.

Estimation of inbreeding depression

Runs of homozygosity (ROH) were used to estimate the inbreeding coefficient (F_{ROH}). ROHs were detected with software PLINK (Purcell et al.2007) with adjusted parameters (--homozyg-density 120 --homozyg-kb 500 --homozyg-snp 0). Each SNP was coded as 0 or 1 based on ROH-status. Chromosomal inbreeding coefficient was calculated as the sum of SNPs in the chromosome divided by the total number of SNPs in the chromosome. For intra-chromosomal inbreeding coefficient a sliding window approach was applied and the regional inbreeding coefficient was calculated as the sum of SNPs in the window divided by the total

number of SNPs in the window. Inbreeding depression (b) was estimated using the mixed model equation (1).

$$y_i = \mu + bF_i + a_i + e_i \,, \quad (1)$$

where covariate F was either chromosomal inbreeding coefficient or intra-chromosomal inbreeding coefficient. Statistical analyses were performed using the DMU-package (Madsen and Jensen, 2000).

Association between a haplotype and AIS

The effect of a haplotype to AIS was evaluated for genomic regions showing association between ROH-based inbreeding coefficient and AIS. A haplotype was considered as a candidate haplotype if the frequency of homozygous individuals was greater than 0.05. Haplotypes of length 1 to 10 SNP were tested to find the optimal length. The association was evaluated using a recessive model equitation (2).

$$y_i = \mu + g_i + a_i + e_i \,, \quad (2)$$

where $g_i = 0$ or 1 (1=homozygous for the haplotype).

Results

Chromosomes 2, 15 and 18 were identified to have statistically significant association with increased number of inseminations (Table 1). Based on the analyses, a 10% increase in F_{ROH} on chromosome 2 or 18 was associated with 0.04 or 0.03 more inseminations for heifers, respectively (P-values < 0.01). Similarly, F_{ROH} estimated on chromosome 15 was associated with 0.05 more inseminations for the second parity cows (P-value < 0.01).

Table 1. Chromosomes associated with inbreeding depression on AIS.

Trait	Chr	b	SE	p-value
AIS0	2	0.41	0.09	1.48e-05
AIS0	18	0.28	0.08	3.07e-04
AIS2	15	0.48	0.16	3.10e-03

Within these three chromosomes the regions showing inbreeding depression lied near the beginning of chromosome 2 (position 16.5 – 47.2 Mb) and towards the ends of chromosomes 15 (position 41.17 – 85.26) and 18 (position 33.08 – 65.98 Mb) (Table 2).

Table 2. Genomic regions associated with inbreeding depression on AIS.

Trait	Chr	Window position	Window size	b (SE)	p-value
AIS0	2	16.5 – 47.2 Mb	30.7 Mb	0.25 (0.06)	3.6e10-5
AIS0	18	33.08 – 65.98 Mb	32.9 Mb	0.15 (0.06)	0.01
AIS2	15	41.17 – 85.26 Mb	44.1 Mb	0.37 (0.12)	0.002

Examination of these regions revealed three common haplotypes that were statistically significantly associated with increased AIS provided that the individual was homozygous for the haplotype (Table 3). On chromosome 2 the heifers, that were homozygous for the haplotype 1, were estimated to require 0.09 more inseminations to conceive than other heifers. Similarly on chromosome 18 the effect of haplotype 2 was 0.10 more inseminations. On chromosome 15 the effect of haplotype 3 was 0.15 more inseminations for second parity cows.

Table 3. Haplotypes associated with inbreeding depression on AIS.

Trait	Haplotype	Chr	Position	Freq. homozyg.[1]	b (SE)	p-value
AIS0	1	2	28.6 – 28.8 Mb	0.10	0.09 (0.03)	9.1e10-4
AIS0	2	15	49.7 – 49.9 Mb	0.15	0.10 (0.02)	1.3e10-6
AIS2	3	18	70.4 – 70.9 Mb	0.18	0.15 (0.04)	2.3e10-4

[1]Frequency of individuals homozygous for the haplotype

Conclusions

In this study we used hierarchical approach to detect homozygosity affecting female fertility. First, we estimated the chromosomal inbreeding using ROHs to detect the approximate location of the regions affected by inbreeding depression. Then we were able to reveal common homozygous haplotypes from these regions that were associated with increased number of inseminations. The fertility-impairing effect of these haplotypes might be due to their impact on, for example, the signs of heat or the regulation of follicular development. However, further examination is needed to better understand the role of inbreeding depression by searching for candidate genes associated with female fertility.

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