

**SeQuaMol**

Qualité Moléculaire de la semence



*Franz Marc, The Red Bull*

# Genetic and non-genetic factors determine DNA methylation patterns in bull spermatozoa

H. Kiefer, August 27, 2019  
EAAP session 24, 'Epigenetics'



ALIMENTATION  
AGRICULTURE  
ENVIRONNEMENT

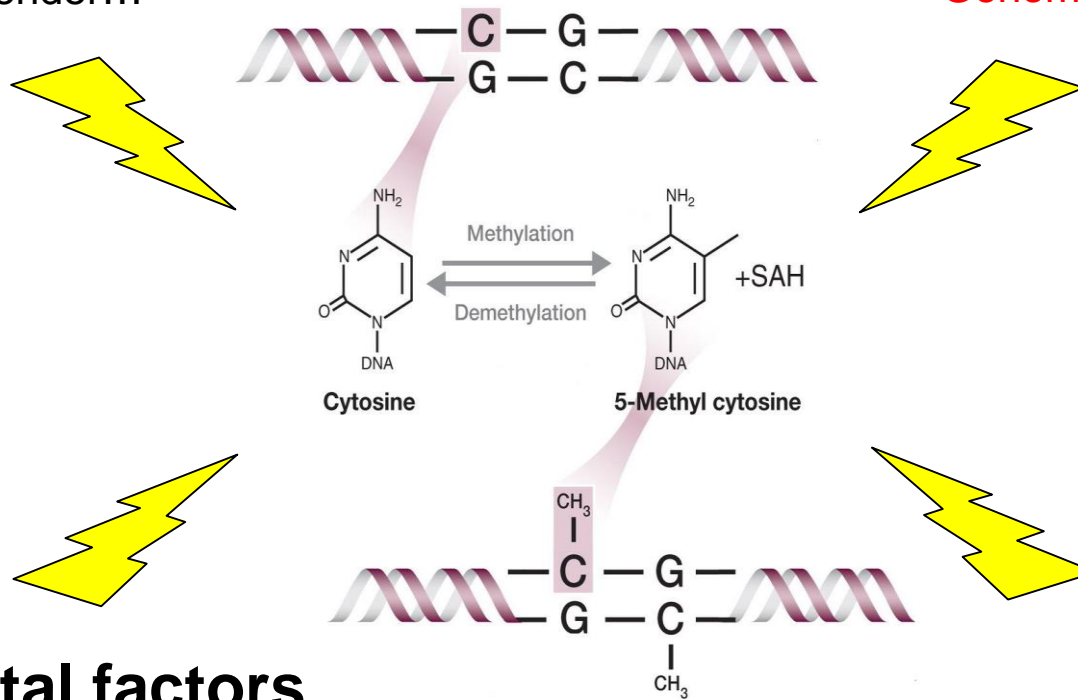
# The determinants of DNA methylation patterns

## Intrinsic factors

Cell type, age, gender...

## Genetic factors

Genomic DNA sequence



## Environmental factors

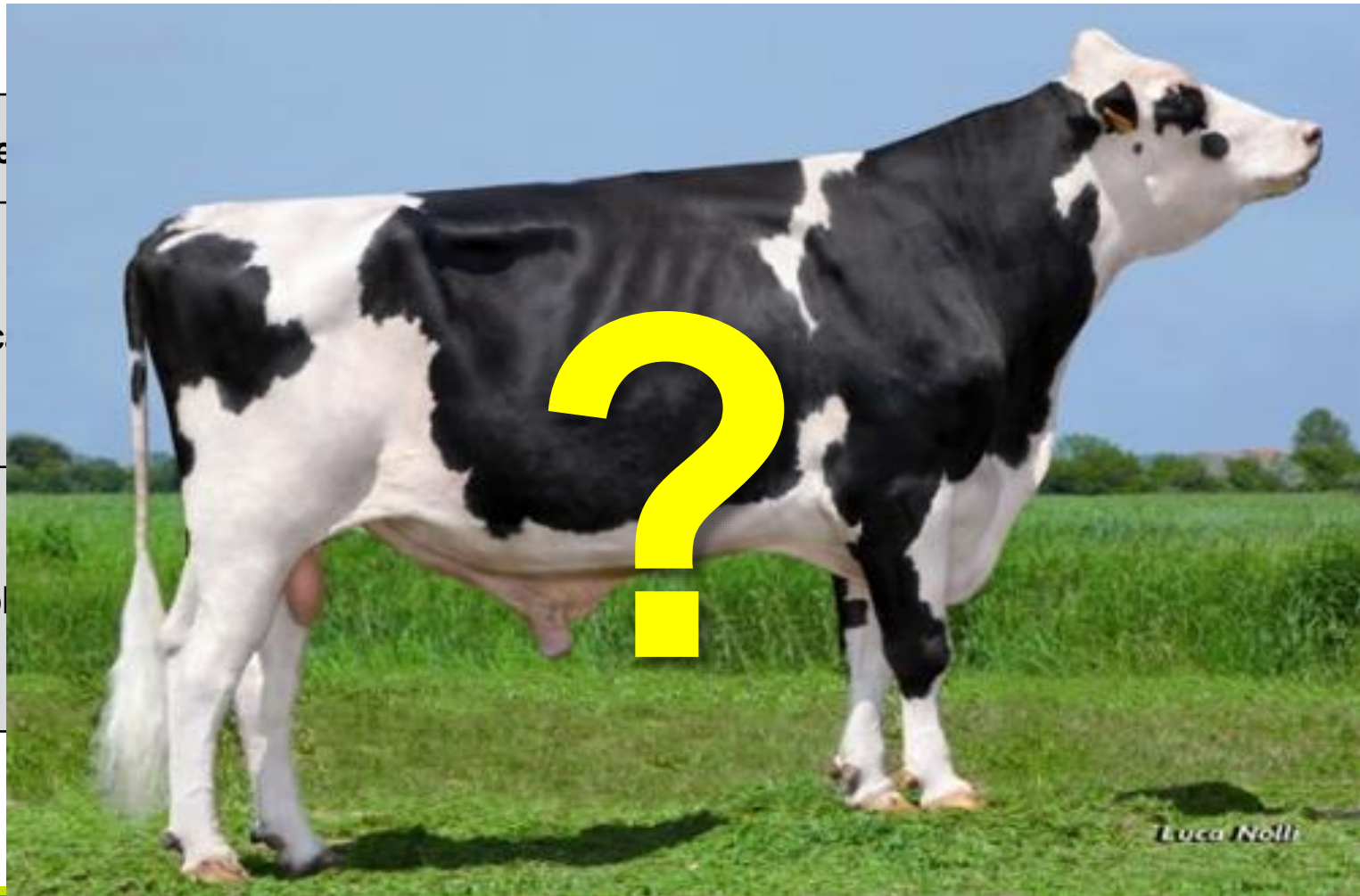
Nutrition, pollution, stress...

