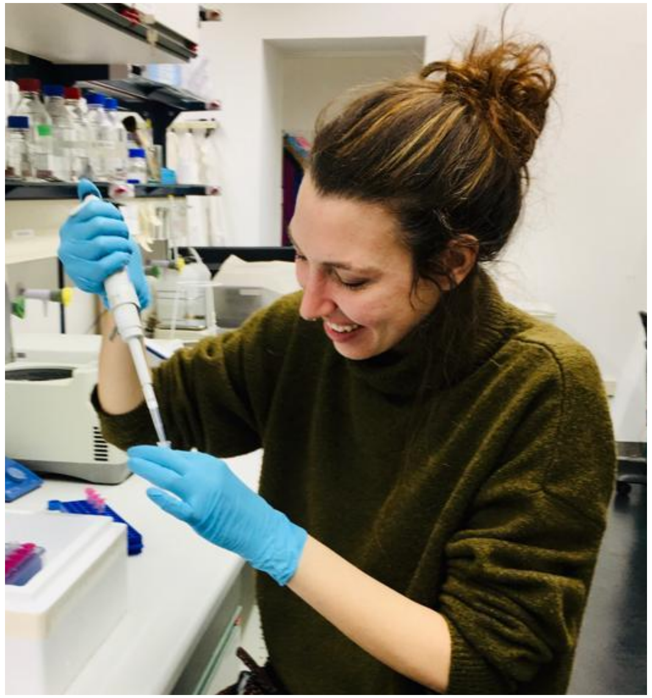
Consiglio Nazionale delle Ricerche

CNR IRET Conference Rome, February 18th-19th, 2025

CNR IRET
Istituto di Ricerca sugli Ecosistemi Terrestri



Intraspecific epigenomics divergence in brown bears (*Ursus arctos*): insights from genome-wide DNA methylation patterns

Laura Gramolini
IRET - Montelibretti

Phenotypic plasticity

Along life cycles

Between individuals from the same species, population or family

Genotype

Environment

Phenotype

+ =

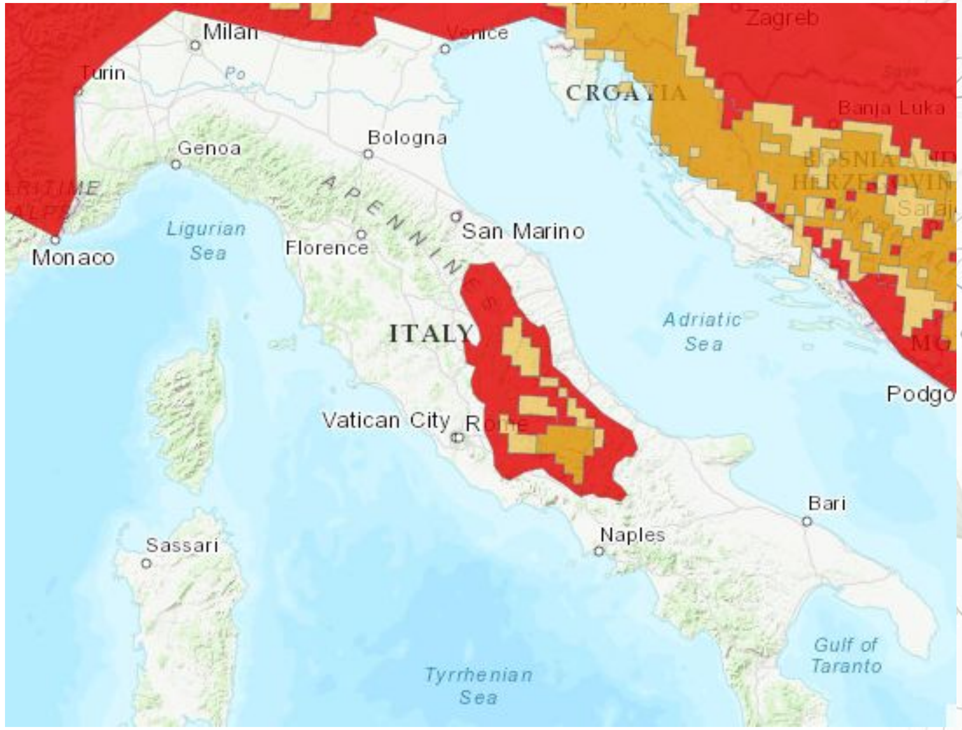
+ =


Marsican bear


Apennine, male


European population


Alps, male


Milan
Venice
Zagreb
Turin
Po
CROATIA
Banja Luka
Genoa
Bologna
Monaco
Ligurian Sea
Florence
APENNINES
San Marino
ITALY
Adriatic Sea
Vatican City
Podgo
Bari
Sassari
Naples
Gulf of Taranto
Tyrrhenian Sea

Differences in:

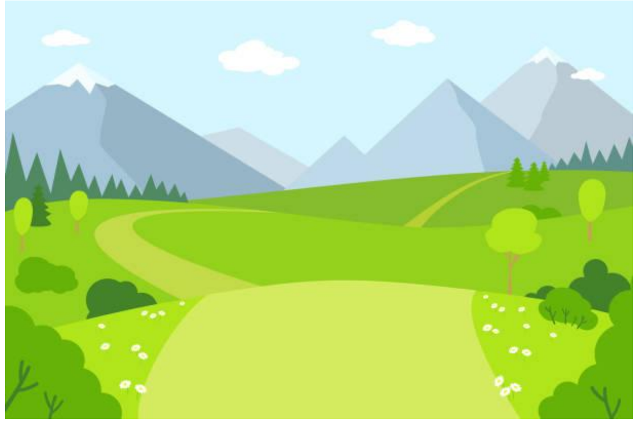
1. skull shape
2. diet
3. behaviour
4. sexual dimorphism
5. colour

