Genetic evaluations in dairy cattle incorporating an epigenetic source of information

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Special thanks to…

Oxford NANOPORE Technologies

Epigenetics effects on the phenotype

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PLOS ONE

Trans-Generational Effect of Maternal Lactation during Pregnancy: A Holstein Cow Model

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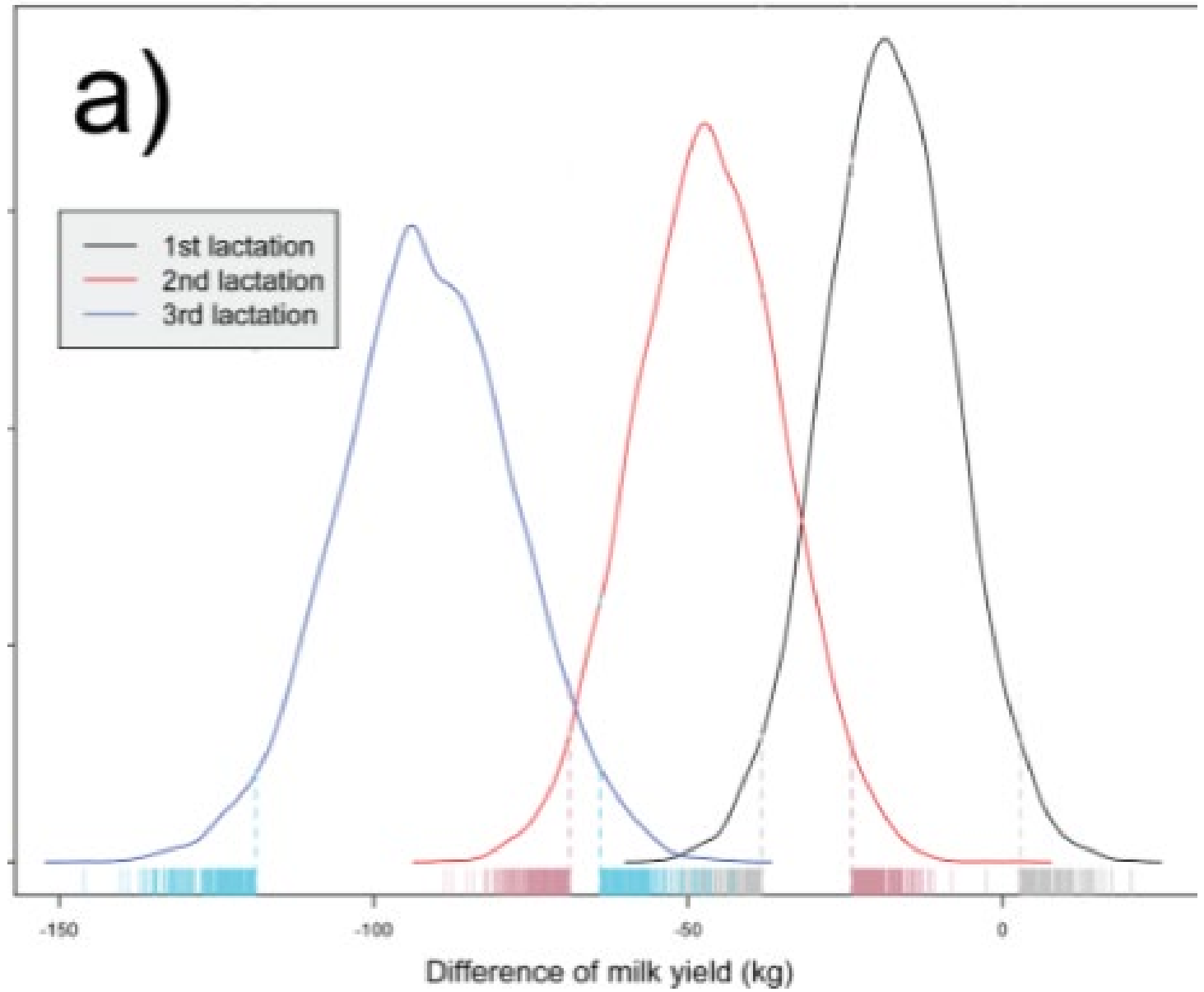
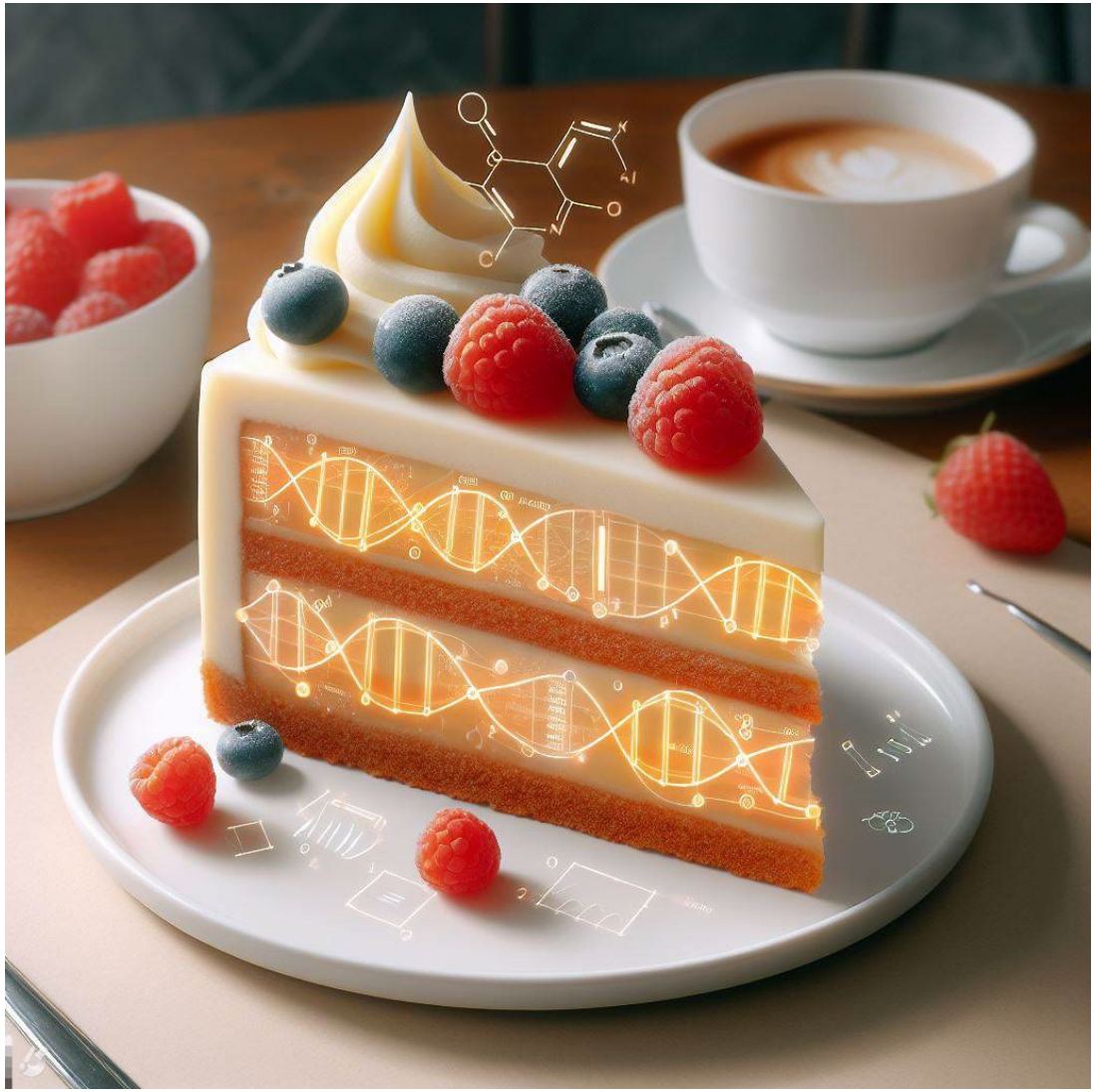
Epigenetics effects on the phenotype



