



Identifying the rules for defining CpG epigenetic bovine diversity

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del Sacro Cuore

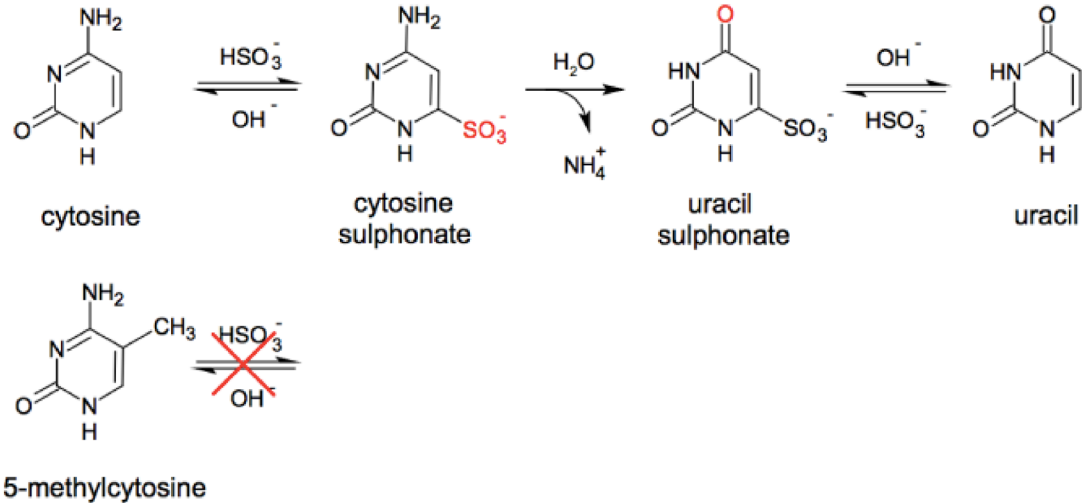
Epigenetic diversity

- Epigenetic mechanisms contribute to phenotypic variability in response to environmental adaptation
 - a result of long-term selection for morphological-adaptive and quantitative traits
 - persisting after embryo epigenetic reprogramming during differentiation
- Partially inherited across generations

Methylation

- Occurs at CpG dinucleotides
- Associated with gene silencing when found at promoters
- Helps to maintain genomic stability
- Laid down by DNA methyltransferases
- Mitotically heritable, due to features of DNMT1
- Can be removed passively, or actively which involves TET proteins
- Essential for viability, as DNMT knockouts die in utero