## Stochastic factors

<https://www.caymanchem.com/images/articles/screen/2153-1.jpg>

# Genetic sources of inter-individual variations in DNA methylation patterns

Effe  
Loc  
Glo



2015  
t al., 2017

Luca Nolli

Do et al., 2017  
Kader & Ghai, 2017  
Lappalainen and Grealley, 2017

# Bull semen is an important product for breeders and artificial insemination (AI) industry...



- 7 millions AI performed in France in 2016 (ruminants), ~100 million worldwide
- AI allows the diffusion of valuable genotypes
- Success of AI = important issue for breeders

## ...But data about genome-wide DNA methylation in bull sperm is still scarce

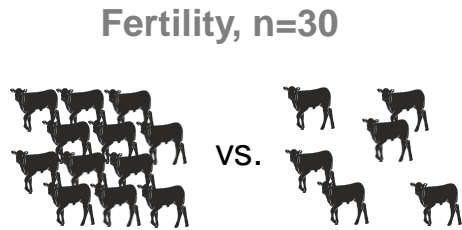
- Inter-individual variations with fertility (Verma *et al.*, 2014; Kropp *et al.*, 2017)
- Inter-individual variations with age (Lambert *et al.*, 2018; Takeda *et al.*, 2017; 2019)
- AI bulls are selected based on their genotype

**Contribution of genetic factors to the sperm methylome of AI bulls**



# Experimental design

## 1 Bull semen

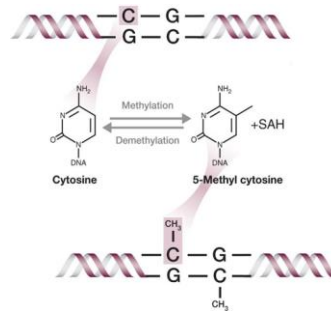


5 breeds, n=35



Total n=55

## 2 Molecular analyses

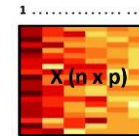


DNA methylation analyses (RRBS)

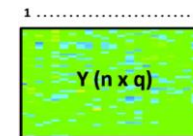


Genotyping (BovineSNP50 BeadChip, Illumina)

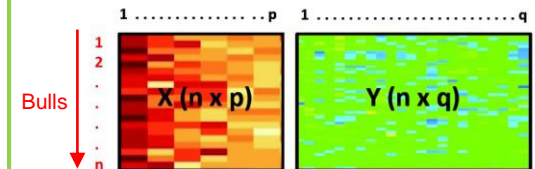
## 3 Data integration



Differentially Methylated CpGs (DMCs)



SNPs



PLS (mixOmics R package)