Genotype

Environment

Phenotype

+

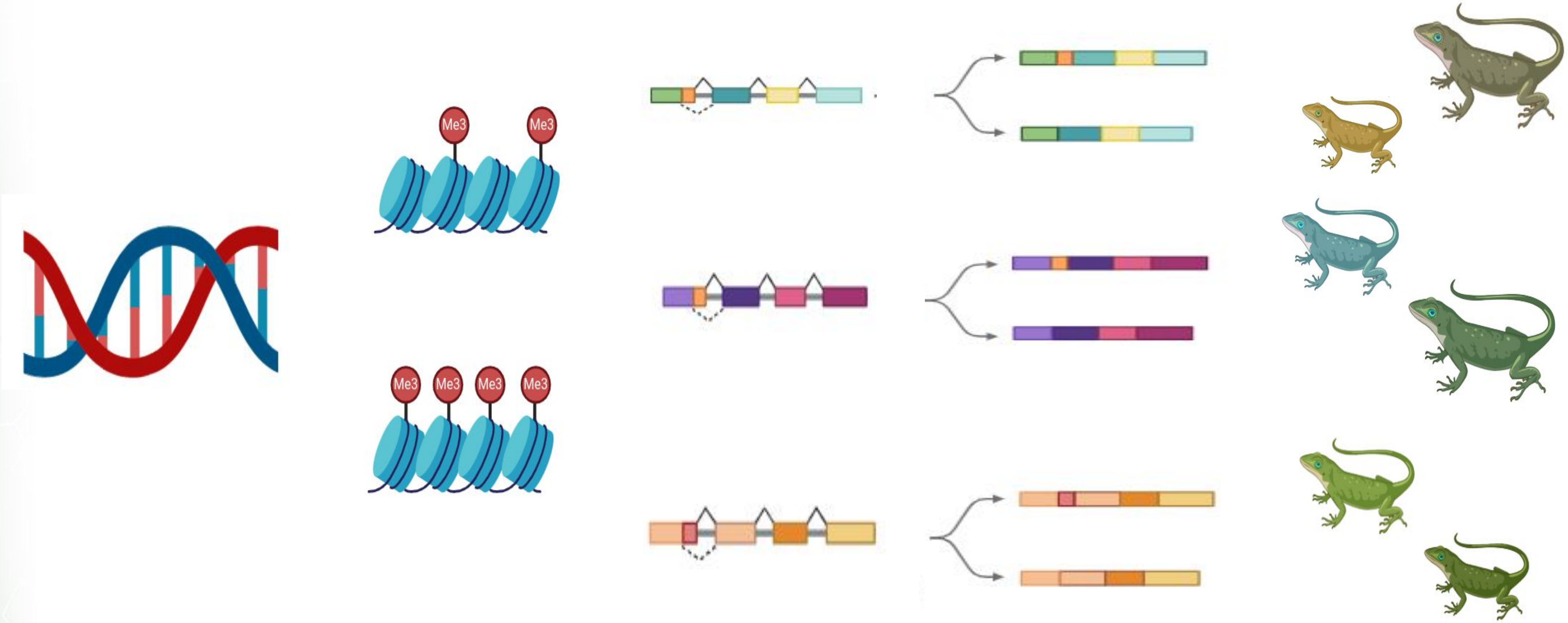


=

+

=

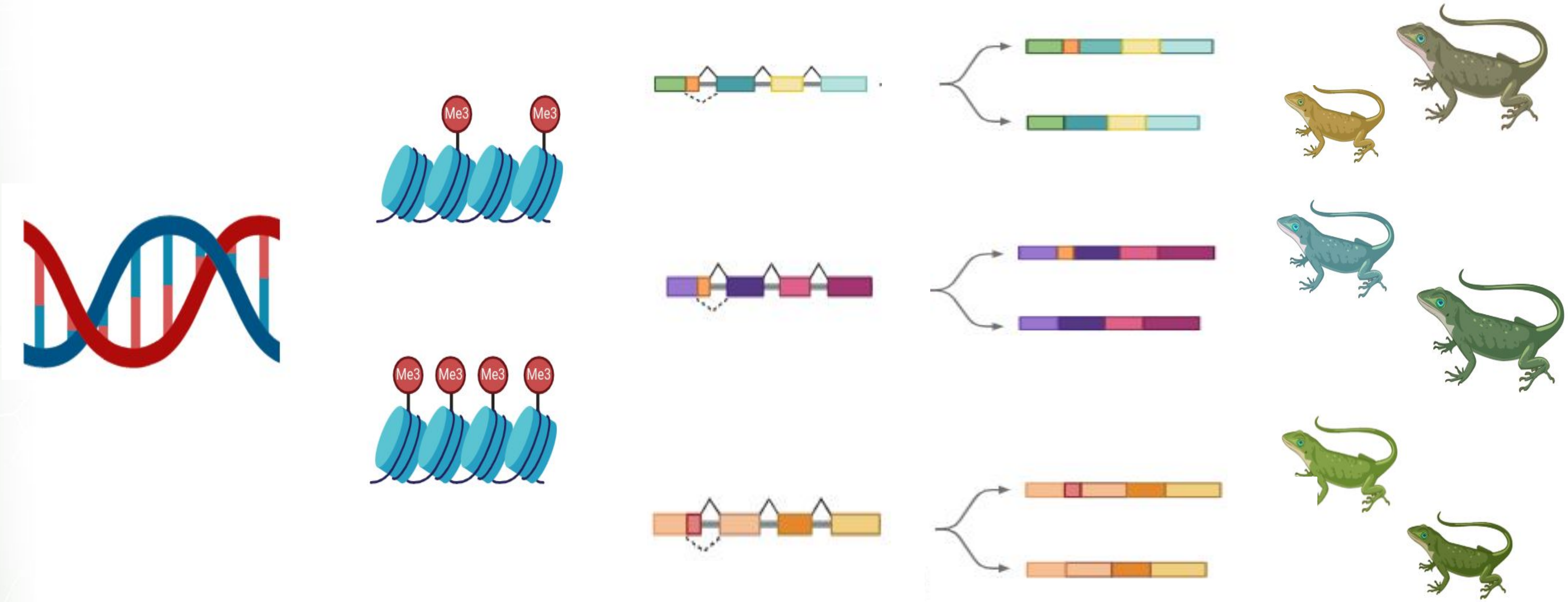
From Genotype → to Phenotype



From Genotype → to Phenotype

Non-genetic variability

From Genotype → to Phenotype

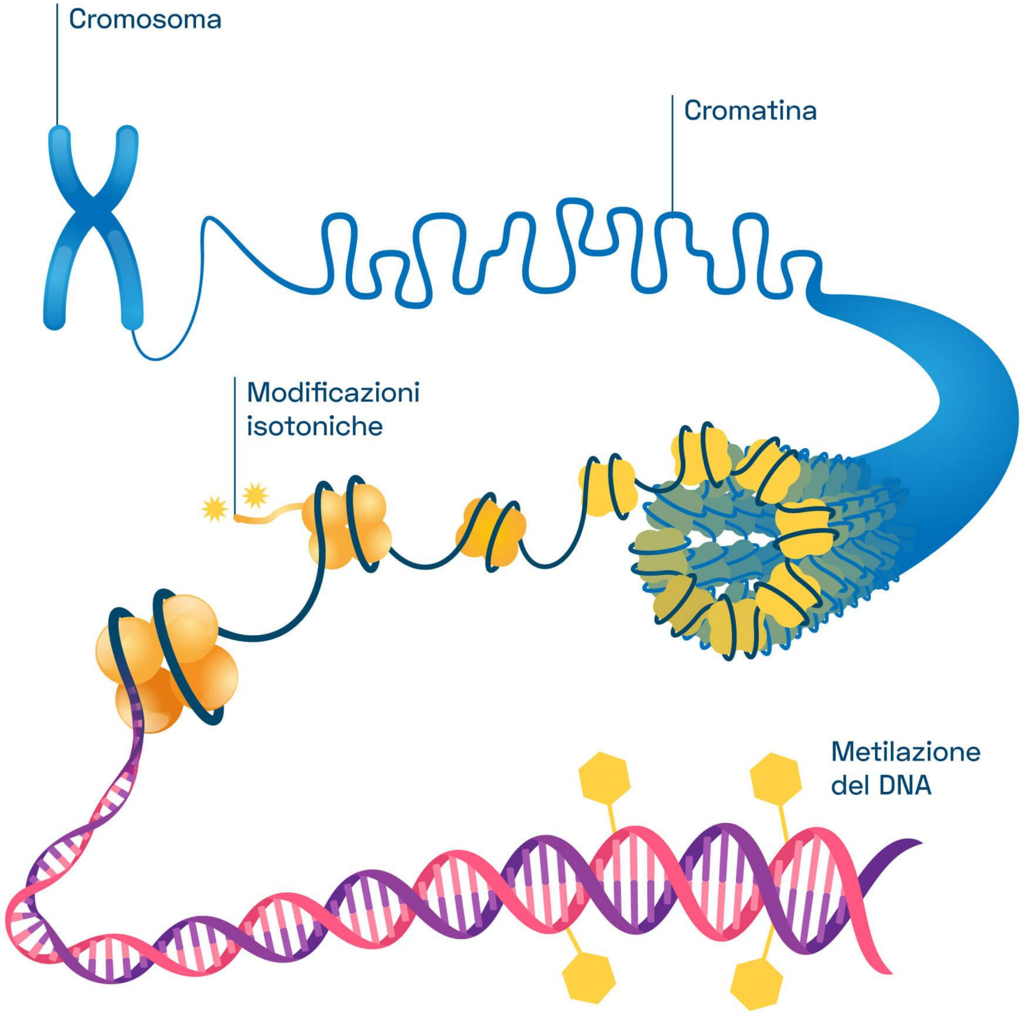


Methylation

Transcription

Alternative splicing

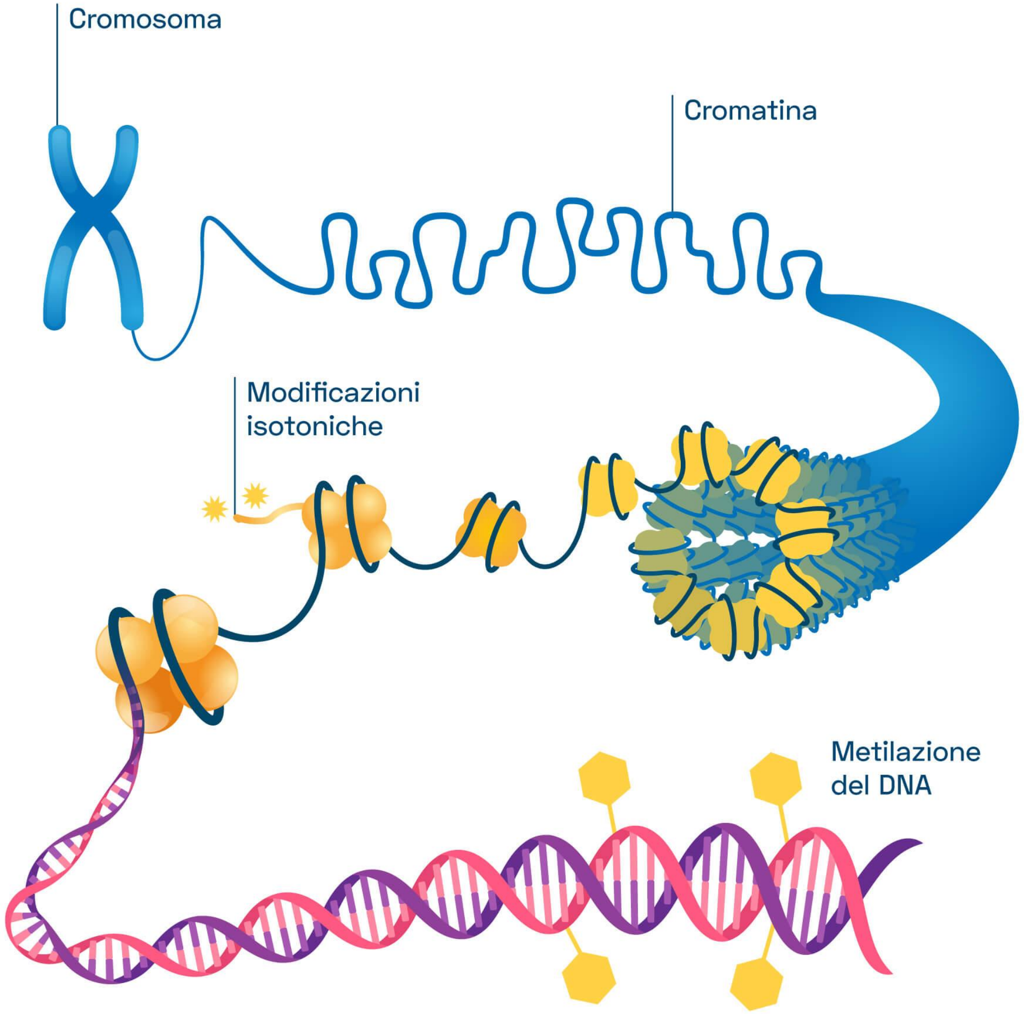
DNA Methylation



CH3 CH3 CH3

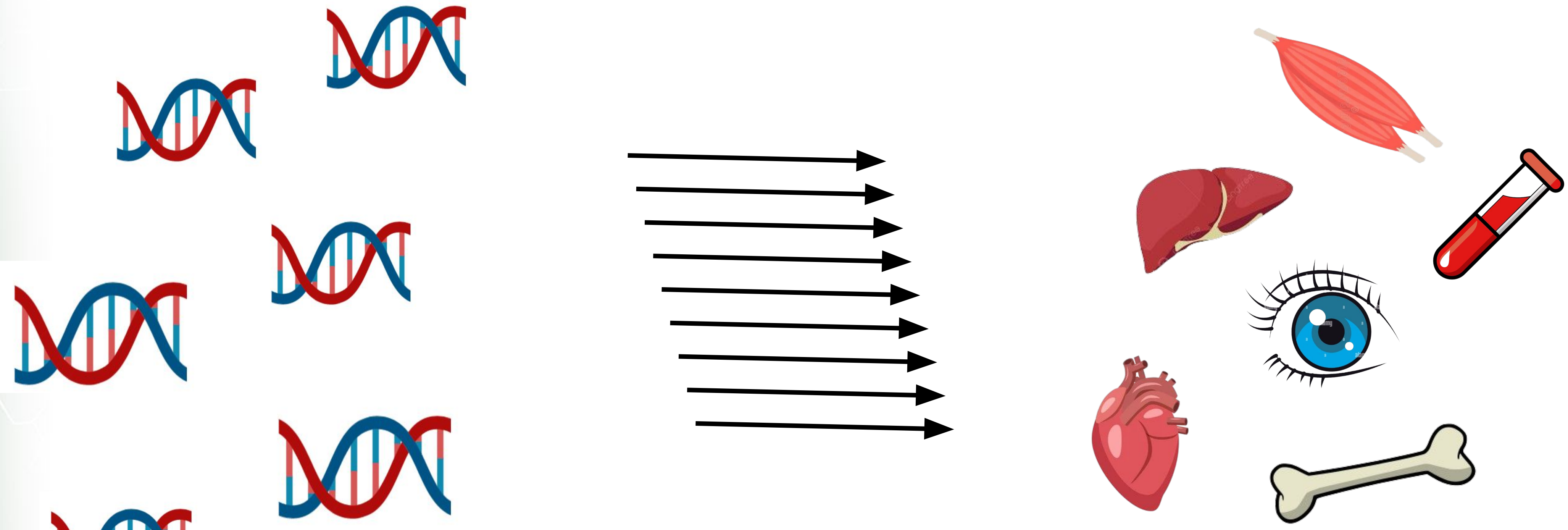
ACCGCTGGATAGCGAGCG

DNA Methylation

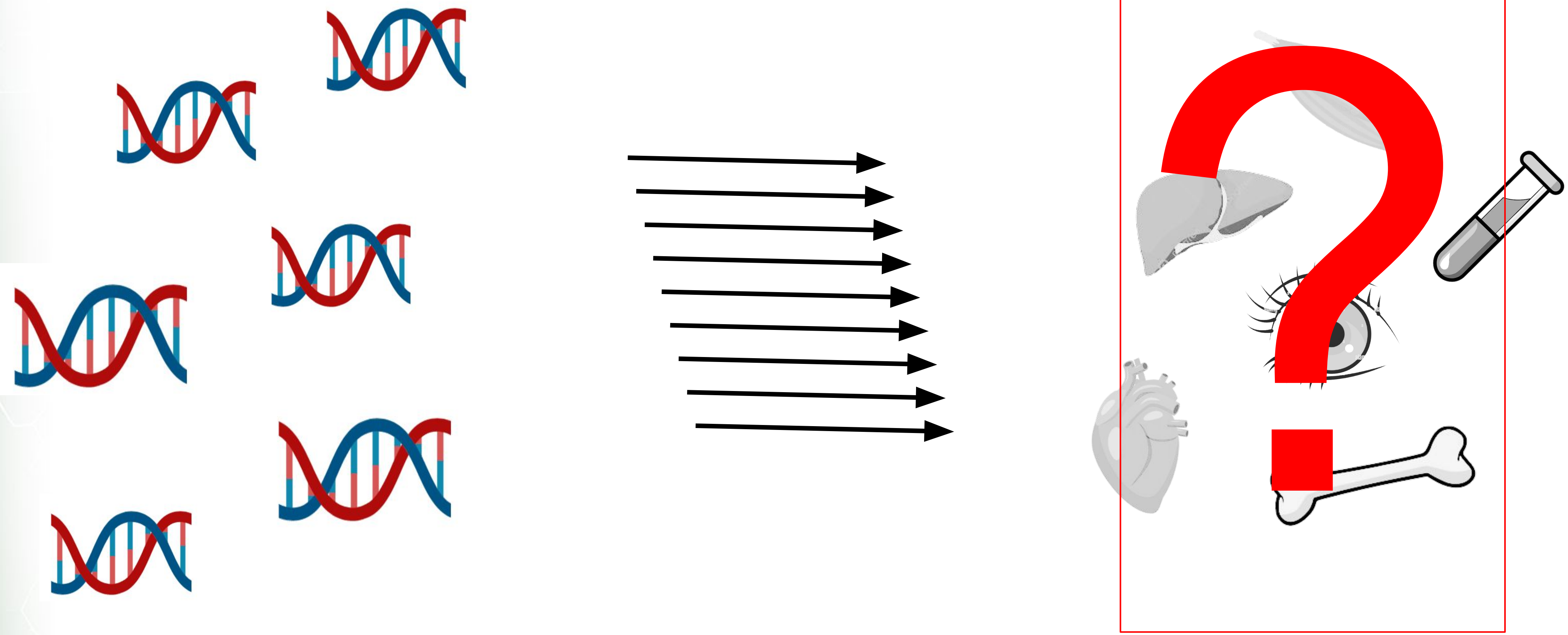


CpG Island