Bisulphite sequencing



TCGAG

Bisulfite
conversion

TUGAG

PCR
amplification

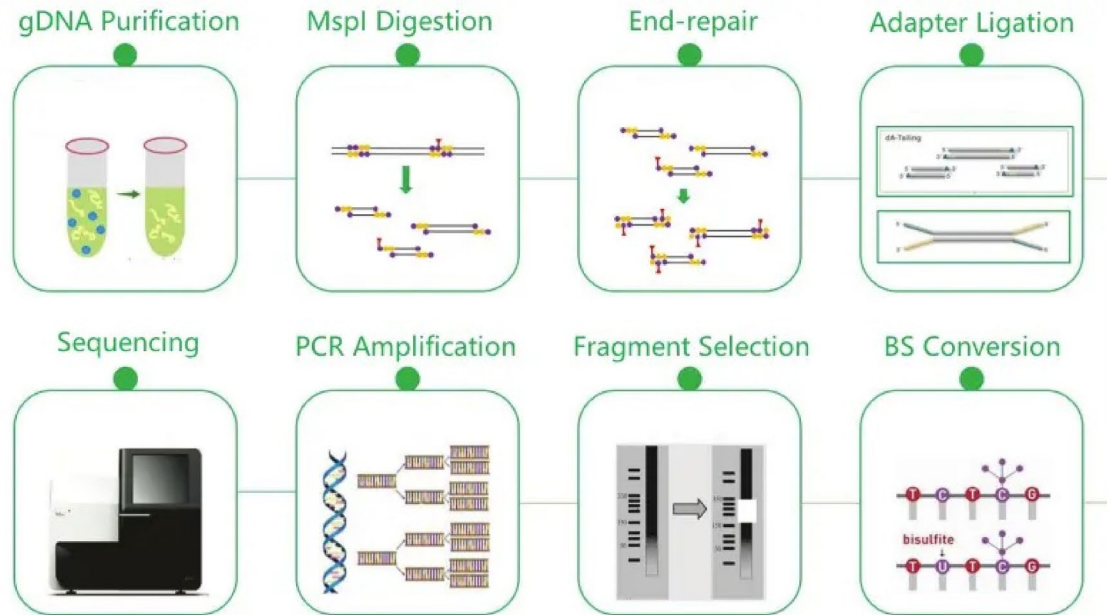
TTGAG