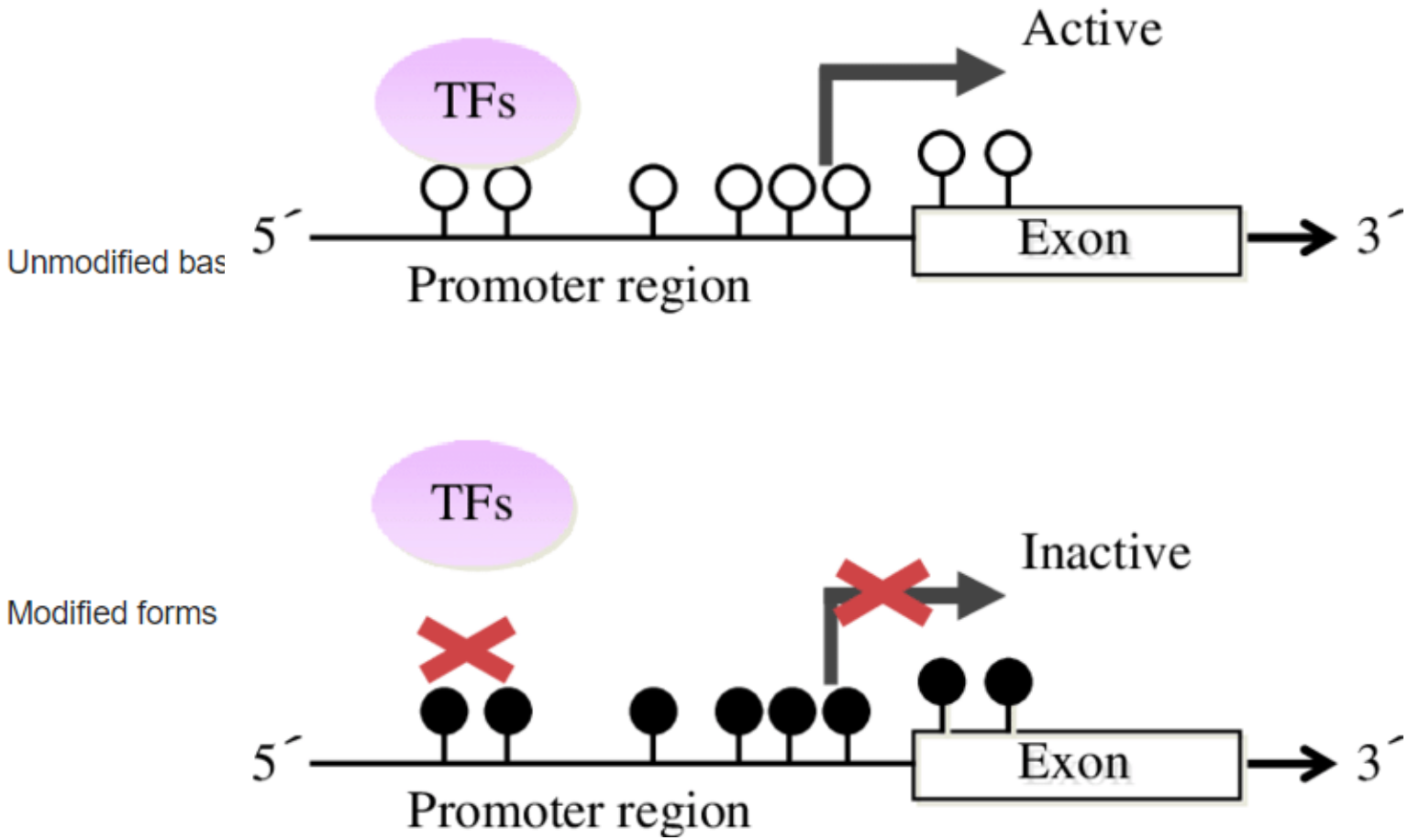
Epigenetics effects on the phenotype

Our DNA is made up of two layers of information:

- Genetics: Resistant to changes and inherited from our parents
- Epigenetics: dynamic and help us adapt to our environment

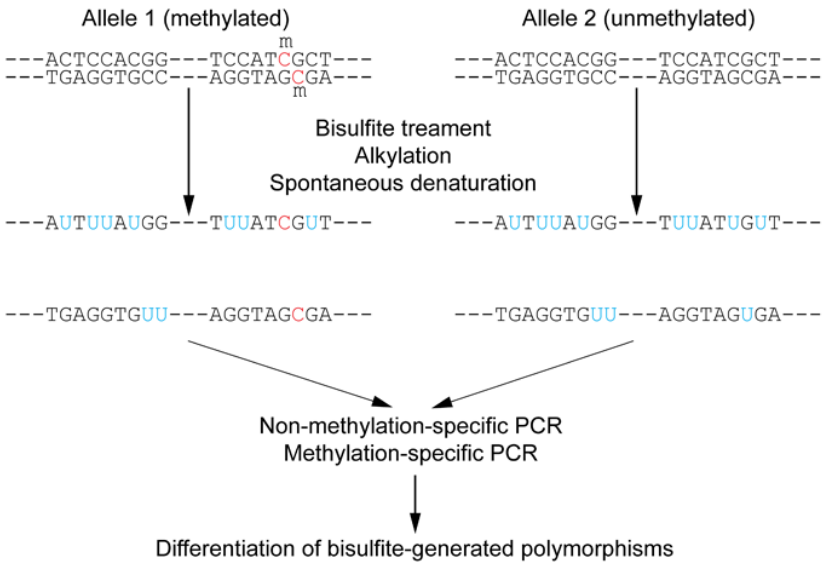


Is methylation altering the phenotypes?



It's 2024..... Post-genomic era. We can sequence the “methylome”

Bisulfite



Nanopore

It's 2024..... Post-genomic era. We can sequence the “methylome”

1. Can we use ONT to detect methylated CpGs?
   - ✓ Comparison with the current gold standard

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Oxford nanopore sequencing as an alternative to reduced representation bisulphite sequencing for the identification of CpGs of interest in livestock populations

Adrián López-Catalina [a,b], Valentin Costes [c], Ramón Peiró-Pastor [a], Hélène Kiefer [d,e], Oscar González-Recio [a,*]

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[c] R&D Department, ELIANCE, 149 rue de Bercy, Paris 75012, France
[d] Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas 78350, France
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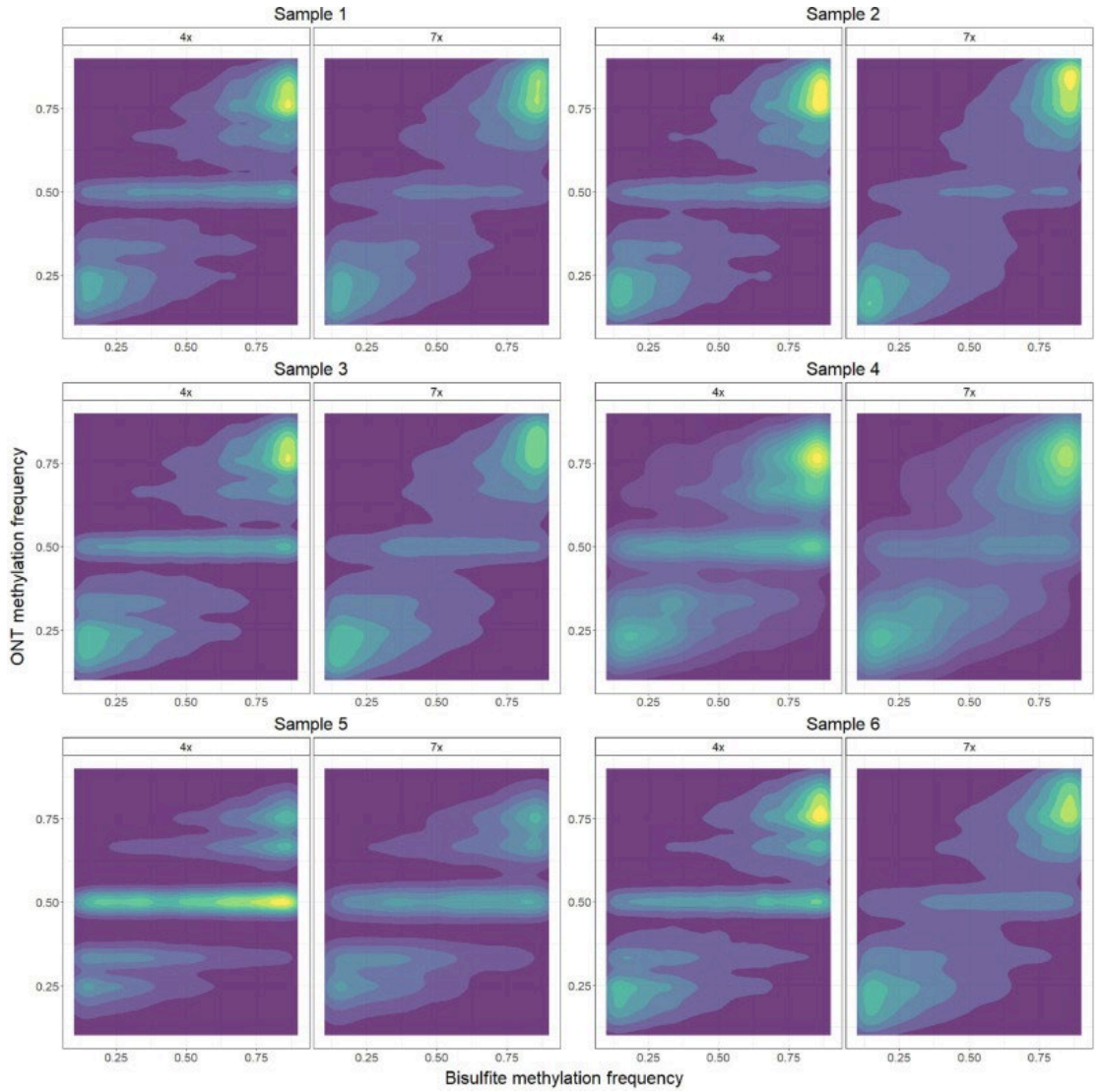
- High correlation between the methylation frequency detected by each technique. Maybe we would need to increase the minimum depth for ONT samples



It's 2024..... Post-genomic era. We can sequence the “methylome”

1. Can we use ONT to detect methylated CpGs?
   - ✓ Comparison with the current gold standard
2. Can the mother's phenotype affect the calf's "epigenome"?
   - ✓ Co-methylation network based on the PCIT algorithm

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RESEARCH ARTICLE

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Stress-induced epigenetic effects driven by maternal lactation in dairy cattle: a comethylation network approach

Adrián López-Catalina [a,b,c], Antonio Reverter[c], Pamela A. Alexandre[c], Loan T. Nguyen[d], and Oscar González-Recio[a]