ACCGCTGGATAGCGAGCG



ACCCTGGATAGCAGC



ACCCTGGATAGCAGC

AIM: Investigate the mechanisms driving phenotypic plasticity

1) Do the populations differ in terms of methylation patterns?

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1) Which is the suitable tissue for this kind of analysis in wild organisms?

AIM: Investigate the mechanisms driving phenotypic plasticity

1) Do the populations differ in terms of methylation patterns?

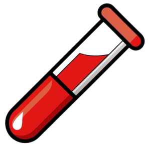
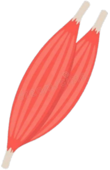
1) Which is the suitable tissue for this kind of analysis in wild organisms?

1) Where do these modifications occur? What are they related to?

Sampling

Laboratory procedures

DNA extraction from two tissues:

Blood  and Muscle 

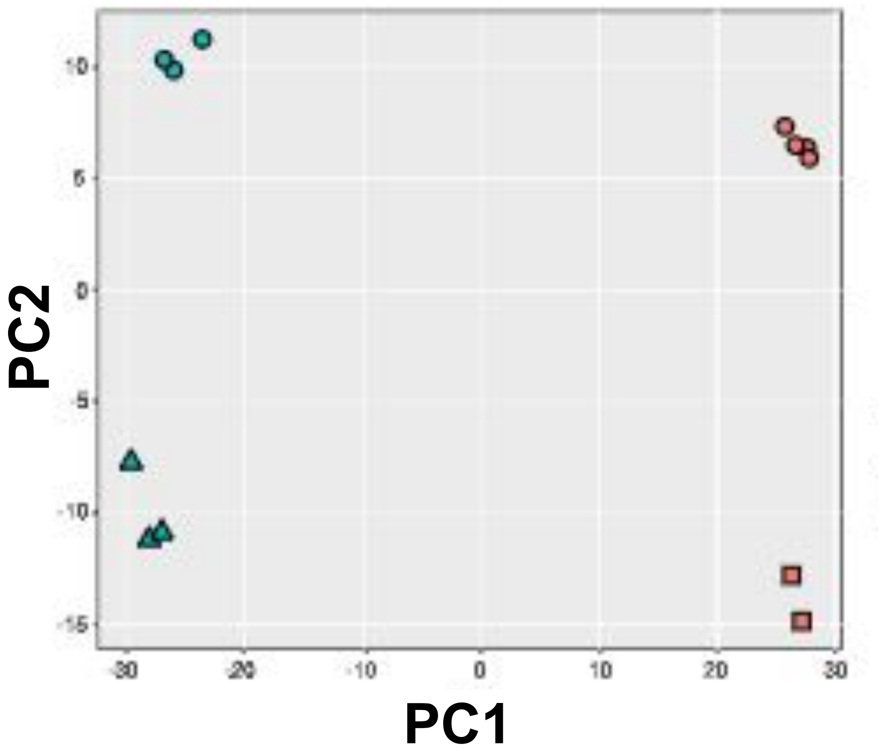
2 X  3 X 

4 X  3 X 

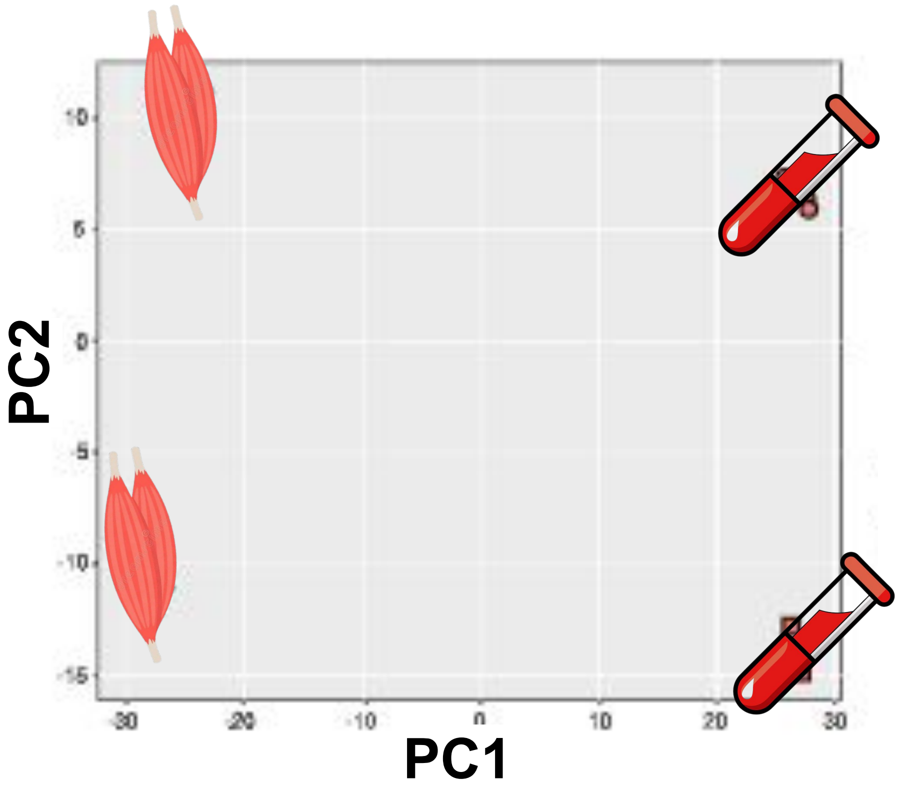
Reduced Representation Bisulfite Sequencing (RRBS)

Data analysis

Results 1) Do the populations differ in terms of methylation patterns?



Results 1) Do the populations differ in terms of methylation patterns?



