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EFFECT OF GENOTYPING DENSITY ON THE DETECTION OF RUNS OF HOMOZYGOSITY AND HETEROZYGOSITY

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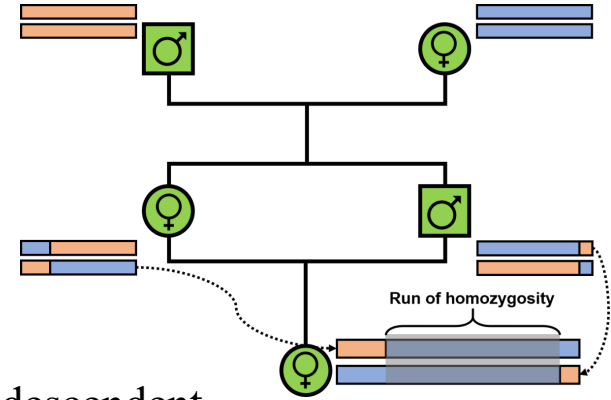
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Introduction

Runs of Homozygosity (ROH)

- Continuous homozygous chromosomal segments identical by descent
- Related to autozygosity → predictor of inbreeding
- Due to either artificial or natural selection → fixation of favorable alleles



Introduction

Runs of Heterozygosity (ROHet)

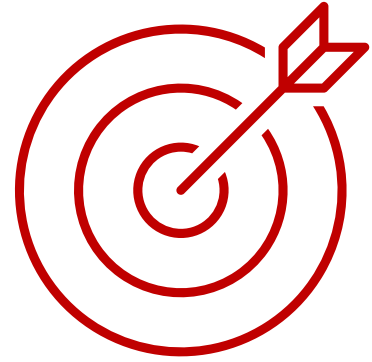
- Heterozygosity-rich regions
- Firstly introduced by Williams et al. (2016)
- ROHet occurrence avoids the deleterious effects of continuous homozygous genotypes
- They might be associated to survival rate, fertility and other fitness traits → balancing selection



Introduction

- Both are a consequence of the selection that shapes the genomic structure of livestock populations
- Lack of consensus in establishing the criteria to define runs
- The genotyping density can introduce bias in the detection

Aim



To investigate:

- The differences in the detection of ROHom and ROHet using three different BeadChip genotyping arrays
- The signals of selection highlighted by the two approaches

Materials and Methods

Italian Simmental

- Farmed mostly in small herds in Northeastern Italy
- Third largest Italian cattle breed
- Dual-purpose
 - Low-density (**LD**; 50k, 397 animals)
 - Medium-density (**MD**; 140k, 348 animals)
 - High-density (**HD**; 800k, 152 animals)
- 897 cows



Materials and Methods

Quality control filter (PLINK v. 1.9):

- ✓ HWE ($P > 1e^{-6}$)
- ✓ Call rate > 0.95
- ✓ MAF > 0.01

After removing SNP unmapped or located on
allosomes (ARS-UCD 1.3):

- 43,431 SNPs for LD
- 113,042 SNPs for MD
- 583,637 SNPs for HD



Materials and Methods

Runs detection

detectruns R package (Biscarini et al., 2018)

- Minimum number of SNPs
- Opposite allowed

Materials and Methods

Runs detection

detectruns R package (Biscarini et al., 2018)

➤ Minimum number of SNPs (Purfield et al., 2012) →

$$n\text{SNP}_{\text{ROHom}} = \frac{\log_e \frac{\alpha}{n_s \cdot n_i}}{\log_e (1 - \text{het})}$$

➤ Opposite allowed

$$n\text{SNP}_{\text{ROHet}} = \frac{\log_e \frac{\alpha}{n_s \cdot n_i}}{\log_e (1 - \text{hom})}$$



Materials and Methods

Runs detection

detectruns R package (Biscarini et al., 2018)

➤ Minimum number of SNPs

➤ Opposite allowed

$$maxOpp = \frac{nSNP_{ROHom}}{nSNP_{ROHet}}$$



Materials and Methods

Runs detection

detectruns R package (Biscarini et al., 2018)

- Minimum number of SNPs
- Opposite allowed

Parameters	ROHom	ROHet
Min. SNP	50	18
Opposite	0	3
Missing	0	0
Min. length	1 Mb	1 Mb
Max gap	1 Mb	1 Mb



Materials and Methods

- ROHom- and ROHet-based coefficients:

$$F_{ROHom} = \frac{\text{total sum of ROHom}}{\text{genome length covered by SNP}}$$

$$D_{ROHet} = \frac{\text{total sum of ROHet}}{\text{genome length covered by SNP}}$$