# Three groups of fertility



n=30 Montbéliarde breed

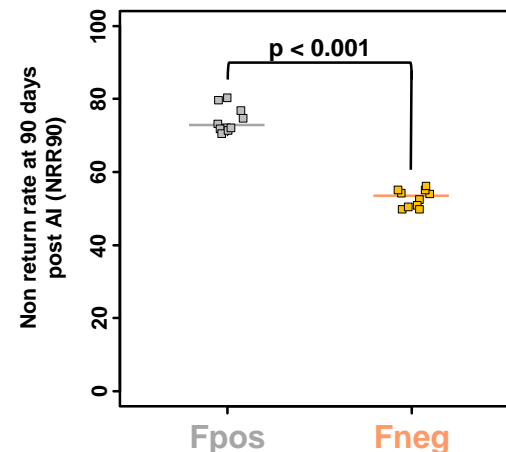
Median age consistent among groups

(17~19 months)

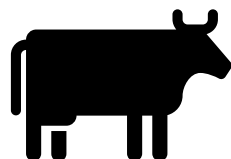
- ❖ n=10 fertile ejaculates (**Fpos**) and n=10 subfertile ejaculates (**Fneg**), based on the results obtained on field



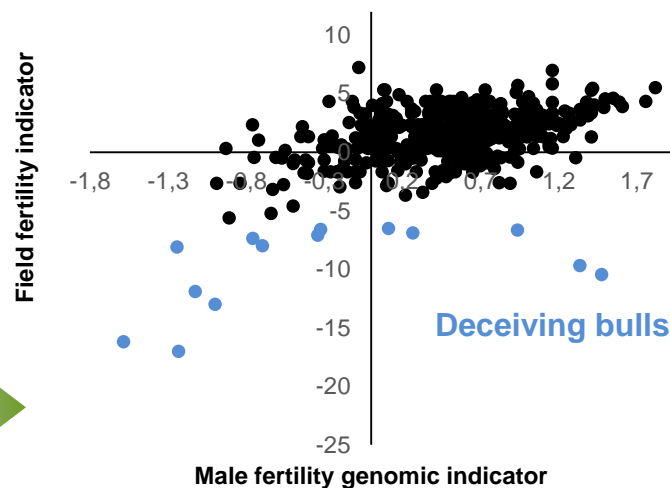
Ejaculate level



- ❖ n=10 deceiving bulls (**De**) based on the inadequation between field fertility indicator and genomic indicator

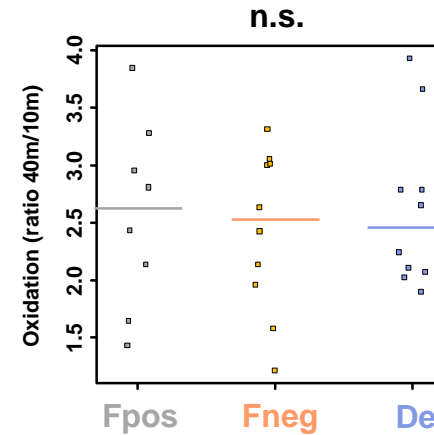
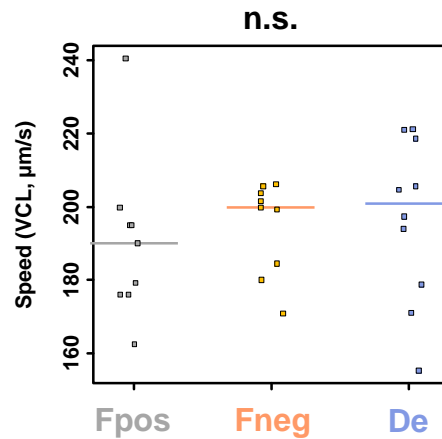
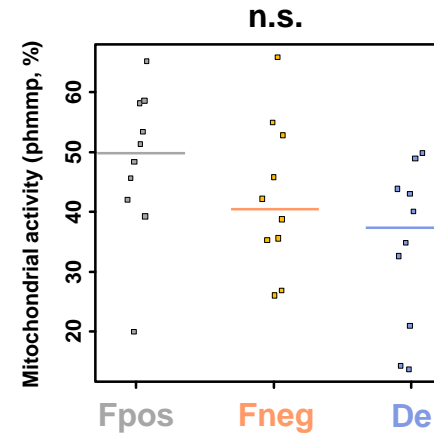
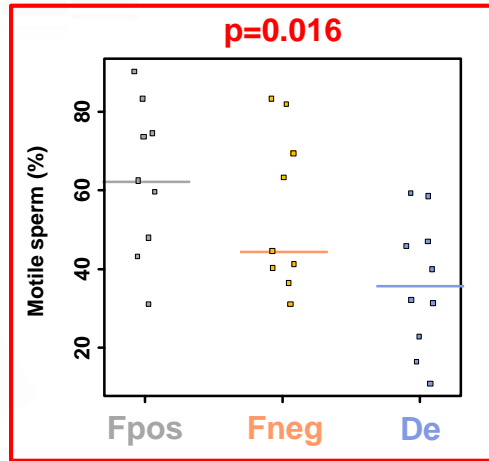