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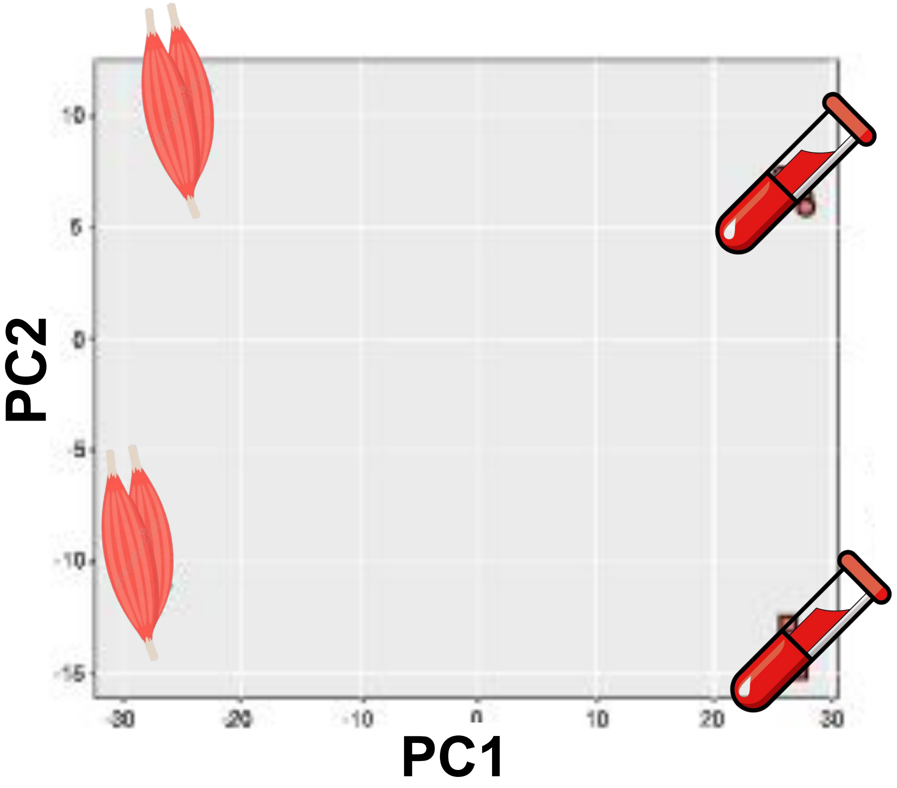
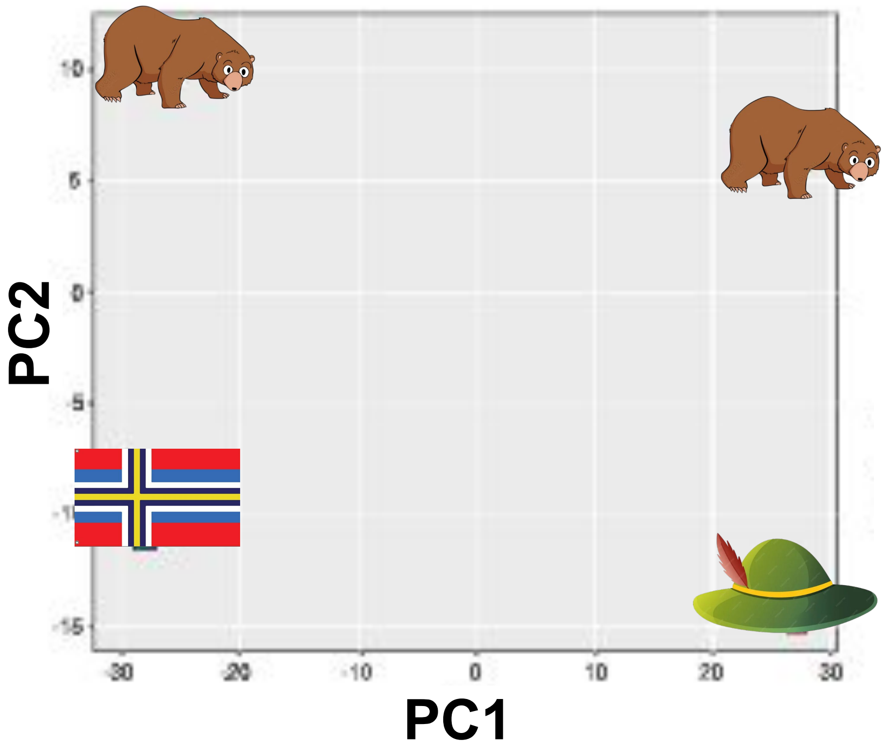
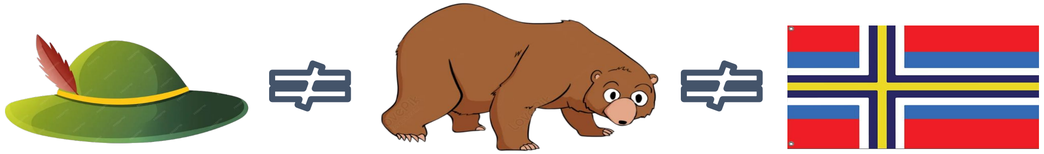


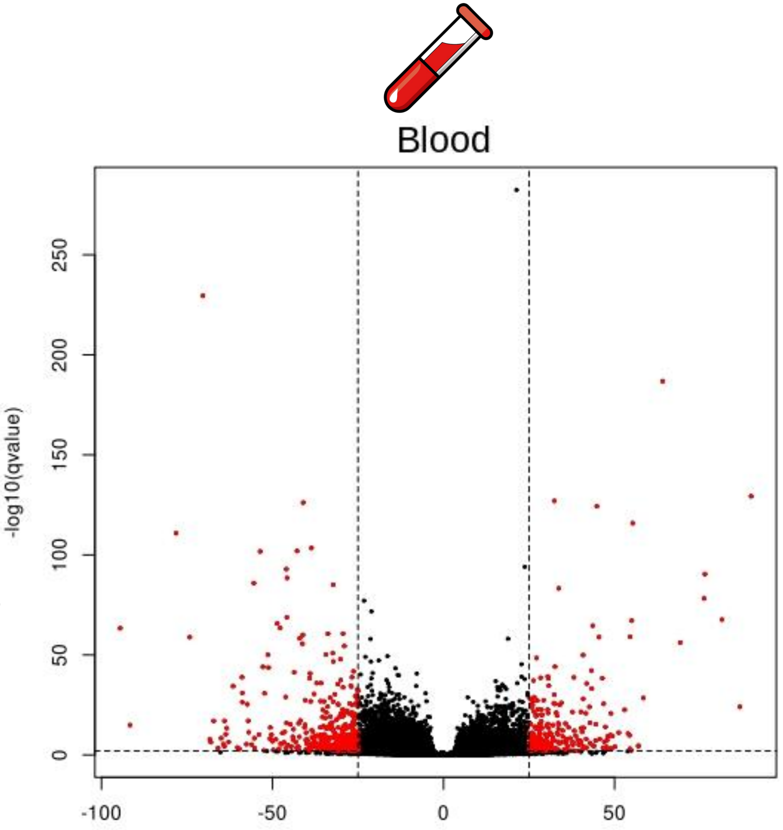


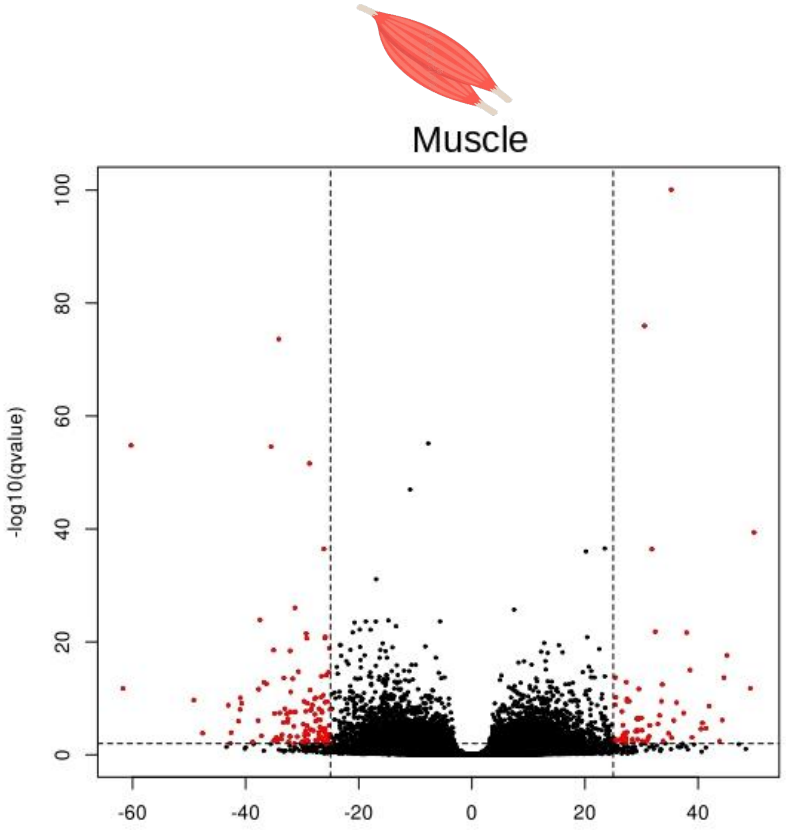
Discussion

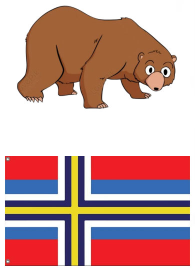
1) Marsican brown bears can be distinguished by the methylation patterns



Results 2) Which is the suitable tissue for this kind of analysis?