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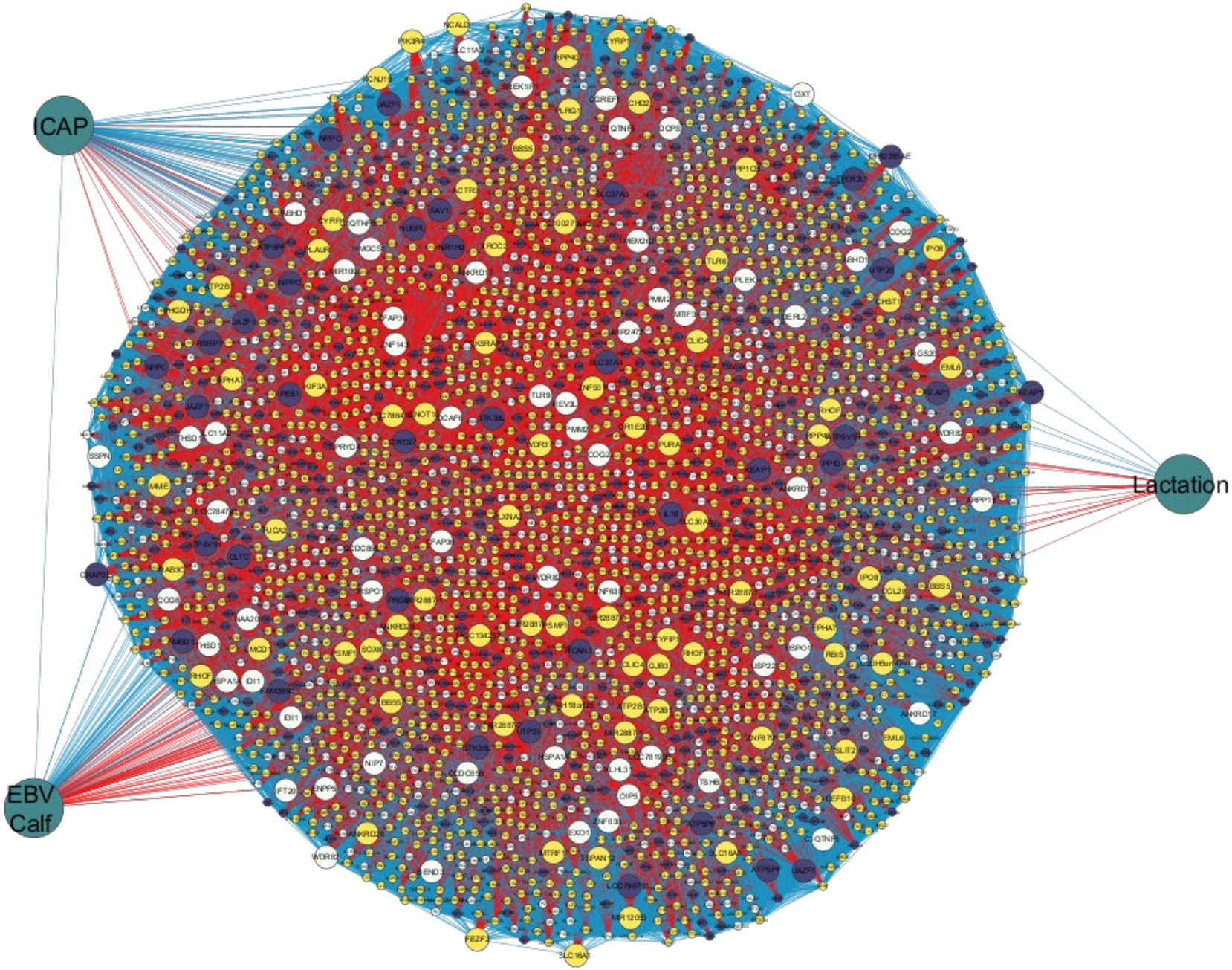
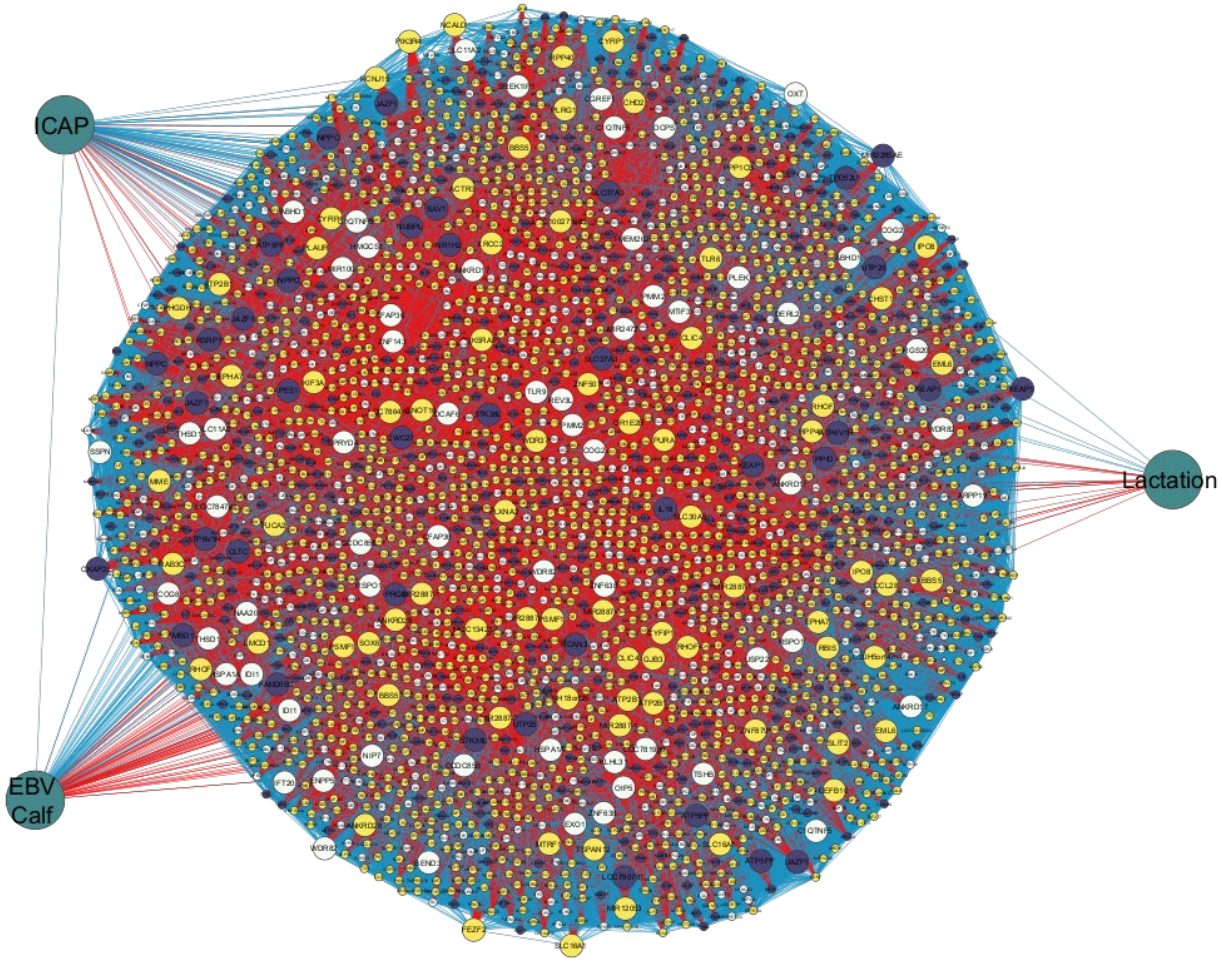
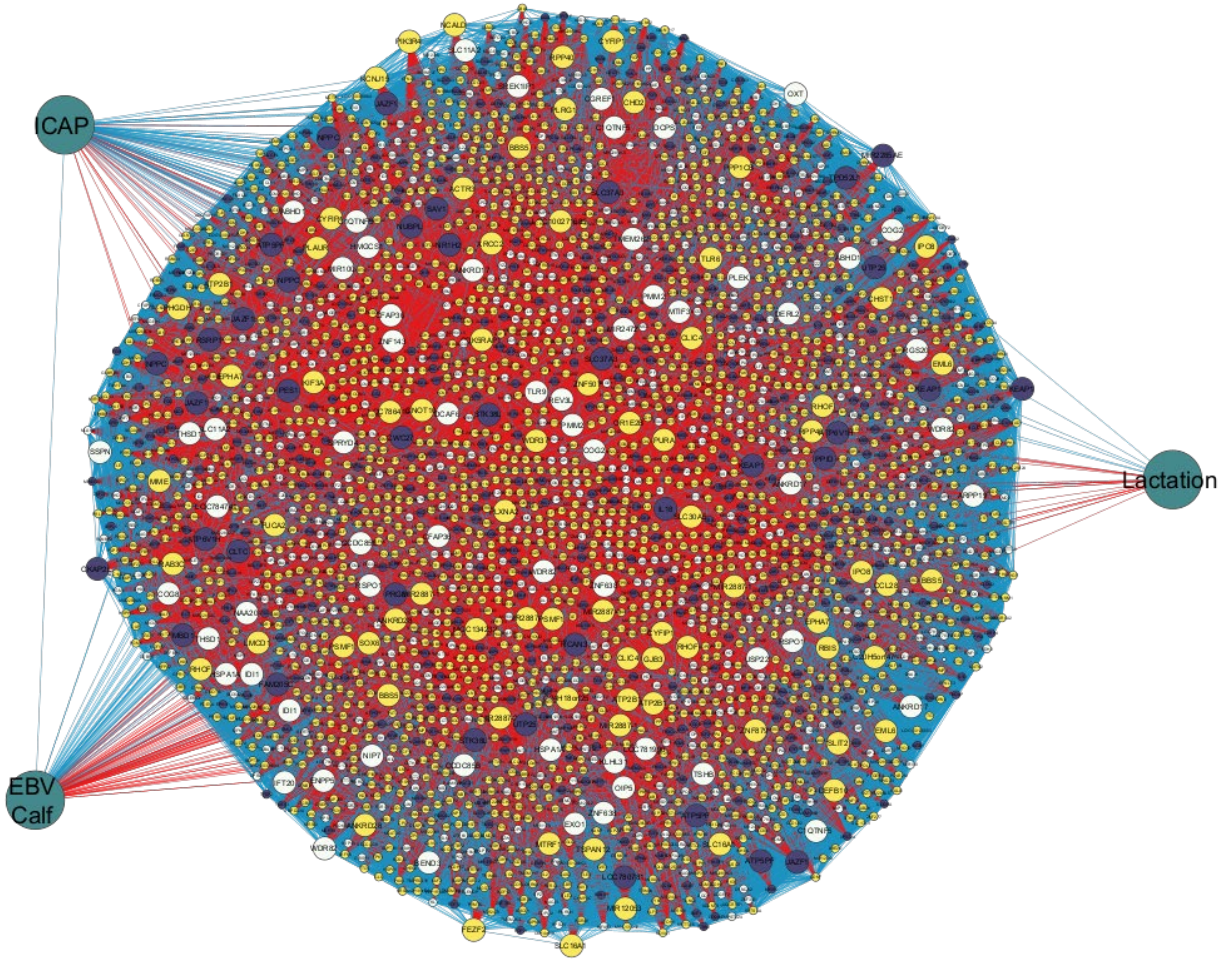