Bull level





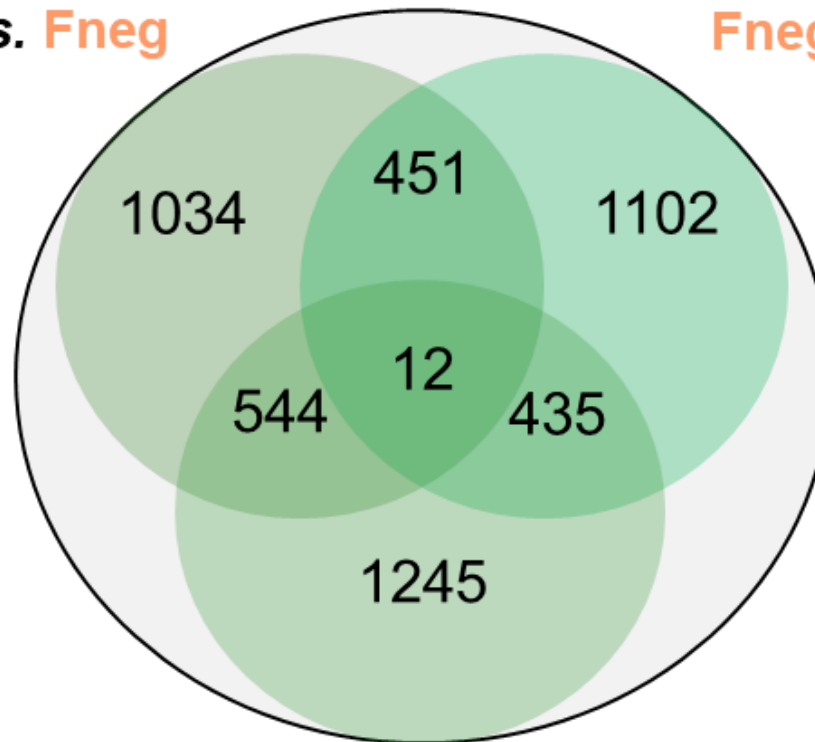
# Semen functional parameters (CASA & flow cytometry)



# Identification of fertility-related DMCs

Fpos vs. Fneg

Fneg vs. De



2,971 DMCs with no missing data

Fpos vs. De

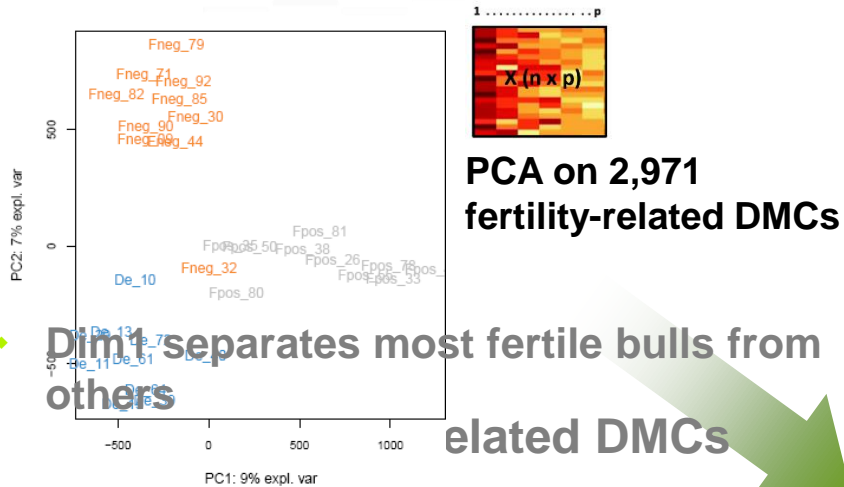
Differential analysis on CpGs covered by  $\geq 10$  reads in at least 4 samples per group  
methylKit software,  $q\text{value} < 0.01$ , methylation difference between groups  $\geq 25\%$