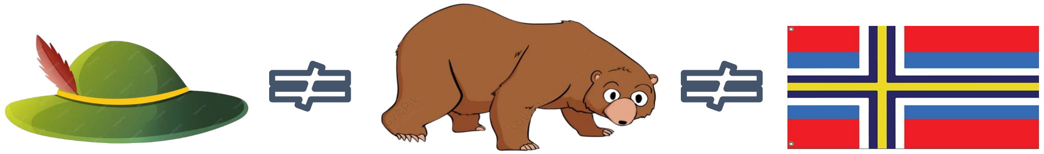
Hypo ⟷ Hyper

Hypo ⟷ Hyper

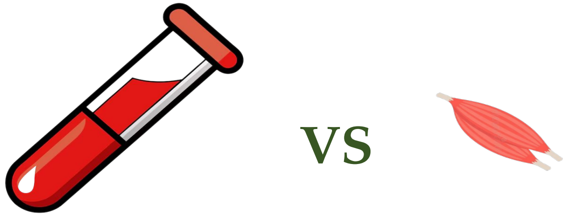
Methylation

Discussion

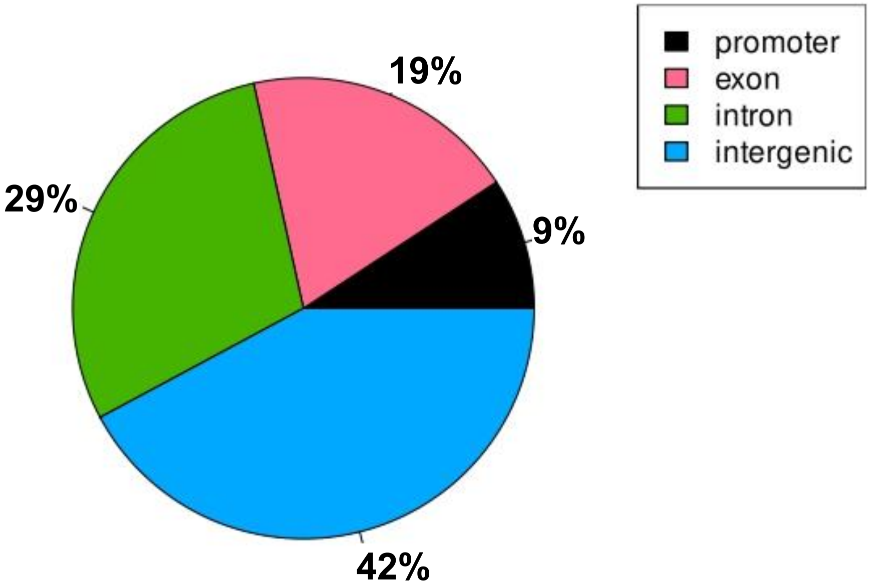
1) Marsican brown bears can be distinguished by the methylation patterns

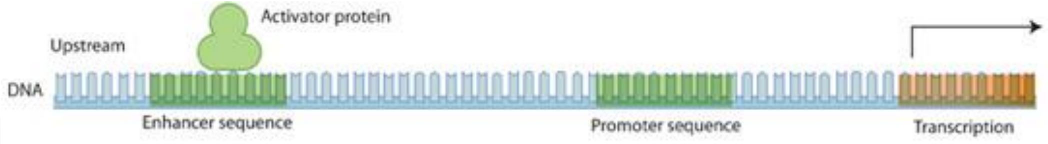


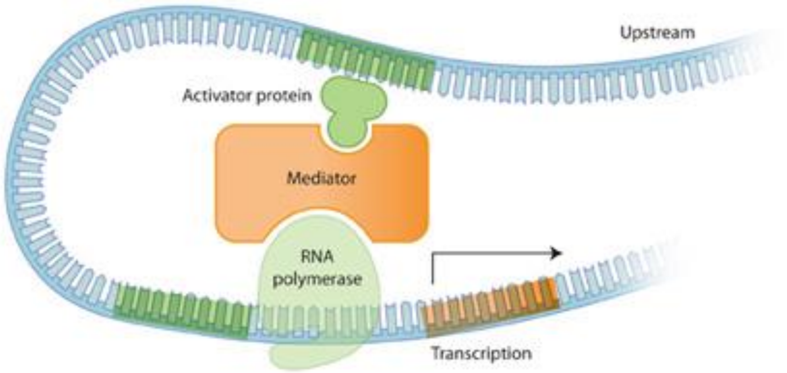
2) Blood is a suitable tissue for this analysis and this organism



Results 3) Where do these modifications occur? What are they related to?





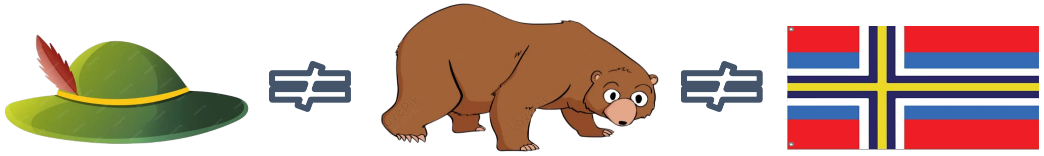


Results 3) Where do these modifications occur? What are they related to?

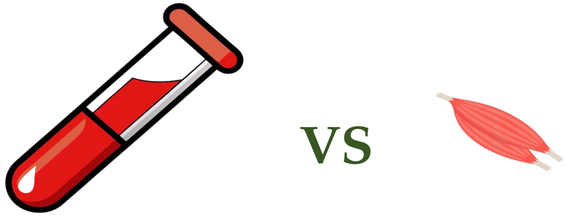
- **Developmental process:** Bone Morphogenetic Protein 7 (**BMP7**) and Homeobox B3 (**HOXB3**)
- **Metabolic pathways**: Galanin Receptor 3 (**GALR3**), Arachidonate 15-Lipoxygenase (**ALOX15**) and LDL receptor related protein 5
- **Immune Response**: Colony Stimulating Factor 3 (**CSF3**), Class II Major Histocompatibility Complex Transactivator (**CIITA**), DLA class I histocompatibility antigen, and Interferon Regulatory Factor 7 (**IRF7**)