- ANOVA to compare number of regions per animal, runs mean length, and runs-based coefficients
- NCBI online database to identify the genes mapped in or close (± 250 kb) the most repeated runs

Results and Discussion

Runs	Density	Animals	Runs				Mean±SD length (number of runs)				
			Total	Unique	Per animal	Average size	1-2 Mb	2-4 Mb	4-8 Mb	8-16 Mb	>16 Mb
ROHom	LD	393	6,288	5,467	16.00±6.52 ^c	6.47±5.62 ^a	1.40±0.28 (306)	3.14±0.51 (2212)	5.52±1.12 (2356)	10.9±2.24 (1031)	23.60±7.81 (383)
	MD	338	16,091	13,646	47.61±19.80 ^a	2.72±2.61 ^b	1.46±0.27 (8925)	2.74±0.54 (4648)	5.43±1.10 (1855)	10.80±2.12 (559)	21.10±6.07 (104)
	HD	151	6,404	5,966	42.41±22.70 ^b	1.59±0.72 ^c	1.33±0.26 (5277)	2.59±0.49 (1028)	4.90±0.89 (97)	11.70±2.21 (2)	—
ROHet	LD	382	1,419	836	3.71±1.89 ^a	1.29±0.88 ^b	1.26±0.22 (1370)	2.27±0.22 (49)		—	
	MD	217	332	147	1.53±0.72 ^b	1.45±0.48 ^a	1.26±0.25 (273)	2.35±0.09 (59)		—	
	HD						—				



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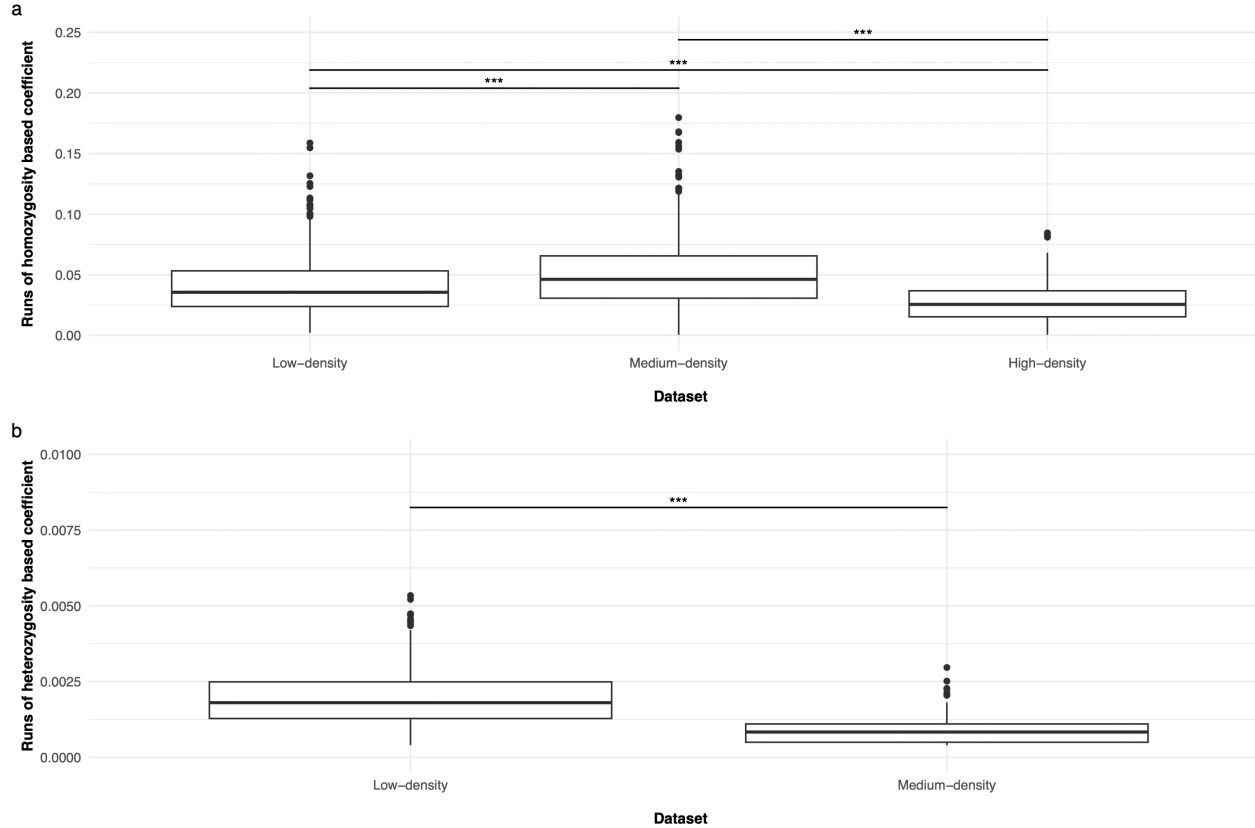