T^{me}CGAG

T^{me}CGAG

TCGAG

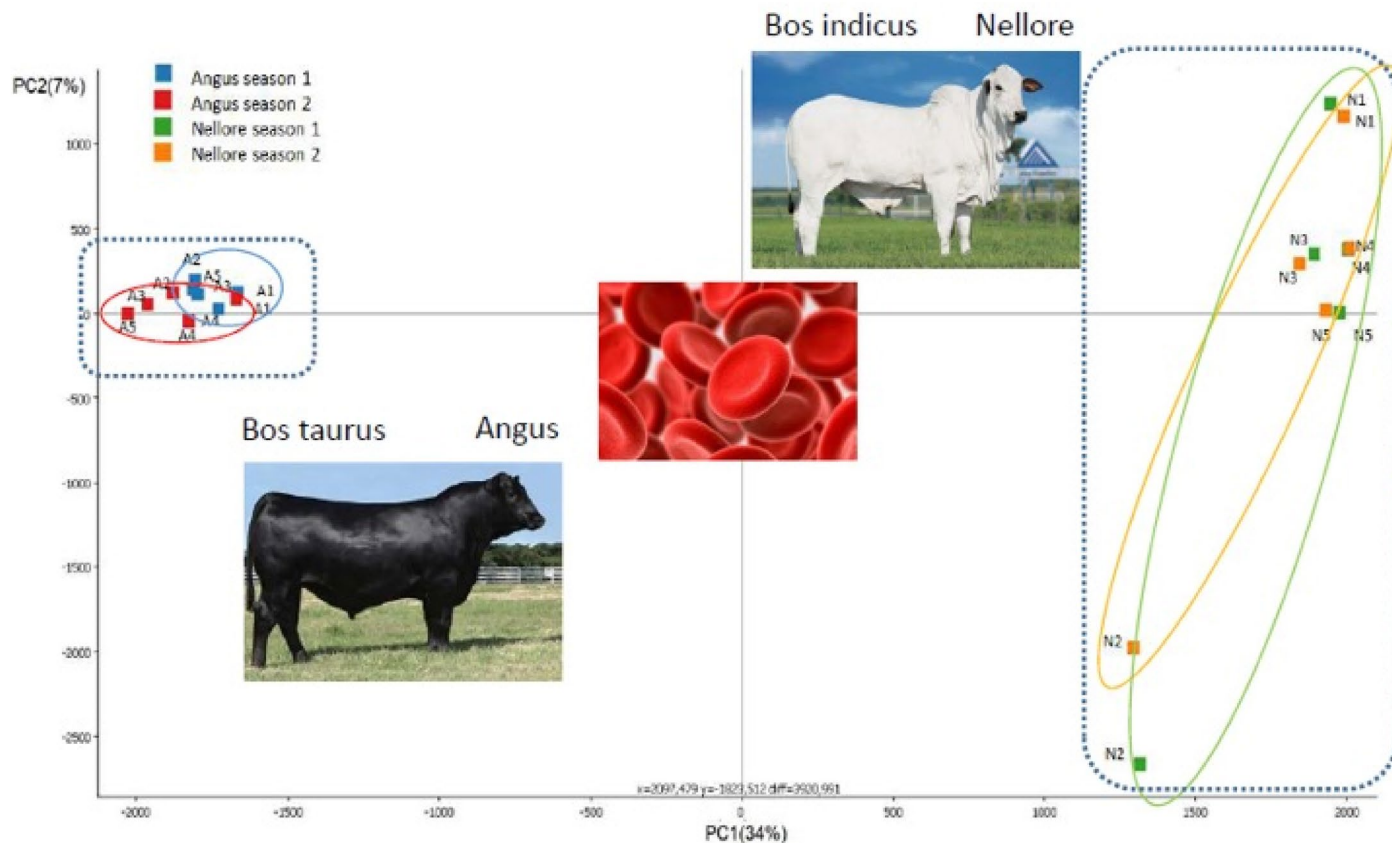
Reduced Representation & Bisulphite sequencing