ICAP
EBV
Calf
Lactation



- The study identified DMRs in calves gestated by heifers and lactating cows, with a comethylation network linking calves' methylation profiles to their ICAP and milk EBV
- Differentially methylated genes in lactating and non-lactating calves were enriched in GO terms related to vasculature, morphogenesis, and signaling, potentially influencing growth, embryo development, and milk yield
- Results suggest that the dam's lactation status affects the methylation profile and development of calves, highlighting the need for further research to integrate these findings into breeding models and assess long-term effects

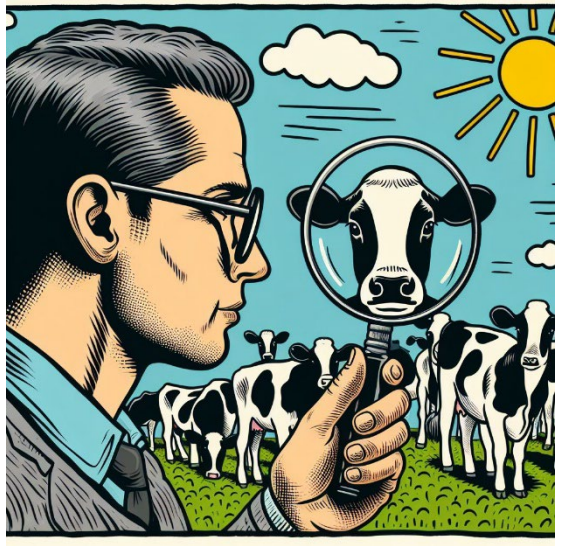

ICAP
Lactation
EBV
Calf

It's 2024..... Post-genomic era. We can sequence the “methylome”

1. Can we use ONT to detect methylated CpGs?
   - ✓ Comparison with the current gold standard
2. Can the mother's phenotype affect the calf's "epigenome"?
   - ✓ Co-methylation Network based on the PCIT algorithm and DMR.
3. How can we make decisions based on these results?
   - ✓ **Quantitative approach** to include methylation in breeding programmes

How can we make use of methylation information from new sequencing and epigenotyping technologies?

- What would happen if we added methylation information to the prediction equations?

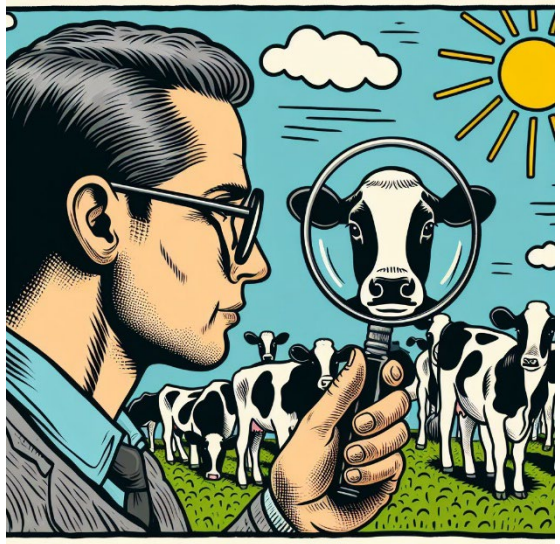


We measure and record phenotypes of interest

And combine them with genomic information

How can we make use of methylation information from new sequencing and epigenotyping technologies?

- What would happen if we added methylation information to the prediction equations?



We measure and record phenotypes of interest

And combine them with genomic information

… and epigenetic information!

Genomic selection

$$y = \mu + Xb + Za + e$$

Genomic selection

$$y = \mu + Xb + Za + e$$

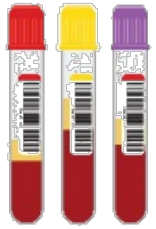
A multi-omics approach

$$y_e = \mu + X\beta + Z\mathrm{M}\alpha + Z a_r + e$$

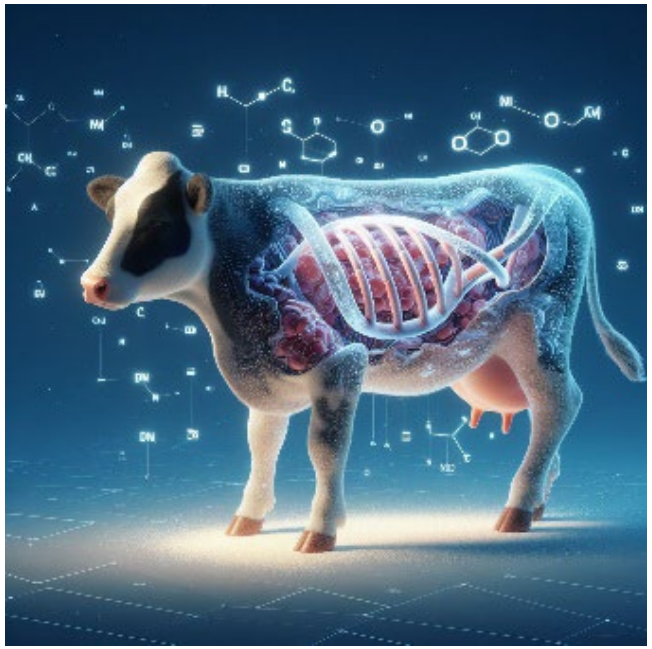
Genetic evaluation including intermediate omics features
Christensen et al. (2021)

Our data

6 blood samples from calves

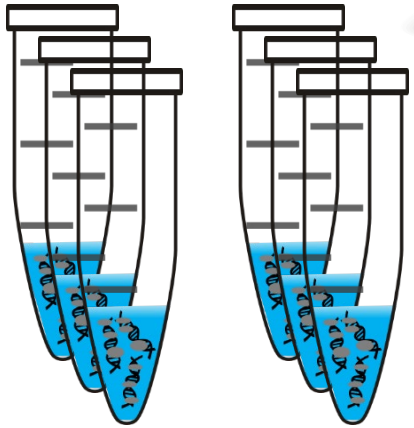


13,183 genotyped cows (CONAFE)



Wet lab – Methods

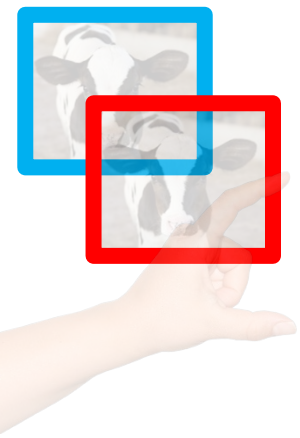
DNA extraction

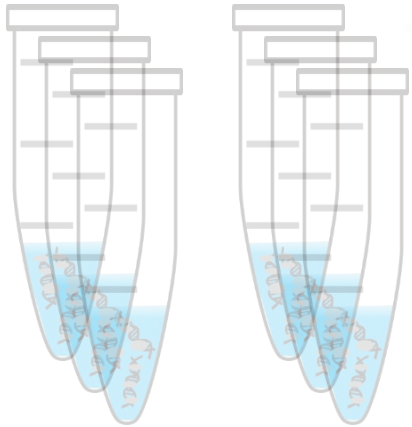


Library preparation

Sequencing

Dry lab – Methods



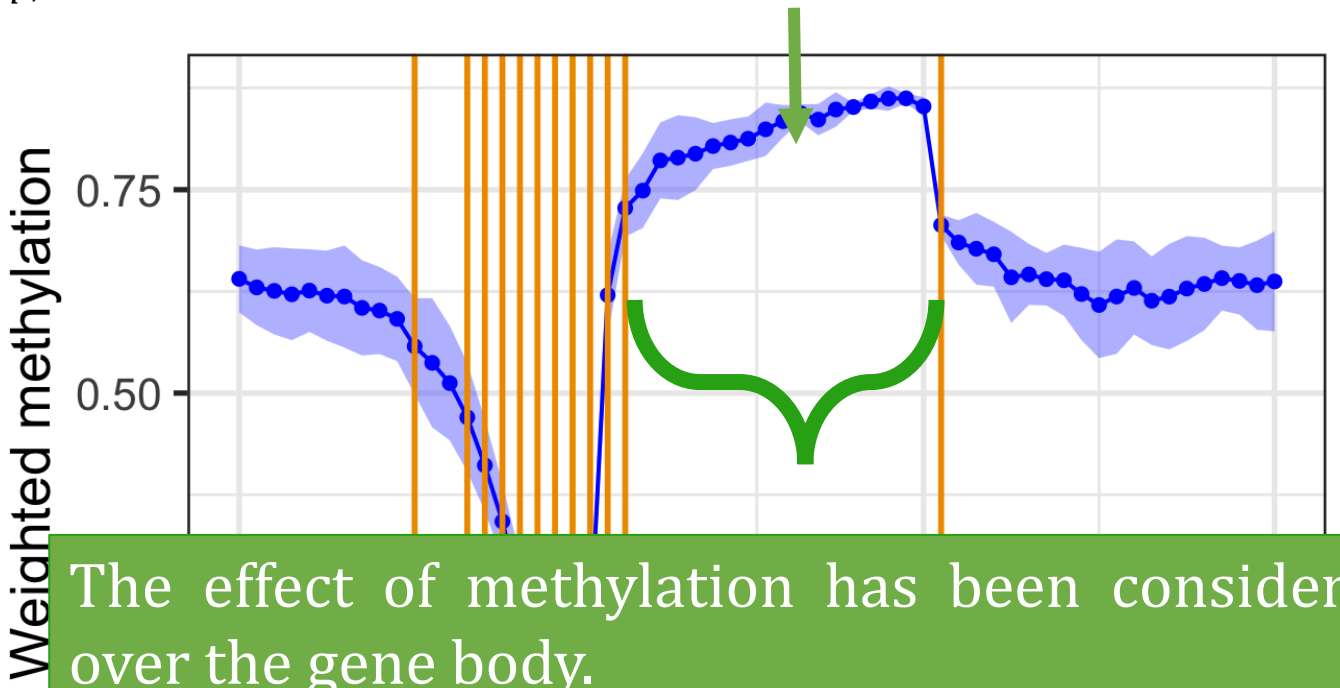


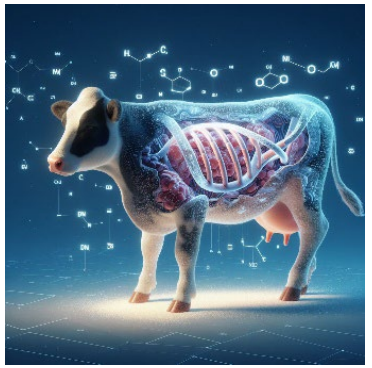
Pipeline R ← Methylation files ← Guppy v.6.3.7 F5C v.1.1 ← Base and methylation calling

A multi-omics approach – Methods

We divided the genic region into 13 methylation windows

- Non-overlapping
- Consecutive
- μ_w, $\sigma^2_{p,w}$ average methylation frequency and variance of each window





The effect of methylation has been considered over the gene body.
If there is methylation in the promoter, it decreases the effect of SNPs in this window.