# Integration of genetic information and DMCs grossly preserves the fertility groups

Fpos  
 Fneg  
 De

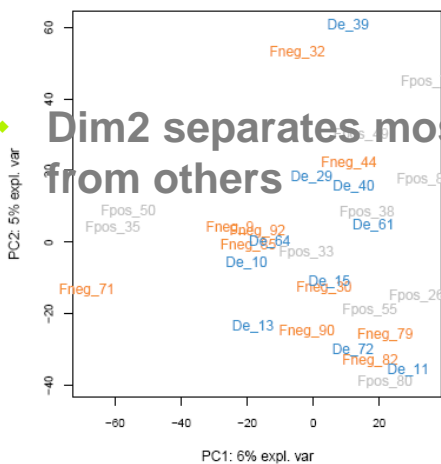


◆ Dim1 separates most fertile bulls from others

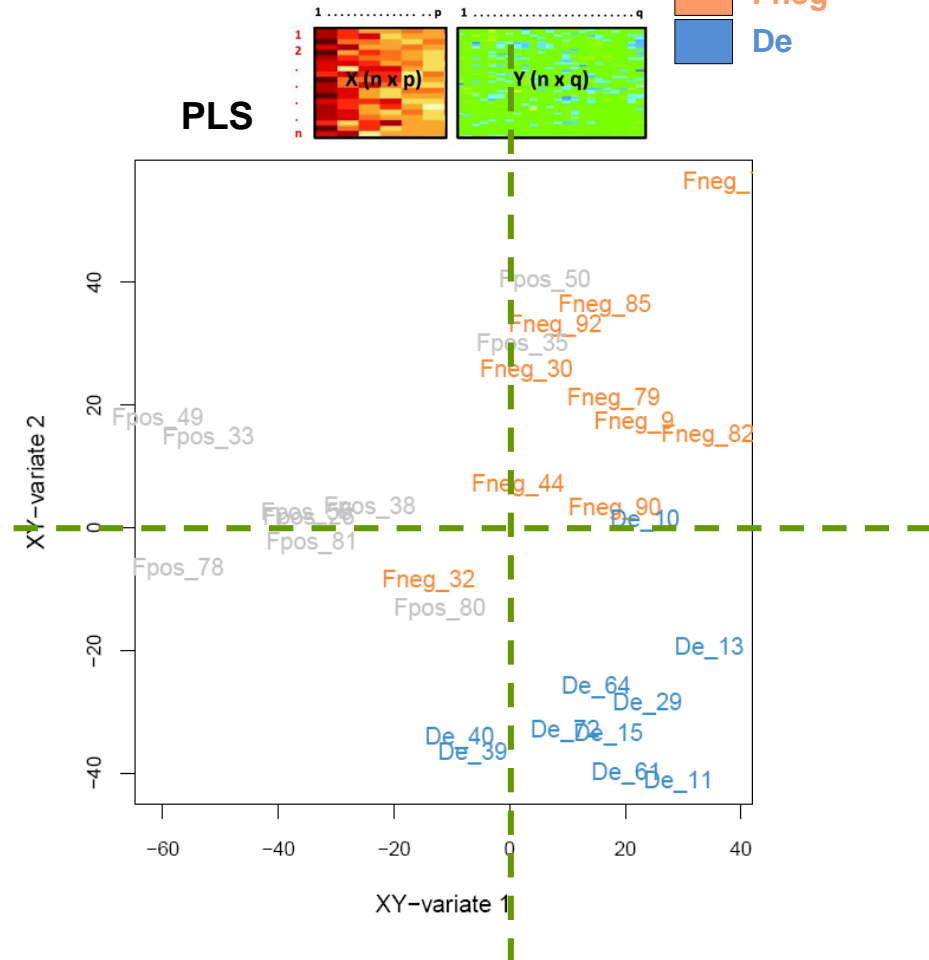
related DMCs

may be underlain by

morphism

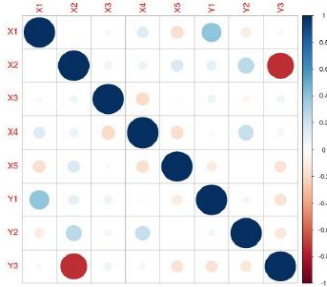


◆ Dim2 separates most deceiving bulls from others