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ROHet	LD	382	1,419	836	3.71±1.89 ^a	1.29±0.88 ^b	1.26±0.22 (1370)	2.27±0.22 (49)	→ 3%	—	
	MD	217	332	147	1.53±0.72 ^b	1.45±0.48 ^a	1.26±0.25 (273)	2.35±0.09 (59)	→ 18%	—	
	HD						—				



Results and Discussion



Results and Discussion

ROHom

- A comparison with other studies on Simmental breed was hard because of the stricter parameters inhere adopted (i.e., 50 minSNP, no heterozygotes or missing allowed)
- In literature, the mean number of ROHom per animal was always greater
- The MD dataset showed the closest values of average ROHom length and F_{ROHom} to those reported in other studies



Results and Discussion

ROHet

- Fewer and shorter than ROHom
- A comparison with other studies was not possible because no studies about ROHet on Simmental cattle were found
- Very low D_{ROHet} values have been reported in literature in pig and goat populations (Bordonaro et al., 2023; Chessari et al., 2024)

Results and Discussion

Runs	Density	BTA	Start (bp)	End (bp)	Animals
ROHom	LD	2	189,886	3,046,092	9
		4	49,651,768	50,796,591	13
		5	92,844,631	93,949,810	9
		14	22,983,665	26,473,490	14
		1	3,023,897	4,834,622	10
	MD	3	113,433,557	114,766,179	12
		4	49,760,465	50,900,429	10
		6	37,896,892	39,216,868	10
		6	38,428,952	39,461,621	27
		6	71,044,403	72,475,809	11
		7	41,565,963	43,126,285	13
		11	60,974,044	62,732,451	10
		12	21,352,699	22,885,975	12
		17	55,454,910	56,587,255	11
		18	39,201,407	40,630,538	10
		21	44,800,371	46,145,471	13
		23	15,894	1,580,636	16
	HD	5	12,426,099	13,789,485	6
		6	33,736,732	35,205,727	9
		6	76,922,031	78,084,721	6
		19	90,671	1,333,831	6
ROHet	LD	21	2,151,256	3,245,487	41
	MD	21	173,023	2,504,481	27



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➤ The most shared **ROHom**, mapped on BTA6:

- Harbored no genes
- Overlapped with 414 QTL, mostly associated with meat and carcass and production traits



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- The most shared **ROHom**, mapped on BTA6:
 - Harbored no genes
 - Overlapped with 414 QTL, mostly associated with meat and carcass and production traits
- The most shared **ROHet**, mapped on BTA21:
 - Harbored four genes: *SNRPN*, *SNURF*, *UBE3A*, and *ATP10A*
 - Overlapped with 762 QTL, mainly related to calving ease (738 QTL)



Results and Discussion

- All the four genes were previously associated with **early pregnancy occurrence** (Irano et al., 2016)
- *SNRPN*, *SNURF*, and *UBE3A* are related to **age at first calving** (Alves et al., 2022)
- *UBE3A* has been found related to **stillbirth and calving ease** (Mészáros et al., 2016)
- *ATP10A* has been associated with **calving ease** (Frischknecht et al., 2017) and **milking speed** (Marete et al., 2018)

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➤ The most shared **ROHet**, mapped on BTA21:

- Harbored four genes: *SNRPN*, *SNURF*, *UBE3A*, and *ATP10A*
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Conclusions

- Genotypes from the Italian Simmental cattle breed were used to investigate the impact of BeadChip density in detecting ROHom and ROHet
- The detection of ROHom is more reliable when the array density increases, whereas an opposite trend was observed for ROHet
- LD and HD could lead to bias in ROHom detection
- LD and HD seem to overestimate and underestimate ROHet, respectively
- MD might represent the best option for the identification of both regions



Conclusions

- Genes and QTL mapped in the highlighted ROHet were mainly associated with reproduction, health, and fitness traits
- Genes and the QTL associated with ROHom were predominantly involved in meat and production traits
- Results of the present study strengthened the usefulness of these genomic regions in investigating the genome and their biological meaning
- Further studies are needed on the comparison between these two techniques and for a deeper analysis of ROHet



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Animal Genetics and Genomics



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