A multi-omics approach – Methods

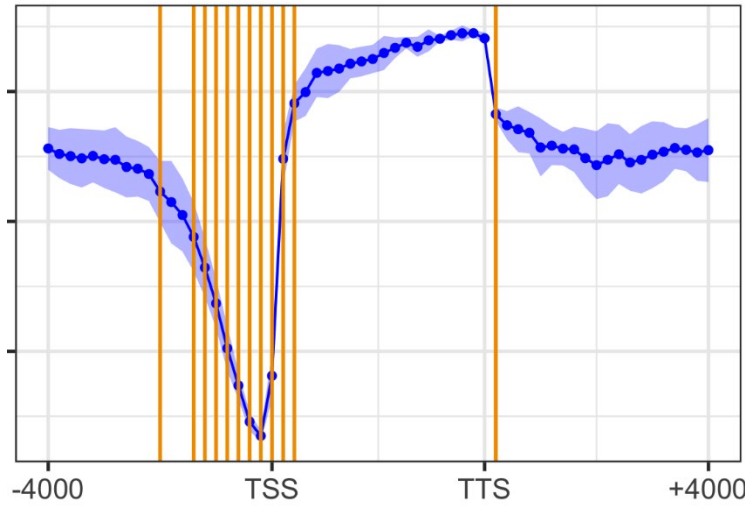
We simulated two traits:

Productive trait

10,000 SNPs with effect

$(\beta) \sim \Gamma(0.42,5.4)$

- 7,500 gene body
- 2,500 outside



Liability to methylation

10,000 SNPs with effect

$(\gamma) \sim \Gamma(0.42,5.4)$

At random 13 windows + intergenic

A multi-omics approach – Methods

Productive trait

10,000 SNPs with effect

$(\beta) \sim \Gamma(0.42, 5.4)$

- 7,500 gene body
- 2,500 outside

Liability to methylation

Simulating the liability to methylation breeding value

$$mBV_i = \sum_{i=1} x_i \cdot \gamma_i$$

A multi-omics approach – Methods

Productive trait

10,000 SNPs with effect

$(\beta) \sim \Gamma(0.42,5.4)$

- 7,500 gene body
- 2,500 outside

Liability to methylation

We simulated the phenotype "methylation frequency"

$$y_{meth_{iw}} = \mu_w + mg_{iw} + e(m)_{iw}$$

- h^2_{meth} = 0.1, 0.3 or 0.8

A multi-omics approach – Methods

Productive trait

10,000 SNPs with effect $(\beta) \sim \Gamma(0.42,5.4)$

- 7,500 gene body
- 2,500 outside

Liability to methylation

We simulated the phenotype "methylation frequency"

$$y_{meth_{iw}} = \mu_w + mg_{iw} + e(m)_{iw}$$

- h^2_{meth} = 0.1, 0.3 or 0.8

Simulation of the epigeneic true breeding value ($epiTBV_i$)

In genic regions, the methylation phenotype affects negatively the breeding value

A multi-omics approach – Methods

Productive trait

Liability to methylation

We simulate the phenotype of the productive trait

$$y_{prod_i} = \mu_{prod} + epiTBV_i + e_i$$

where $e \sim N(0, \sigma_e^2)$ and σ_e^2 is obtained from a traditional model to estimate the productive trait without considering methylation

$$h^2_{trait} = 0.3$$

A multi-omics approach – Methods

Multi-omics model **(GOBLUP)**

(Christensen et al., 2021):

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_m}\mathbf{y}_{meth_w}\boldsymbol{\alpha} + \mathbf{Z}\mathbf{a_r} + \mathbf{e}$$

$$\mathbf{y_{meth}}_w = \mathbf{X_m\beta_m} + \mathbf{Z'}\mathbf{g}_{m_w} + \mathbf{e_w}$$

Epigenetic Estimated Value (EEV) = $a_r + \sum g_m * \boldsymbol{\alpha}$

A multi-omics approach – Results

Trait	$h^2_{simulated\ methylation}$	$\hat{\sigma}^2_{gen\ prod.\ trait}$	$\hat{\sigma}^2_{res\ prod.\ trait}$	$\hat{h}^2_{prod.\ trait}$
Traditional BLUP	0.10	1.17 (0.99-1.34)	6.63 (6.44-6.81)	0.15 (0.13-0.17)
	0.30	1.41 (1.22-1.60)	6.49 (6.31-6.69)	0.18 (0.16-0.20)
	0.80	2.00 (1.78-2.22)	6.16 (5.98-6.35)	0.25 (0.22-0.27)

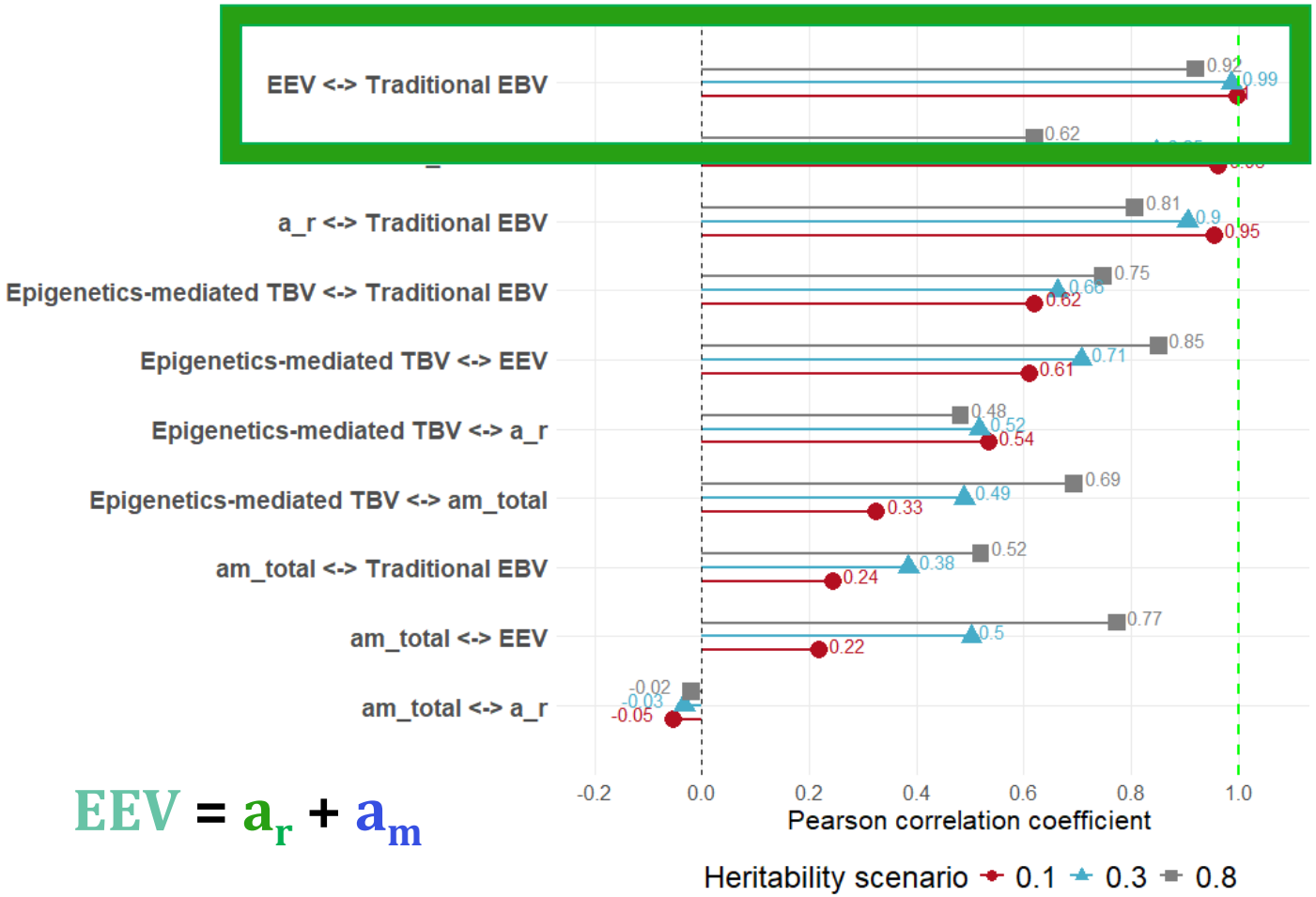
- The estimated heritability increased when the simulated heritability for liability to methylation increased