Discussion

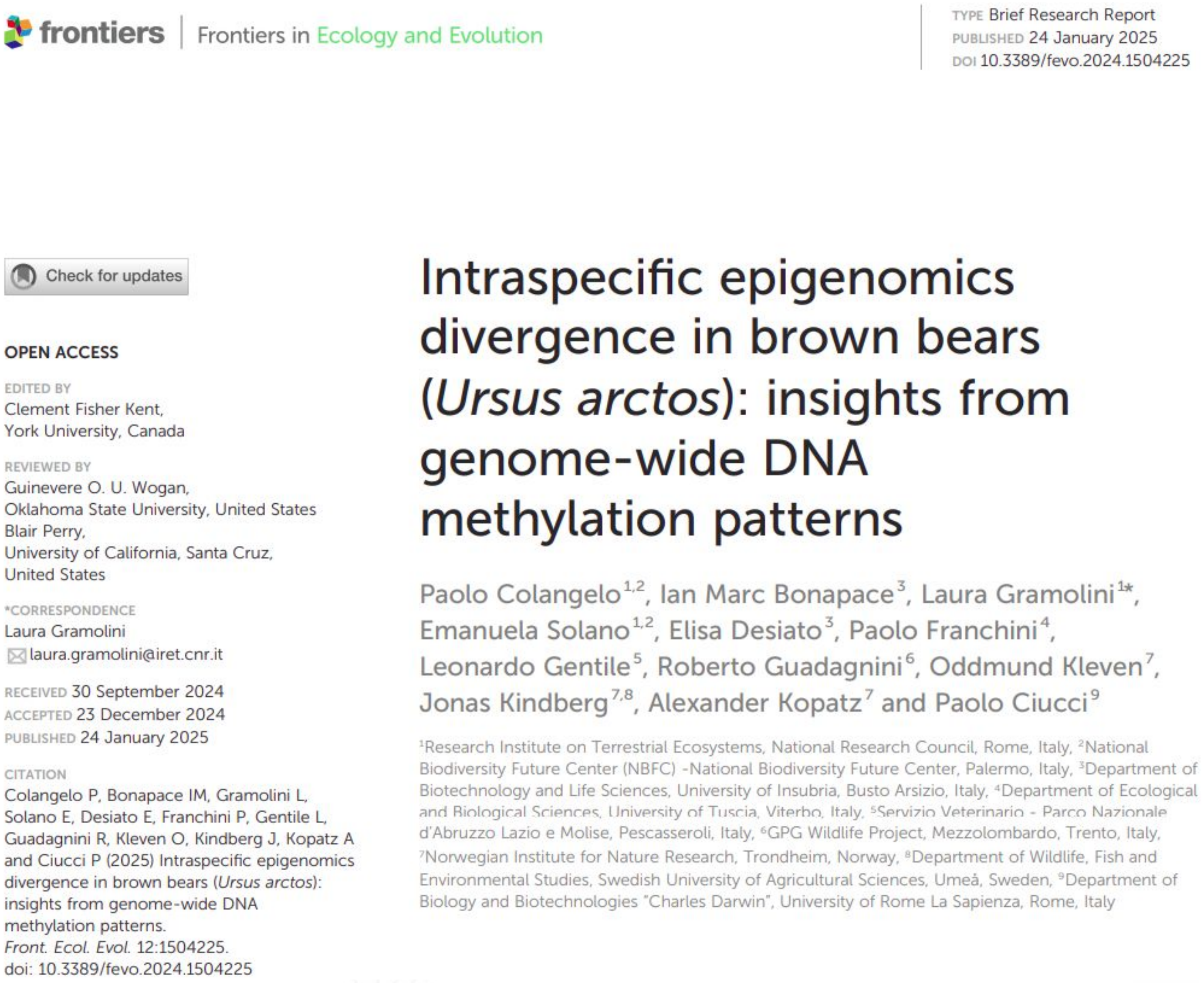
1) Marsican brown bears can be distinguished by the methylation patterns



2) Blood is a suitable tissue for this analysis and this organism



3) Differences in the regulation of developmental processes are consistent with the emergence of novel phenotypes



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Intraspecific epigenomics divergence in brown bears (*Ursus arctos*): insights from genome-wide DNA methylation patterns

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Thank you!

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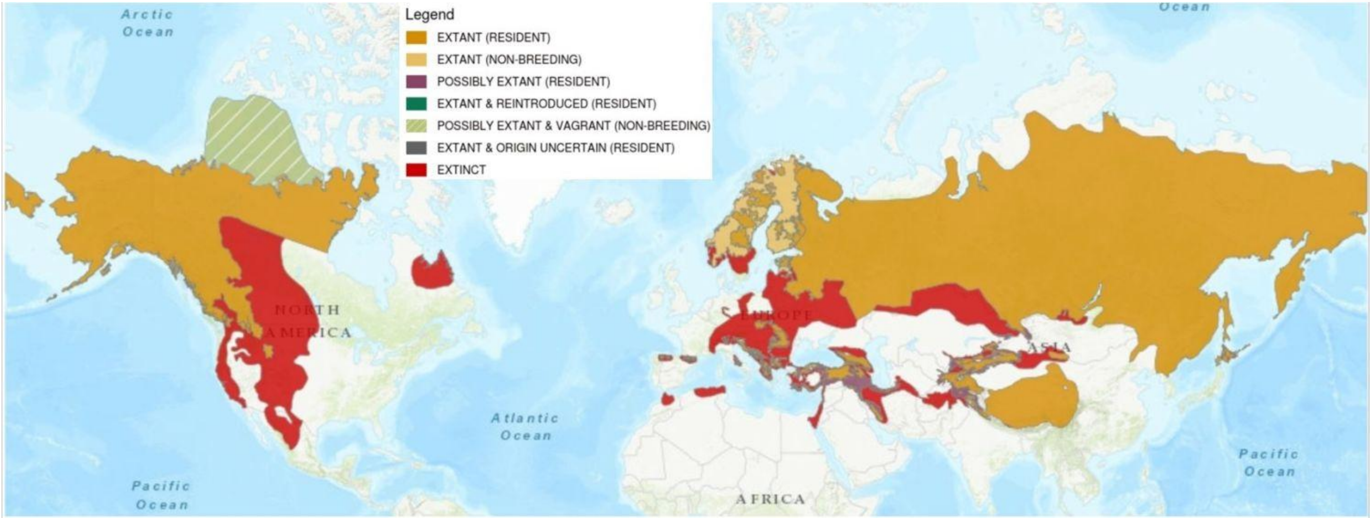
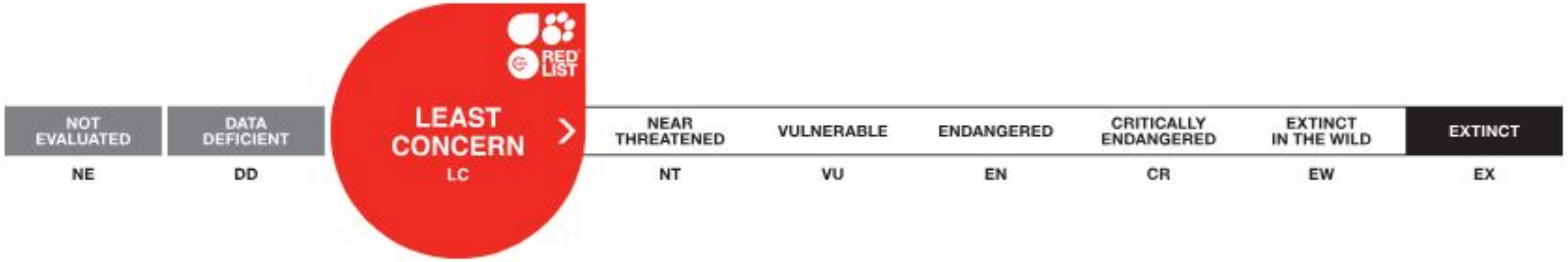
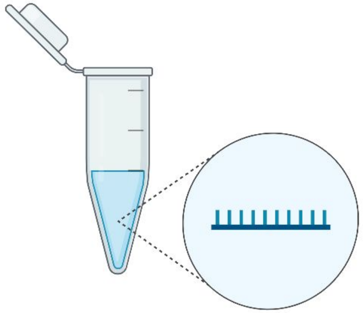