Our goals

- Define rules and build a bioinformatic pipeline for RRBS data analysis
 - Set coverage thresholds
 - Correct for genomic variation (SNP)
- Investigate the relationship between epigenetic and genomic variation
- We investigated breed-specific epigenetic signatures in:
 - blood of Angus versus Nellore
 - sperm of Holstein versus Montbéliarde

Changes in DNA methylation in blood from different breeds

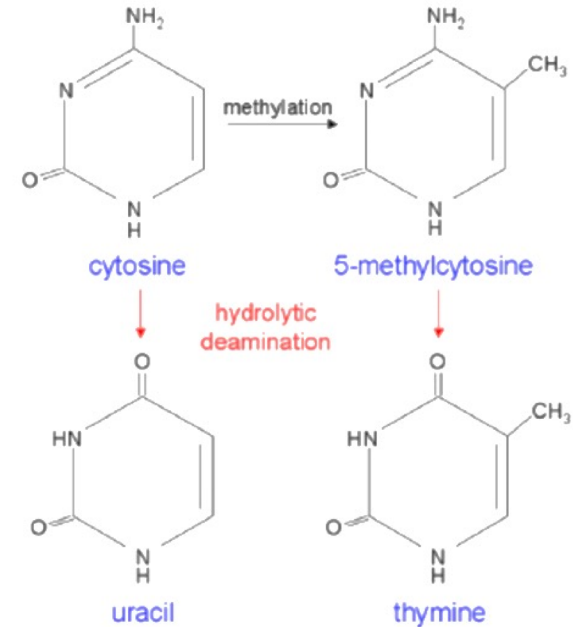


Methylome Patterns of Cattle Adaptation to Heat Stress

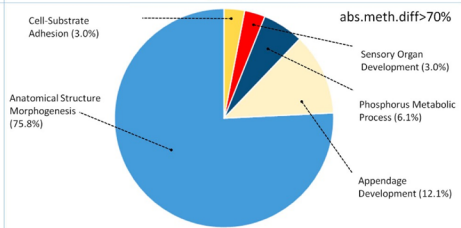
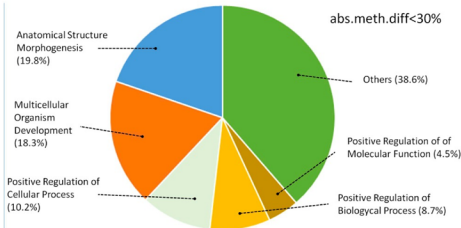
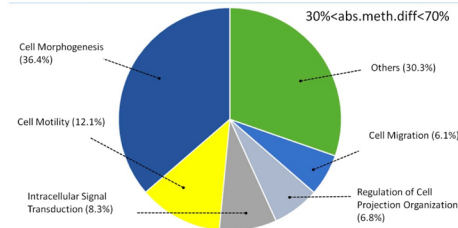
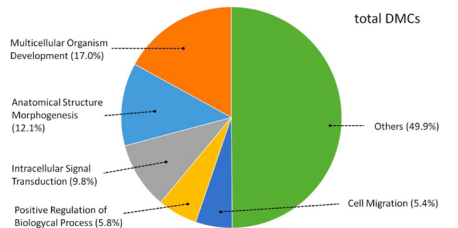
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Need for WGS