A multi-omics approach – Results

Trait	$h^2_{simulated\ methylation}$	$\widehat{h^2}_{methylation\ GOBLUP}$	$\hat{\sigma}^2_{gen\ prod.\ trait}$	$\hat{\sigma}^2_{res\ prod.\ trait}$	$\hat{h}^2_{prod.\ trait}$
Traditional BLUP	0.10	-	1.17 (0.99-1.34)	6.63 (6.44-6.81)	0.15 (0.13-0.17)
	0.30	-	1.41 (1.22-1.60)	6.49 (6.31-6.69)	0.18 (0.16-0.20)
	0.80	-	2.00 (1.78-2.22)	6.16 (5.98-6.35)	0.25 (0.22-0.27)
Epigenetic model (GOBLUP)	0.10	0.102 ± 0.01	1.01 (0.86-1.17)	6.09 (5.92-6.26)	0.14 (0.12-0.16)
	0.30	0.315 ± 0.01	1.01 (0.86-1.17)	6.09 (5.92-6.26)	0.14 (0.12-0.16)
	0.80	0.846 ± 0.01	1.01 (0.86-1.17)	6.09 (5.91-6.25)	0.14 (0.12-0.16)

- GOBLUP separates the heritability to liability to methylation from the estimated heritability of the productive trait

A multi-omics approach – Results

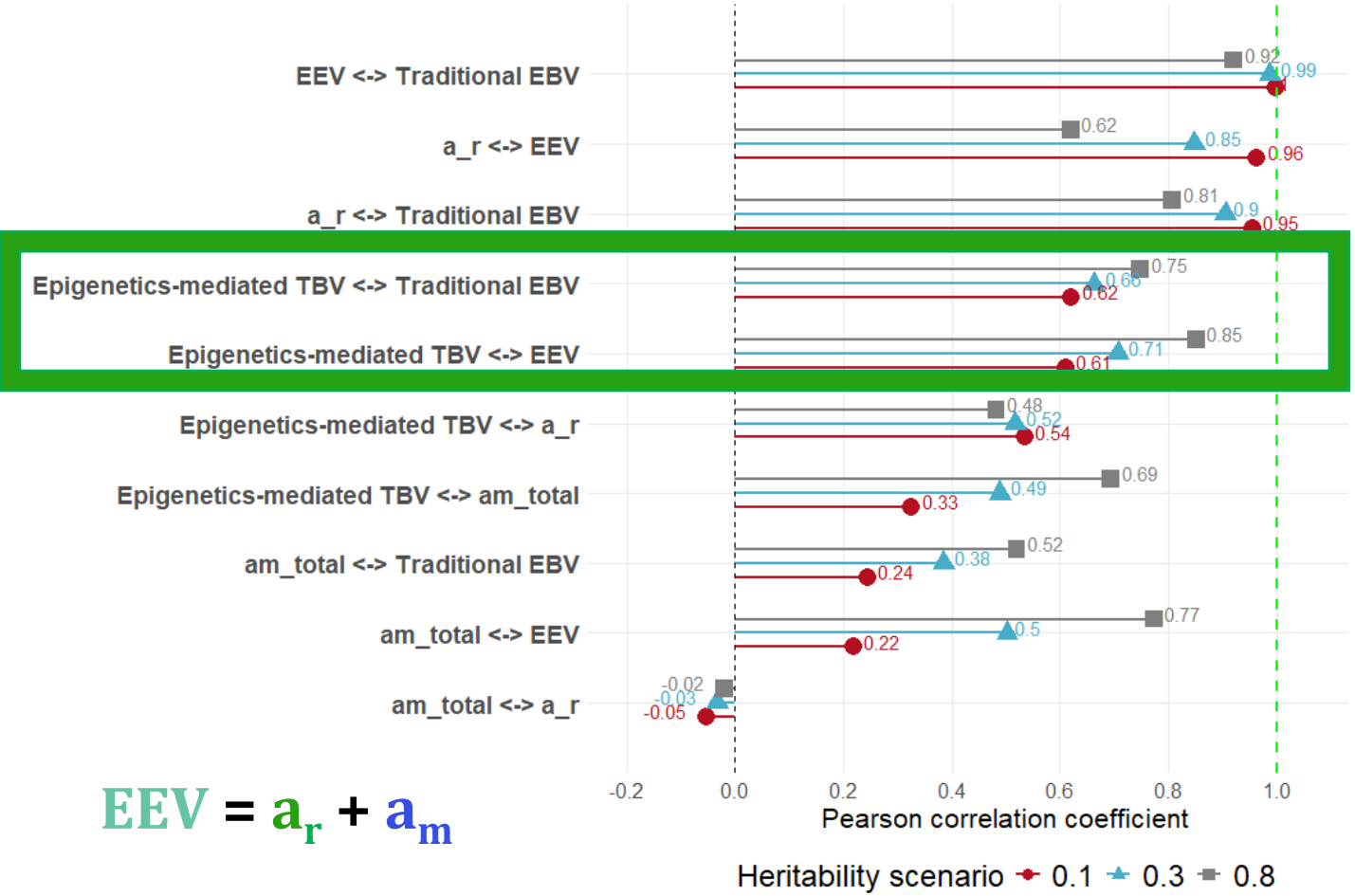


$$EEV = a_r + a_m$$

Large correlation across scenarios between EEV and the traditional EBV

EEV can be decomposed into two different genetic values. Different selection intensity

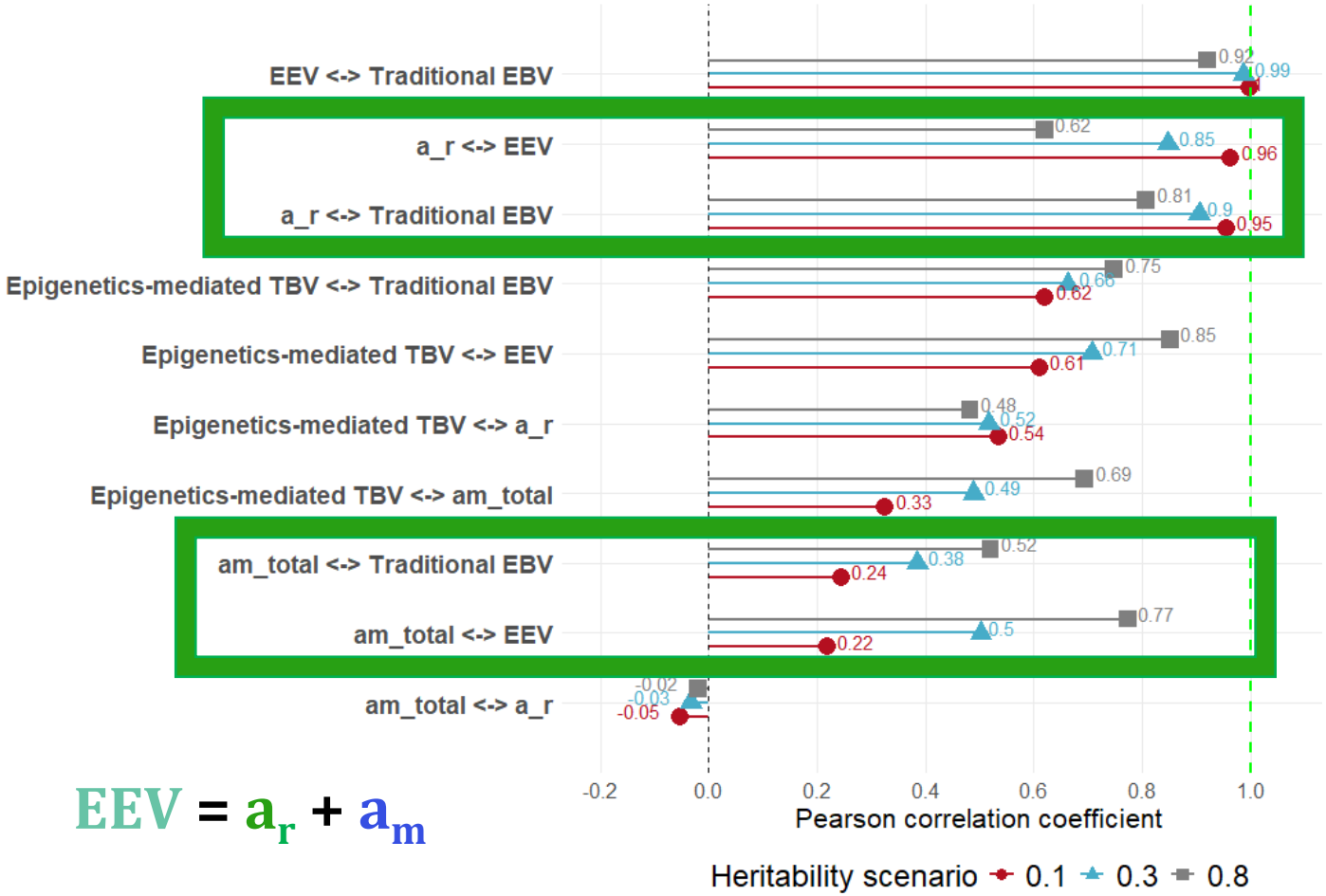
A multi-omics approach – Results



$$EEV = a_r + a_m$$

The EEV showed a stronger correlation with the TBV than EBV, especially when the heritability of liability to methylation was moderate to high. In scenarios with low heritability, correlations between EEV and EBV were similar