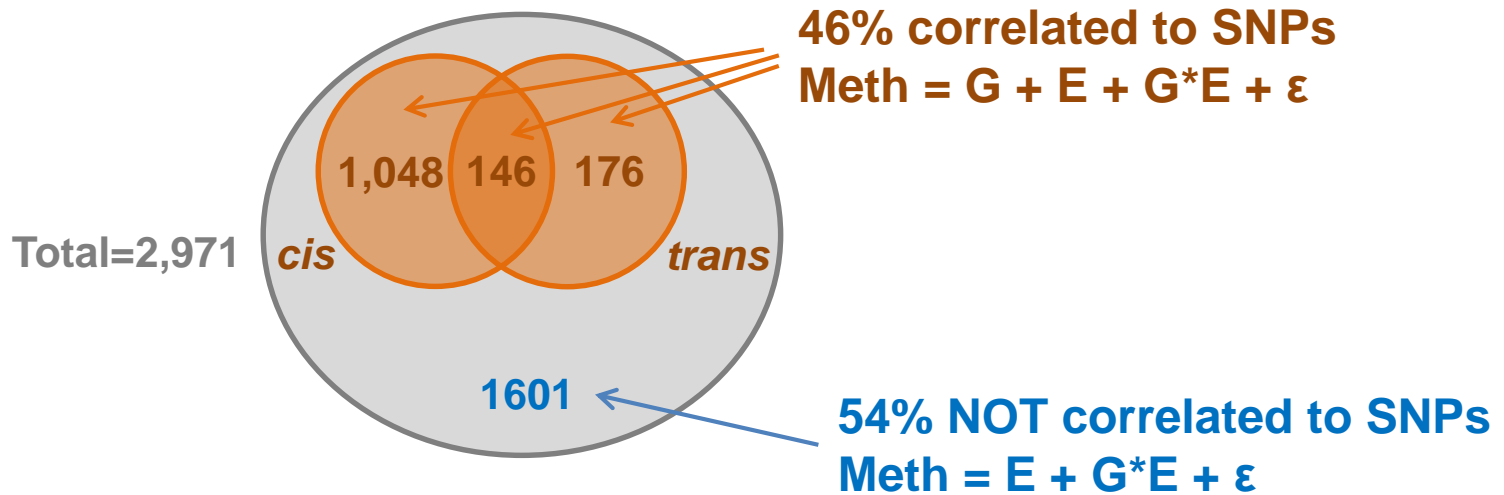




# Which proportion of fertility-related DMCs may be underlain by sequence polymorphism?

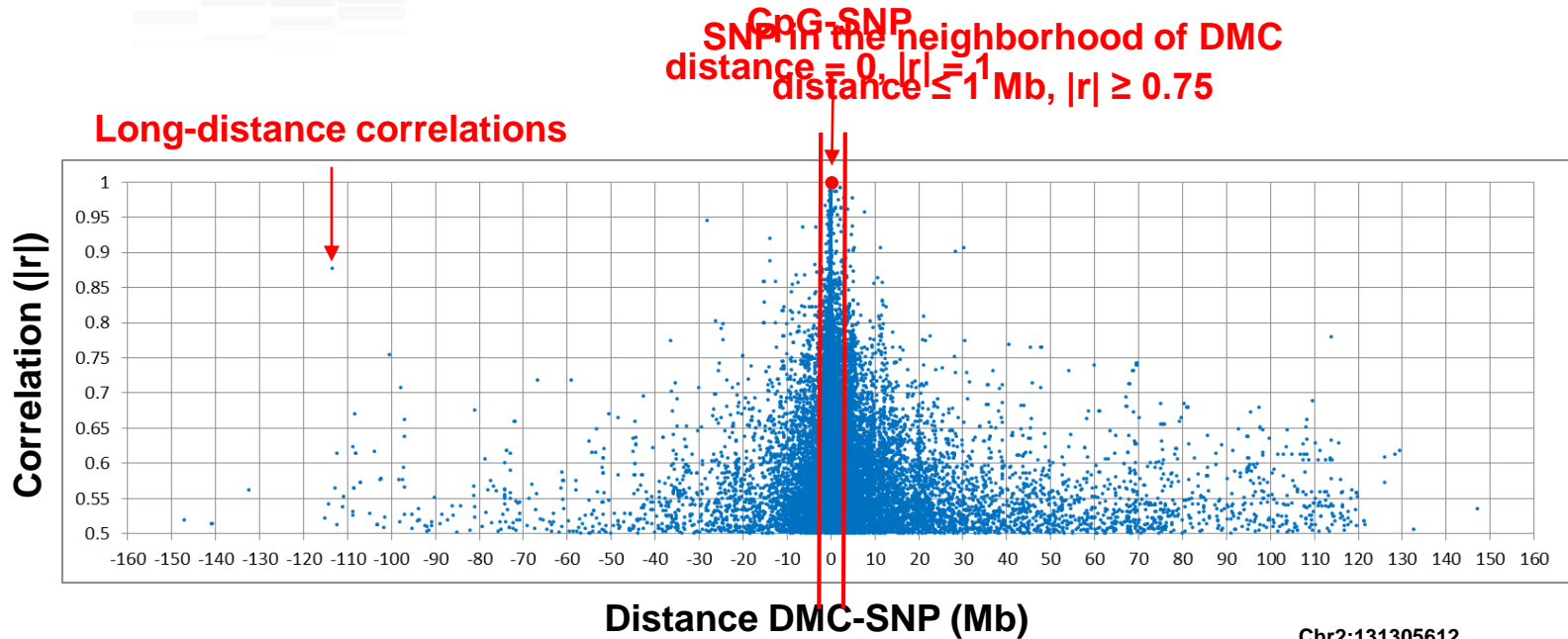


- Selection of 4000 SNPs highly contributing to the PLS results
- Correlation matrix between these SNPs and 2971 fertility-related DMCs
- A pair of SNP-DMC was considered as correlated if  $|r| > 0.75$