- C-T transition are by far the most frequent mutations in mammals
- Deamination of C produces U promptly corrected by repair systems
- Deamination of 5m-C produces T, allowed in DNA
- To distinguish with confidence between genomic thymine and unmethylated cytosines (which are read as thymines in bisulfite sequencing), we also included WG sequences



Contrasting Nellore and Angus



Cell-Substrate Adhesion (3.0%)

Anatomical Structure Morphogenesis (75.8%)

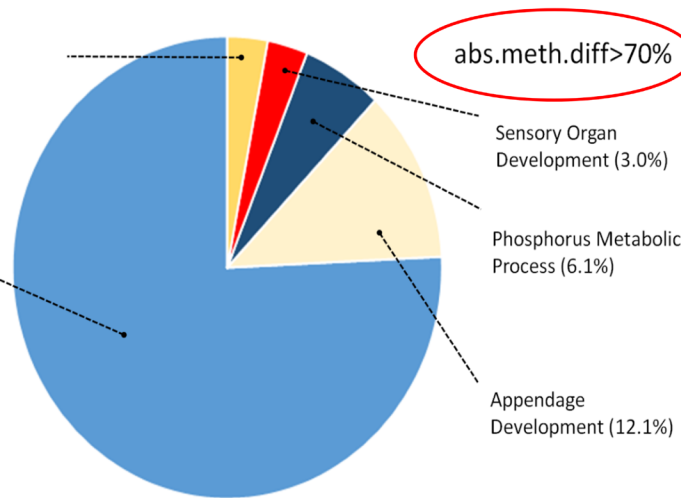


Epigenetics

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Comparison between indicine and taurine cattle DNA methylation reveals epigenetic variation associated to differences in morphological adaptive traits

E. Capra, B. Lazzari, M. Milanesi, G. P. Nogueira, J. f. Garcia, Y.T. Utsunomiya, P. Ajmone-Marsan & A. Stella



abs.meth.diff > 70%

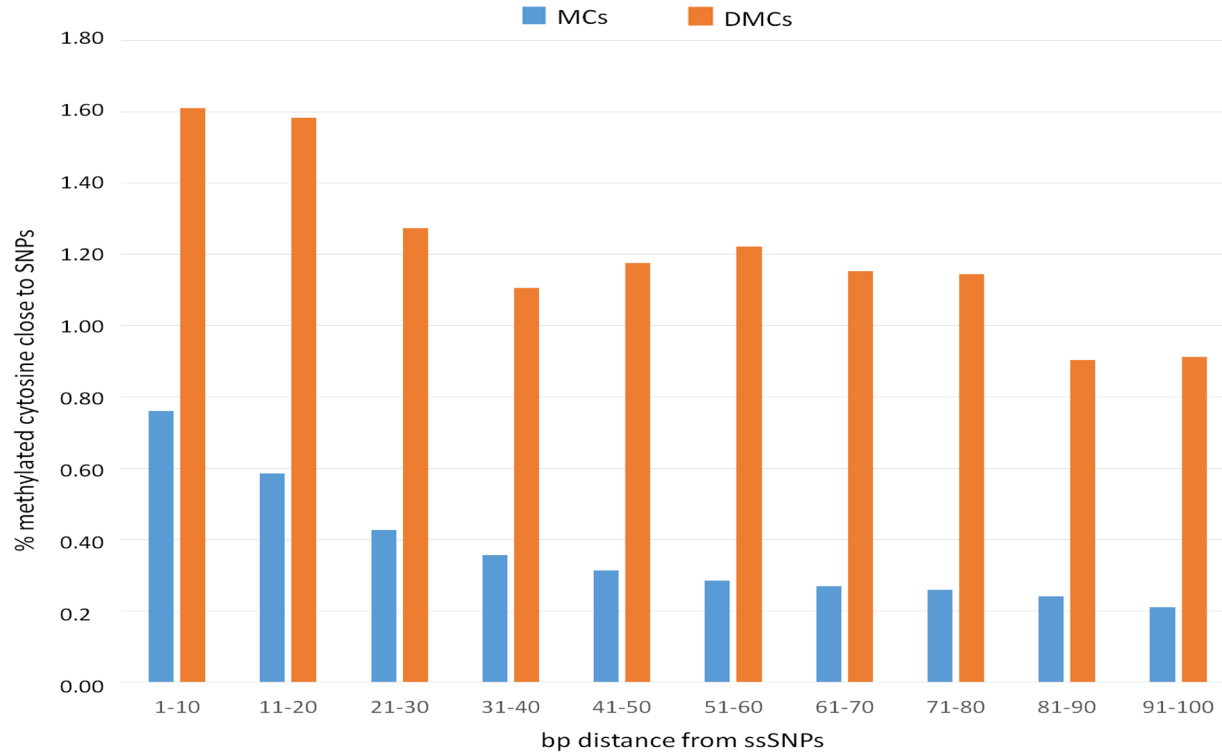
Sensory Organ Development (3.0%)