A multi-omics approach – Results

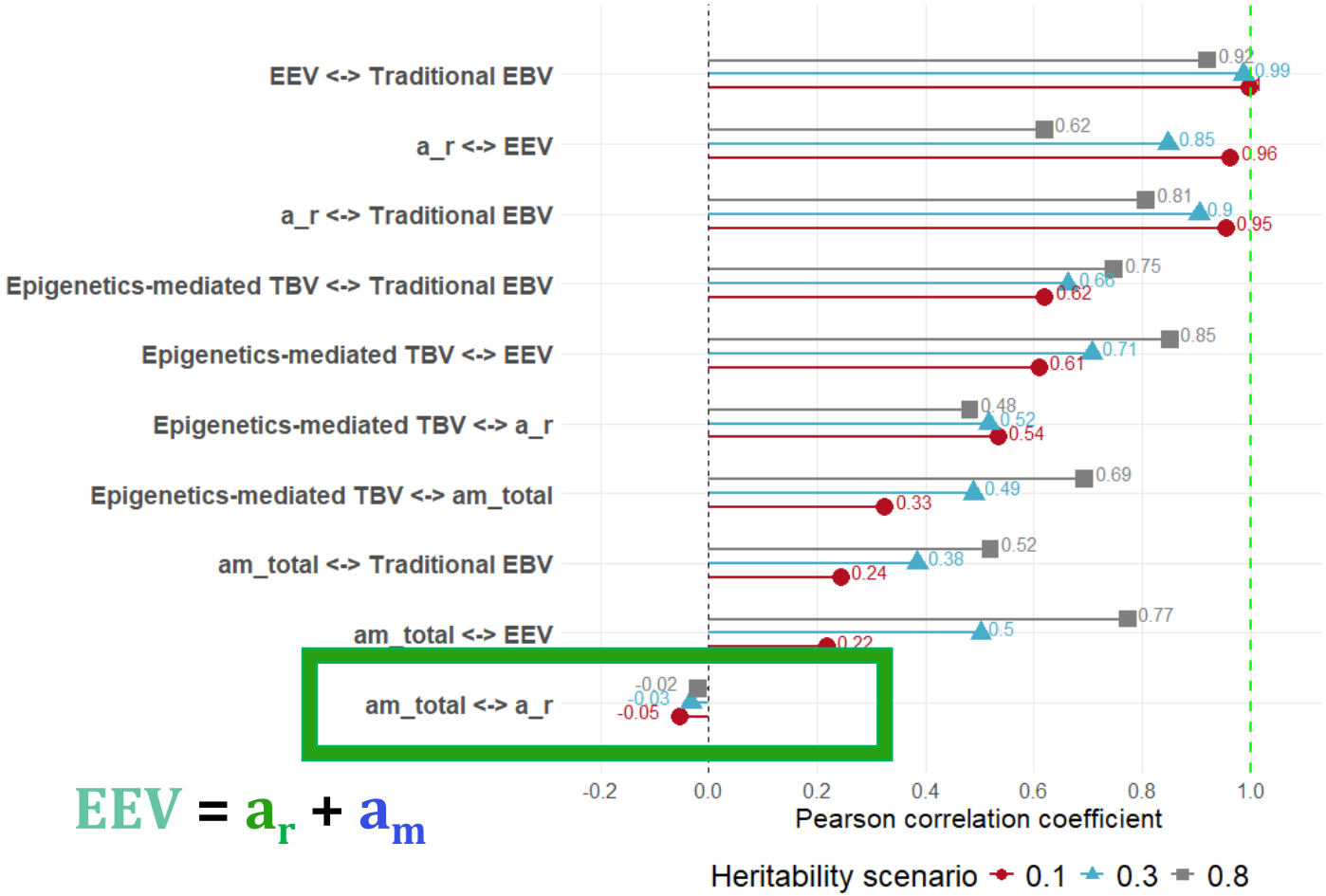


$$EEV = a_r + a_m$$

With higher heritability of liability to methylation, a_m is more correlated with both the EBV and the EEV than a_r

The opposite happens in low heritability scenarios

A multi-omics approach – Results

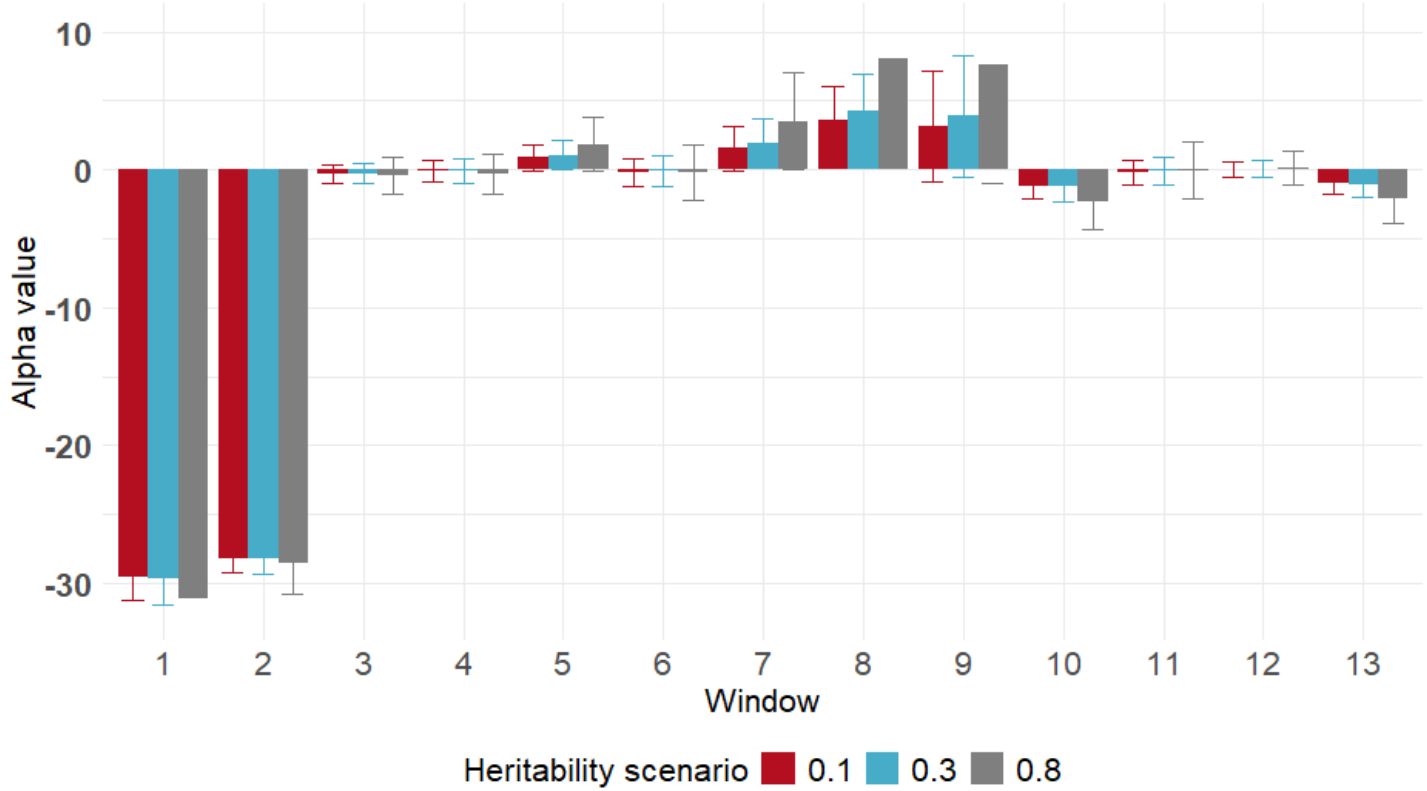


$$EEV = a_r + a_m$$

The correlation between a_r and a_m was approximately zero across all scenarios, indicating that these traits are independent

A multi-omics approach – Results

Effect of the methylation on the phenotype



A multi-omics approach – Limitations

- The data are simulated by simplifying the biological reality. We do not account for methylation in distal intergenic regions
- We have not considered a possible positive effect on the gene body
- We have assumed independence between susceptibility to methylation and environmental methylation

A multi-omics approach – Take home messages

- Traditional EBV and the epigenetic EEV show very high correlations in all three scenarios (0.92-1). However, the epigenetic EEV allows us to select additive and epigenetic effects independently

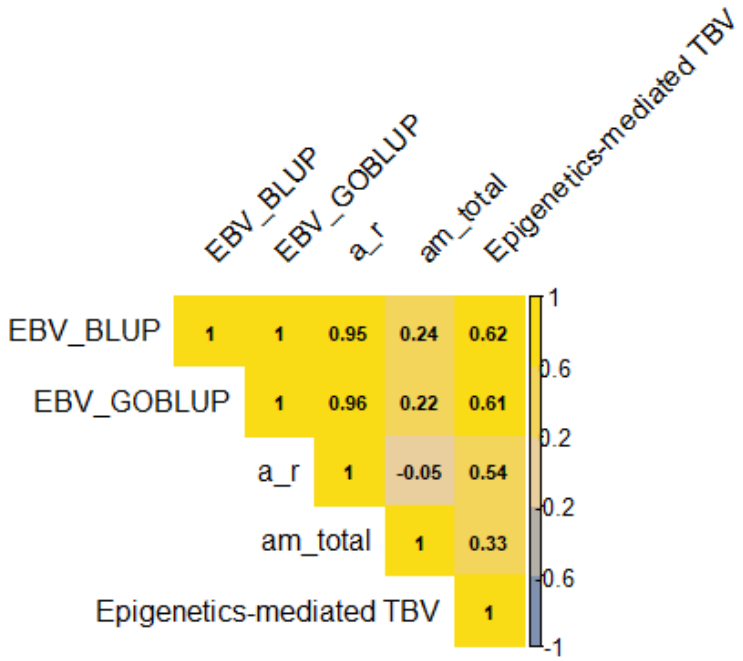
- The variance components recovered for the productive trait of interest was consistent with the simulated values when using GOBLUP. Contrary to the traditional model which captured part of the epigenetic variance as additive

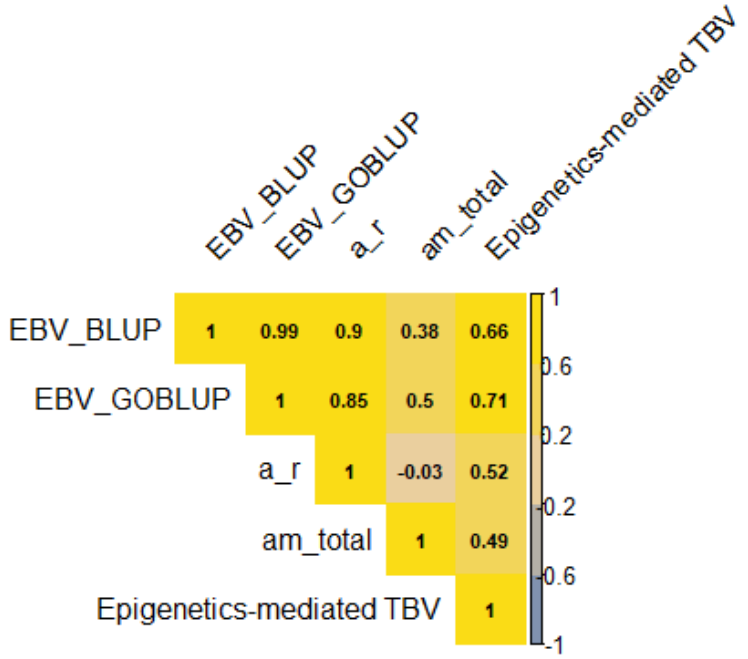
- The missing h^2 is not explained by epigenetics, as it had been captured as an additive effect