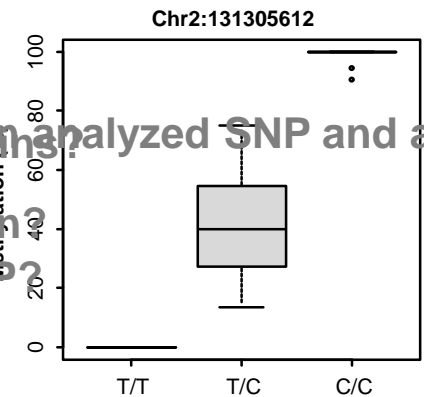




# Correlations *in cis* between DMCs and SNPs are distance-dependent



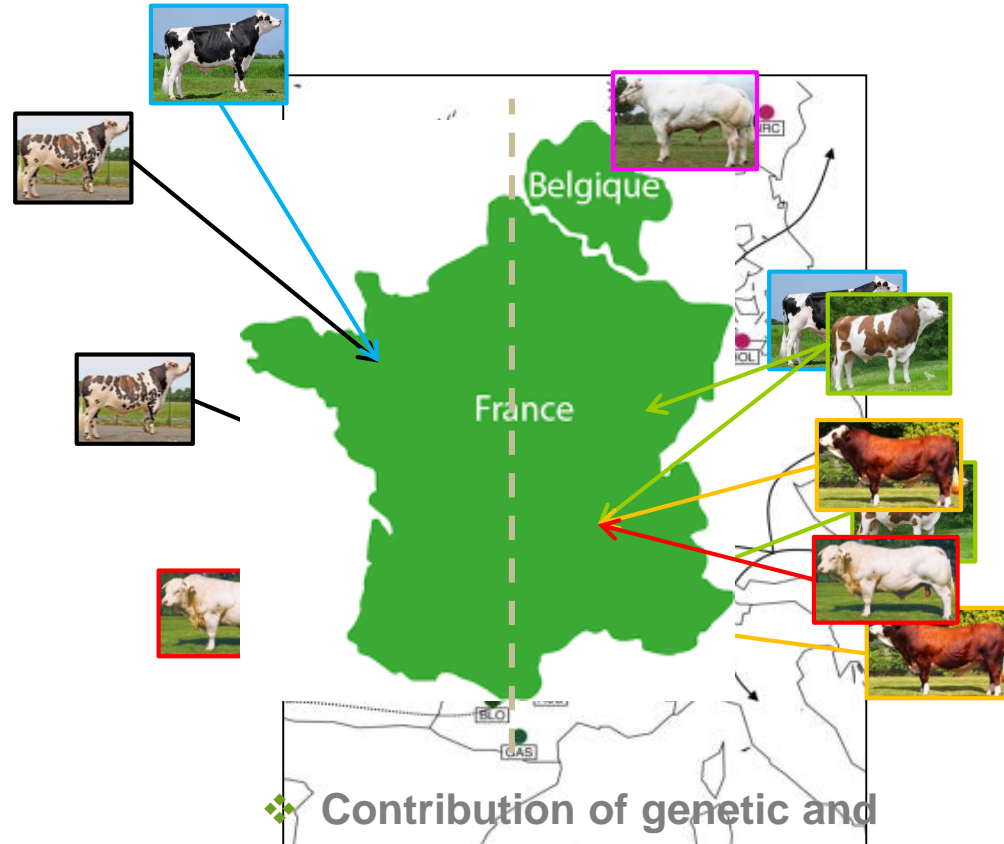
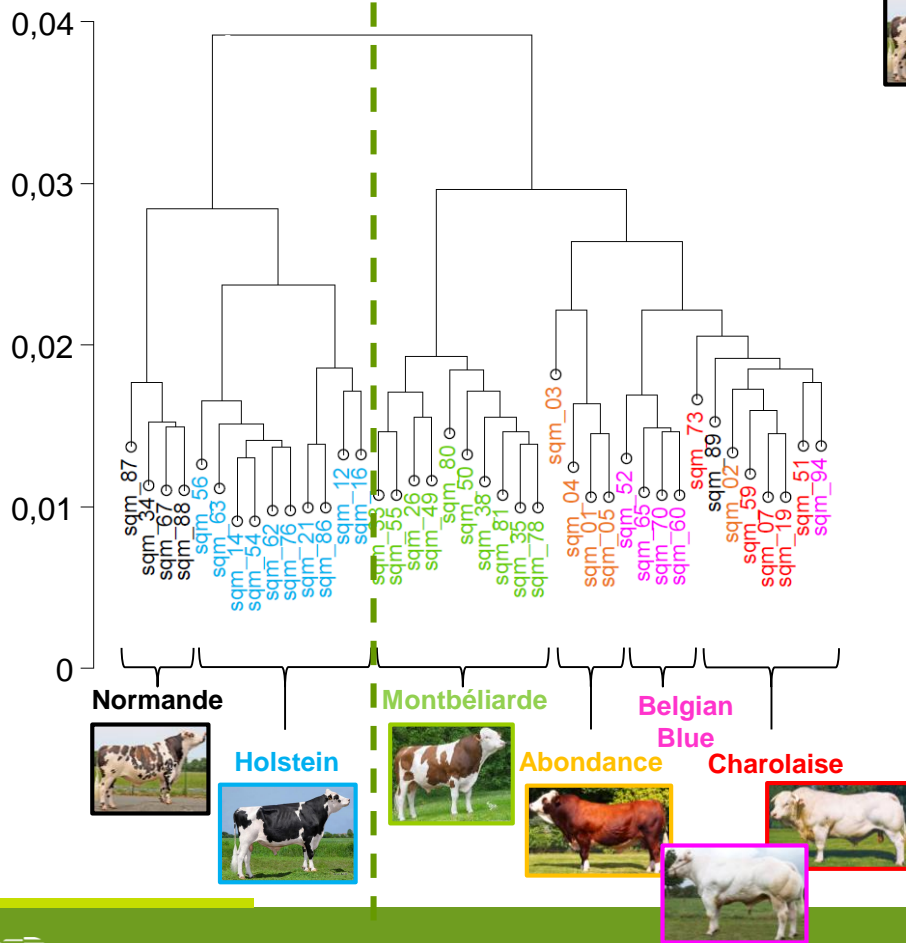
- ❖ Only 10 fertility-related DMCs are confounded with CpG-SNPs on the genotyping array
- ❖ Long-range interactions between chromosome domains putative CpG-SNP?
- ❖ By-products of the genetic structure of our population?
- ❖ The distribution of methylation at these DMCs is disrupted or explained by the SNP methylation at these DMCs