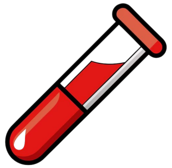



Arctic Ocean
Legend
EXTANT (RESIDENT)
EXTANT (NON-BREEDING)
POSSIBLY EXTANT (RESIDENT)
EXTANT & REINTRODUCED (RESIDENT)
POSSIBLY EXTANT & VAGRANT (NON-BREEDING)
EXTANT & ORIGIN UNCERTAIN (RESIDENT)
EXTINCT
Ocean
NORTH AMERICA
EUROPE
ASIA
Atlantic Ocean
Pacific Ocean
AFRICA
Pacific Ocean

Laboratory analyses

DNA extraction from two tissues:



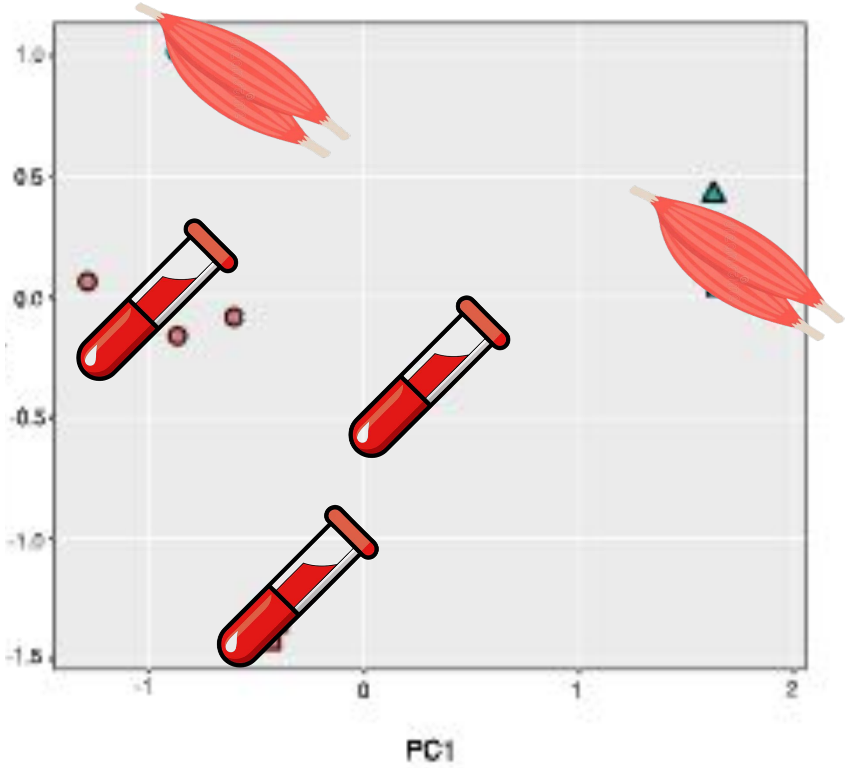
Blood  and Muscle 

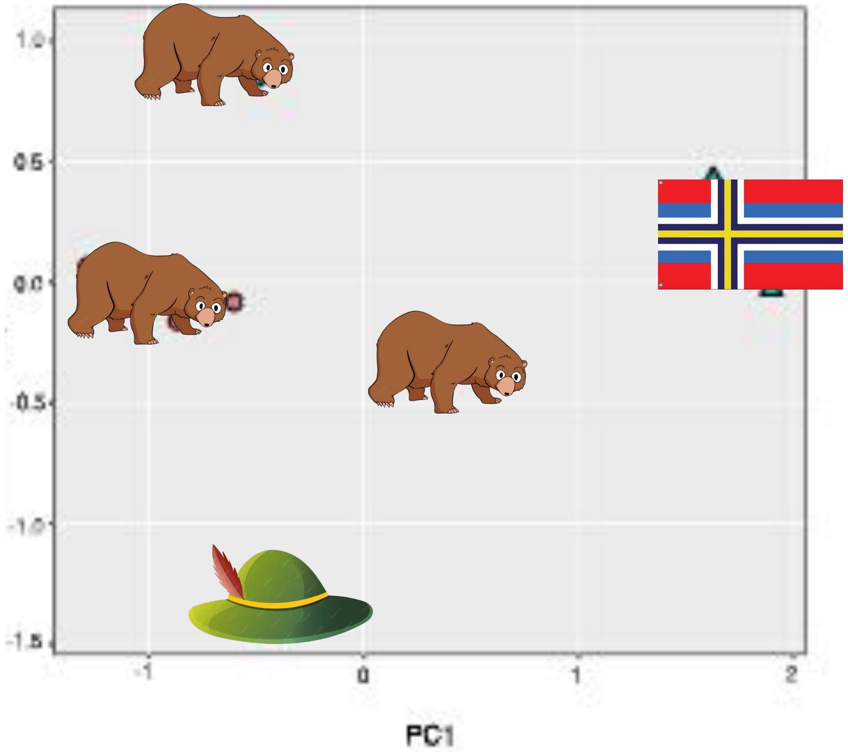
Normal genome sequencing: **ACCCTGGATAGCAGC**

Reduced Representation Bisulfite Sequencing (RRBS):
When a **C** is not methylated it is sequenced as **Uracile** (U).

ACUCTGGATAGUAGU

Results (1): Chromosome X



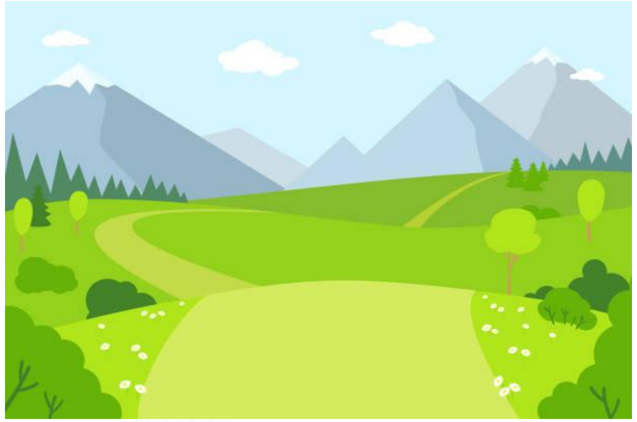


Genotype

Environment

+

+



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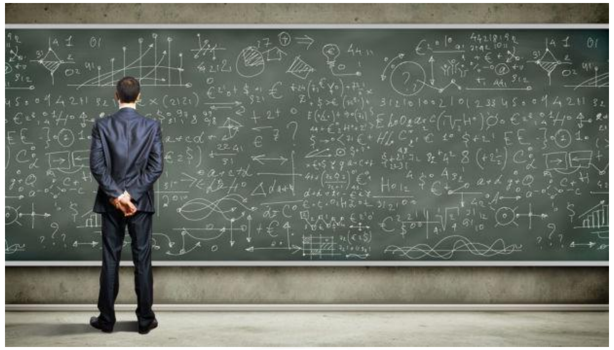
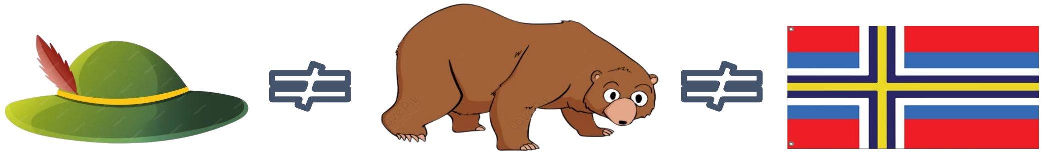


Discussion

1) Populations can be distinguished by looking at the methylations patterns



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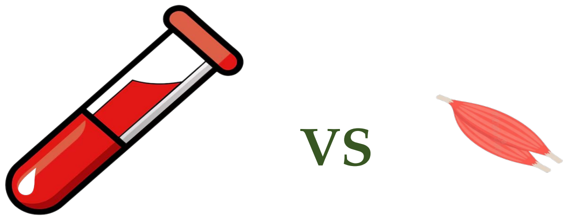

3) Developmental processes are the more diff methylated and the regulative regions