Phosphorus Metabolic Process (6.1%)

Appendage Development (12.1%)

Genes differentially methylated in Angus and Nellore

Contrasting Nellore and Angus

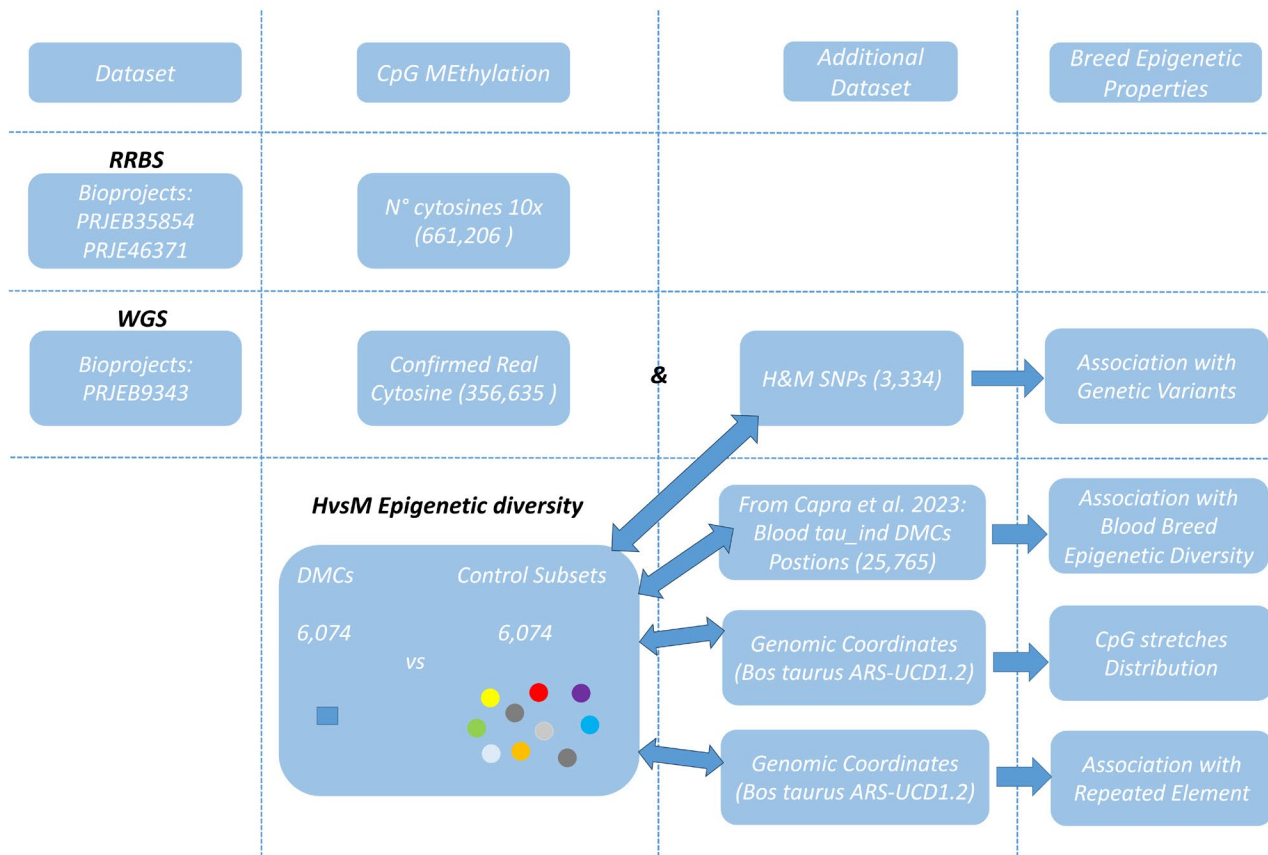


SNP are particularly abundant within differentially methylated regions

Data Retrieval and Filtering Criteria

- Data Source
- Fastq sequences were retrieved from the NCBI Bioproject database.
 - Filtering Criteria:
 - Library Preparation Method: Reduced Representation Bisulfite Sequencing (RRBS).
 - Sample Availability: High number of samples ($n > 8$).
 - Sequencing Coverage: RRBS > 20 million reads.
- Selected samples:
 - PRJEB46371: Montbéliarde
 - Semen from 9 bulls, collected at 74-82 weeks
 - PRJEB35854: Holstein
 - Semen from 9 bulls, collected at 69-71 weeks

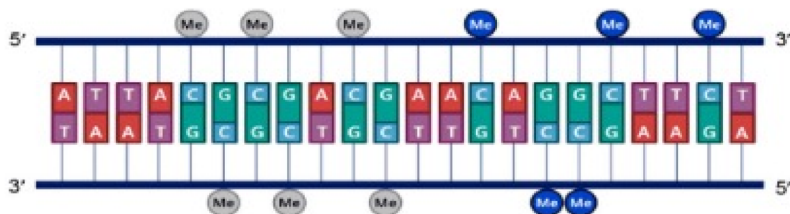
Study workflow



CpG Methylation

- RRBS reads were mapped against Bos taurus genome (ARS-UCD1.2).
 - A cutoff of 10X coverage was applied
 - Average Reads per Sample: 32.2M (range: 25.5M–40.7M)
- Within the CpG enriched regions represented in RRBS an average of 30.2% were methylated
 - 5,182,326 cytosines within the CpG context with 10X coverage per position in at least one sample
 - 661,206 CpGs with at least 10X coverage in all samples