# Bull sperm methylome is shaped by the breed



**Hierarchical clustering  
(1 310 963 CpGs)**

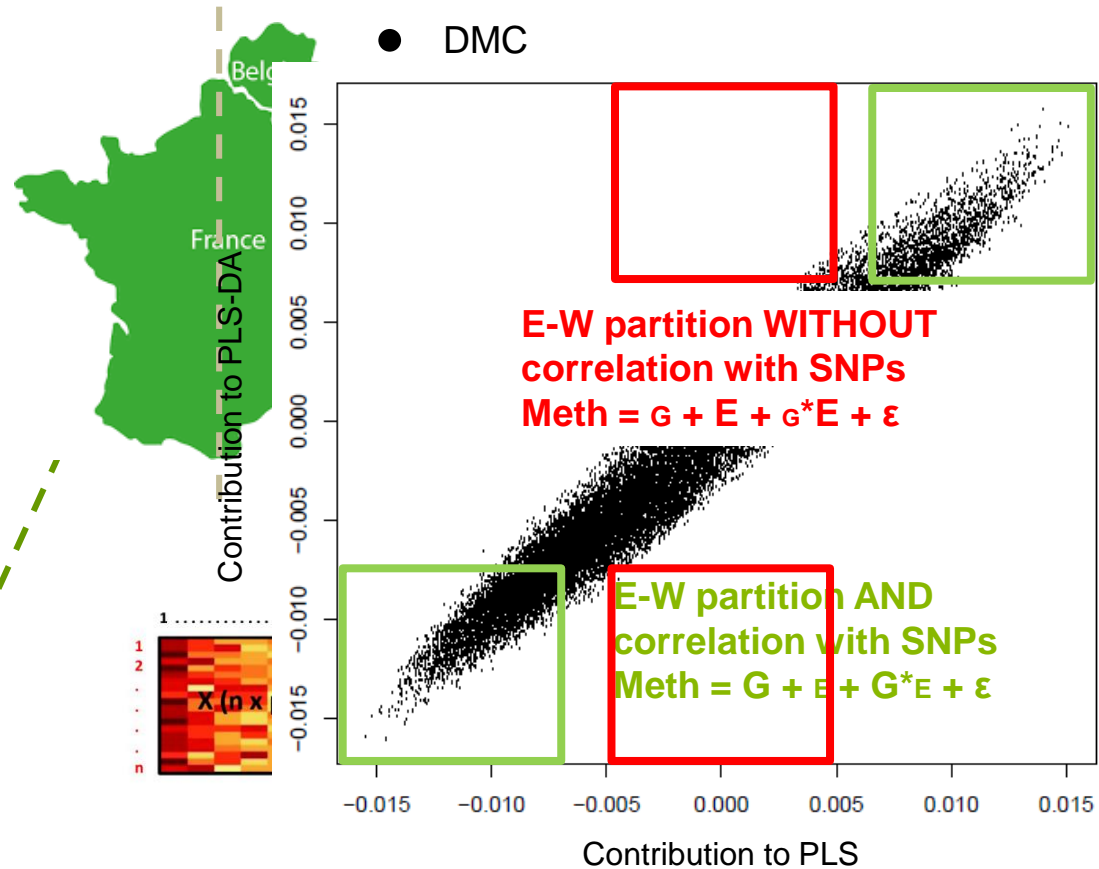


Contribution of genetic and environmental factors to the genetic history of french cattle from dense SNP data (Gautier et al., 2019)  
**breed-related differences?**

# Breed-related DMCs are mostly determined by inter-breed genetic polymorphism



Comparison	DMCs
NO vs. CH	10 656
NO vs. AB	13 959
NO vs. MT	11 029
HO vs. MT	10 898
HO vs. CH	8 969
HO vs. AB	11 298
HO vs. NO	9 386
MT vs. CH	7 606
MT vs. AB	7 352
CH vs. AB	7 628
Total DMCs	98 581
Unique DMCs	37 962



# Conclusion

- ❖ We identified CpGs displaying variations of methylation with fertility, that could be used as potential **fertility biomarkers**
- ❖ **46%** of these biomarkers were associated to **DNA polymorphism**. But this proportion is much more important when breed-related methylation differences are considered
- ❖ Inter-dependencies between the genome and the epigenome strongly suggest the presence of **methylation QTLs** in the cattle genome
- ❖ Provides new opportunities for **genomic selection** → animals with greater **genome plasticity**, able to **adapt to environment** by adjusting methylation to the environmental conditions

Modulation  
of DNA  
methylation



Modulation  
of  
phenotypes



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