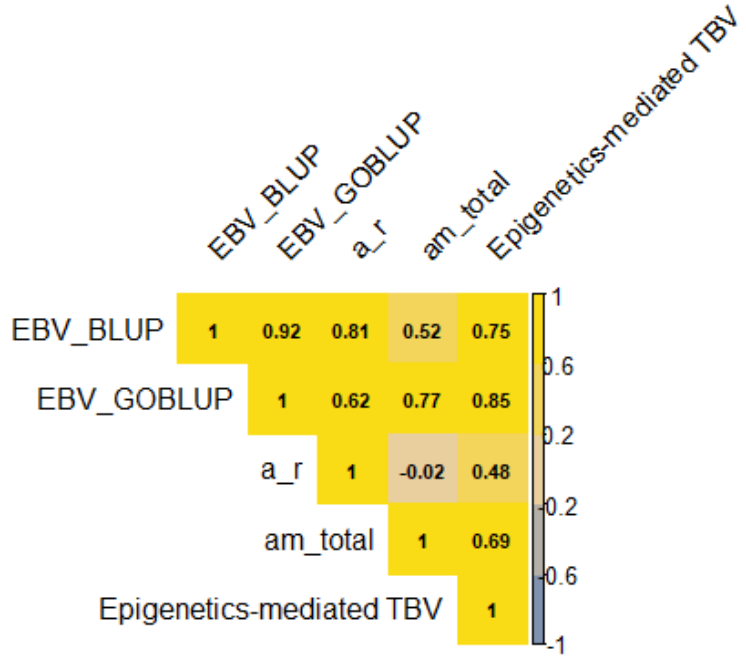
- We are starting to work with epiChips. "Epigenotyping" chips that have been developed jointly with INRAE and QGG. We have just received the epiChips and will have more data to validate these models after the summer. There is a one-year embargo for non-partners

Rumigen

Thank you for your attention!


EBV_BLUP
EBV_GOBLUP
a_r
am_total
Epigenetics-mediated TBV
1 1 0.95 0.24 0.62
1 0.96 0.22 0.61
1 -0.05 0.54
1 0.33
1
1
0.6
0.2
-0.2
-0.6
-1

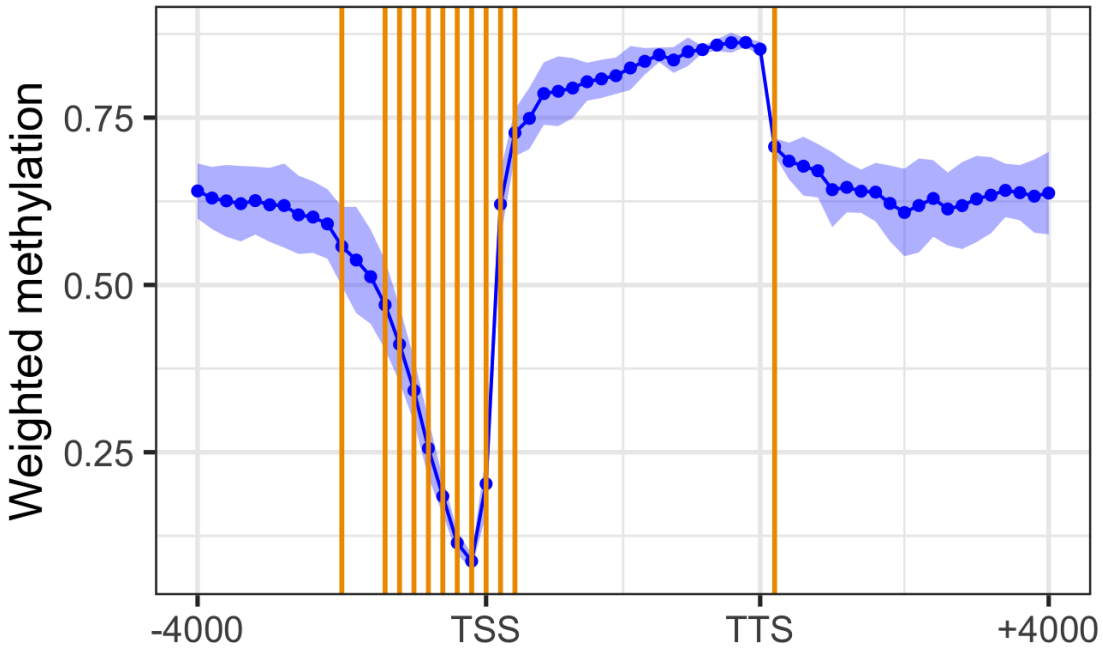

EBV_BLUP
EBV_GOBLUP
a_r
am_total
Epigenetics-mediated TBV
1 0.99 0.9 0.38 0.66
1 0.85 0.5 0.71
1 -0.03 0.52
1 0.49
1
1
0.6
0.2
-0.2
-0.6
-1


EBV_BLUP
EBV_GOBLUP
a_r
am_total
Epigenetics-mediated TBV
1 0.92 0.81 0.52 0.75
1 0.62 0.77 0.85
1 -0.02 0.48
1 0.69
1
1
0.6
0.2
-0.2
-0.6
-1

Una aproximación multiómica – Métodos

Simulación

1. 10,000 SNP effects para un caracter de producción (β) ~ Γ(0.42,5.4) (Hayes and Goddard, 2001)
   - 7,500 en las ventanas 10, 11 and 12 (cuerpo del gen)
   - 2,500 fuera de las ventanas



Una aproximación multiómica – Métodos

2. 10,000 “SNP effects” de metilación (γ) ~ Γ(0.42,5.4)
   - Aleatoriamente distribuidas a lo largo de las 13 ventanas más las regiones intergénicas

3. Simulación del valor genético para susceptibilidad a metilación (mBV)
   - $mBV_i = \sum_{i=1} x_i \cdot \gamma_i$

4. Simulación del fenotipo de “frecuencia de metilación” $y_{meth_{iw}} = \mu_w + mg_{iw} + me_{iw}$:
   - $mg_{iw} = mBV_i * \sqrt{\sigma_w^2 * h_{meth}^2}$ and
   - $me_{iw} = \mathrm{N}(0,1) * \sqrt{\sigma_w^2 * (1 - h_{meth}^2)}$ h_{meth}^2 = 0.1, 0.3 or 0.8

Una aproximación multiómica – Métodos

6. Simulación del valor epigenético (u_{epi})

- $$u_{epi_i} = u_{epi_{core_i}} + u_{epi_{coat_i}}$$

- $$u_{epi_{core_i}} = \sum_{i=1} x_{i,j} * [\beta_j * (\frac{1}{2} y_{meth_{i,1}} + \frac{1}{2} y_{meth_{i,2}})]$$

- $$u_{epi_{coat_i}} = \sum_{i=1} x_{i,j} * \beta_j$$

- $y_{meyh_{i,1}}$ and $y_{meth_{i,2}}$ representan las regiones promotoras, donde la metilación tiene un efecto negative sobre la expresión génica

Una aproximación multiómica – Métodos

7. Simulación del fenotipo :

   - $y_{prod_i} = \mu_{prod} + u_{epi_i} + e$ con $e \sim N(0, \sigma_e^2)$

   - donde σ_e^2 se obtiene de un modelo tradicional para estimar el caracter sin tener en cuenta metilación con una $h^2_{caracter} = 0.3$

8. Implementation of the multiomics model (Ole F Christensen et al., 2021):

   - $\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_m y}_{meth_w}\boldsymbol{\alpha} + \mathbf{Za_r} + \mathbf{e}$
   - $\mathbf{y_{meth}}_w = \mathbf{X_m\beta_m} + \mathbf{Z'g}_{m_w} + \mathbf{e_w}$

EBV_GOBLUP = a_r + a_m

Una aproximación multiómica – Métodos

2. 10,000 “SNP effects” de metilación (γ) ~ Γ(0.42,5.4)
   - Aleatoriamente distribuidas a lo largo de las 13 ventanas más las regiones intergénicas

3. Simulación del valor genético para susceptibilidad a metilación (mBV)
   - $mBV_i = \sum_{i=1} x_i \cdot \gamma_i$

4. Simulación del fenotipo de “frecuencia de metilación” $y_{meth_{iw}} = \mu_w + mg_{iw} + me_{iw}$ where:
   - $mg_{iw} = mBV_i * \sqrt{\sigma_w^2 * h_{meth}^2}$ and
   - $me_{iw} = \mathrm{N}(0,1) * \sqrt{\sigma_w^2 * (1 - h_{meth}^2)}$

h_{meth}^2 = 0.1, 0.3 or 0.8

Una aproximación multiómica – Métodos

6. Simulación del valor epigenético (u_{epi})

- $$u_{epi_i} = u_{epi_{core_i}} + u_{epi_{coat_i}}$$

- $$u_{epi_{core_i}} = \sum_{i=1} x_{i,j} * [\beta_j * (\frac{1}{2} y_{meth_{i,1}} + \frac{1}{2} y_{meth_{i,2}})]$$

- $$u_{epi_{coat_i}} = \sum_{i=1} x_{i,j} * \beta_j$$

- $y_{meyh_{i,1}}$ and $y_{meth_{i,2}}$ representan las regiones promotoras, donde la metilación tiene un efecto negative sobre la expresión génica

Una aproximación multiómica – Métodos

7. Simulación del fenotipo :

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EBV_GOBLUP = a_r + a_m