Methylation analysis



High quality
trimmed FastQ

**Bismark &
Bismark Methylation Extractor**
Alignment and Methylation call

Aligned
sequences

Methylation
calls

SNP list

Custom scripts
Methylation calls
cleaning

Seqmonk
Methylation
analysis

Output
visualization

FastQ

TrimGalore
RRBS mode

FastQC
Quality check

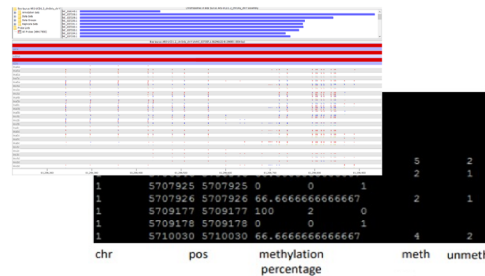
WGS

FastQC: quality check

TrimGalore: sequence trimming

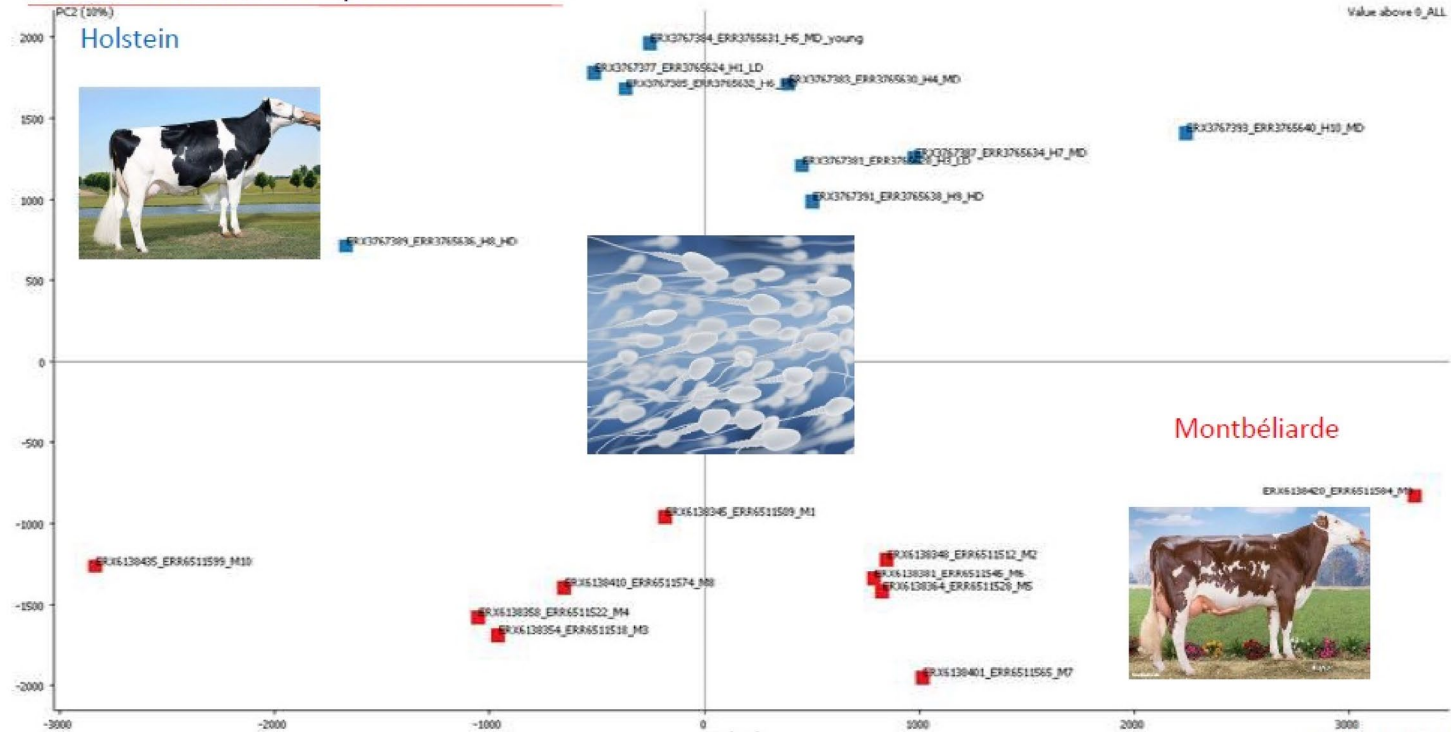
BWA: sequence alignment

Freebayes: variant detection

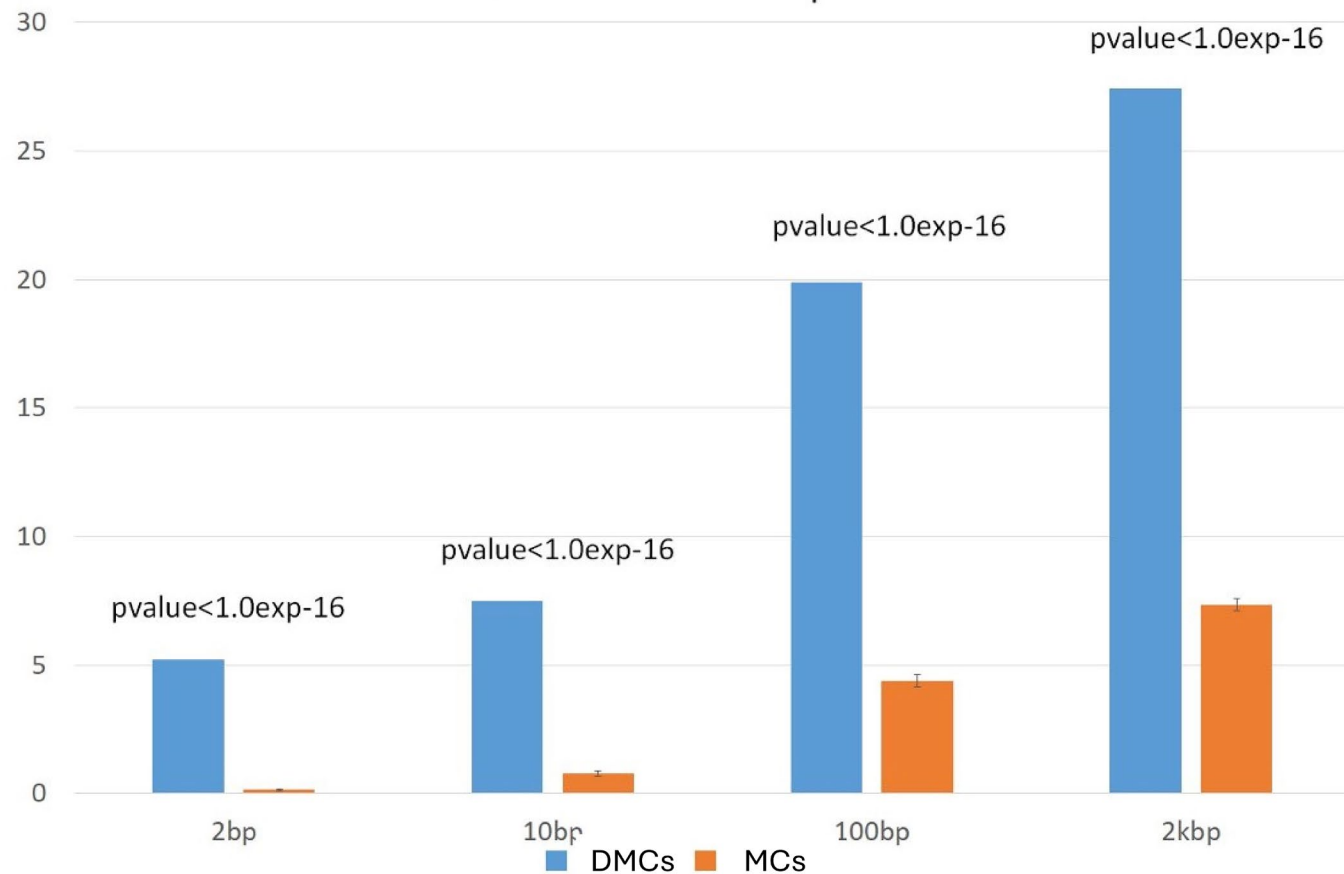


CpG methylation breed diversity

Public NCBI data from sperm



SNPs occurrence near CpGs



Conclusions and perspectives

- Differential methylation is associated to morphological traits relevant for adaptation
- SNP occurrence is consistently higher in differentially methylated regions
- Is epigenetic diversity more closely associated with genetic variation or with environmental variability?

