

EPIGENETICS IN EVOLUTIONARY ECOLOGY

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2007 – 2017

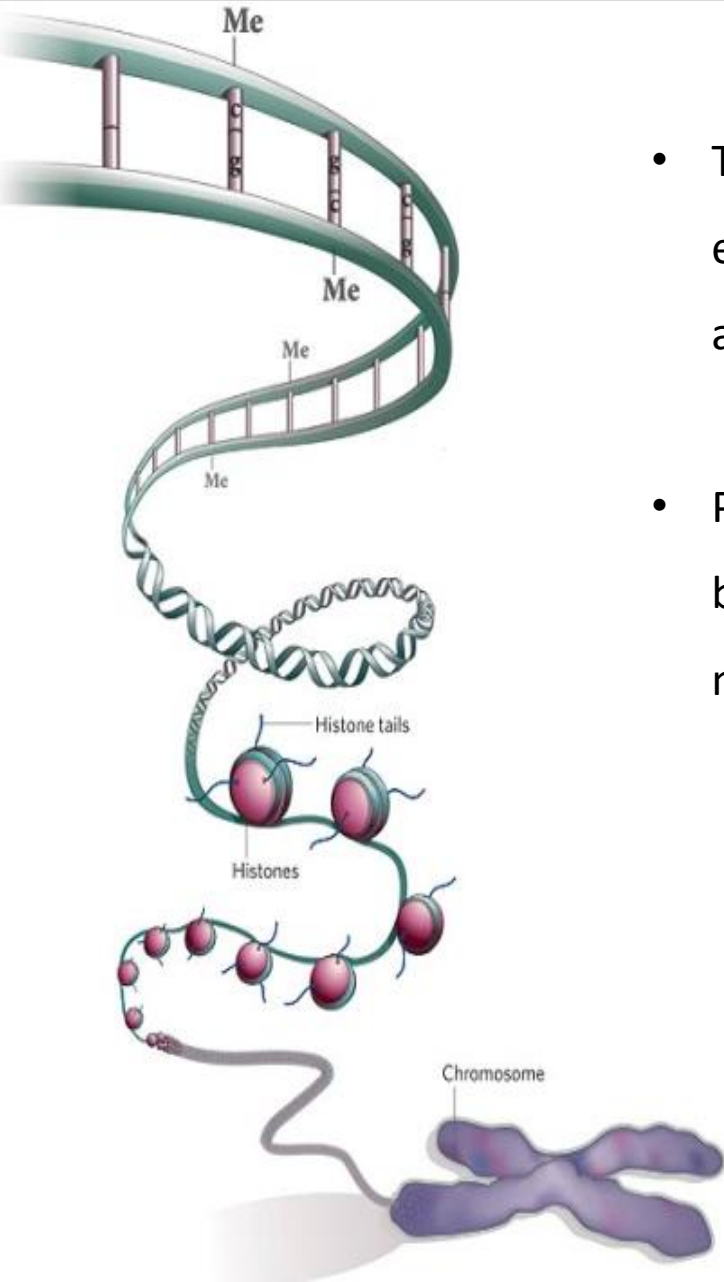
- **Water and soil pollution** – effects on invertebrates and vertebrates at different level of biological organisation (molecular to population level)
- Effects of toxicants on genotoxicity, genetic and epigenetic structure of organisms

University of Innsbruck, Institute of Zoology

2015 – 2016; 2019 – today

- **Soil pollution** – effects on earthworms at the molecular and cell level
- Effects at the epigenetic level (DNA methylation)

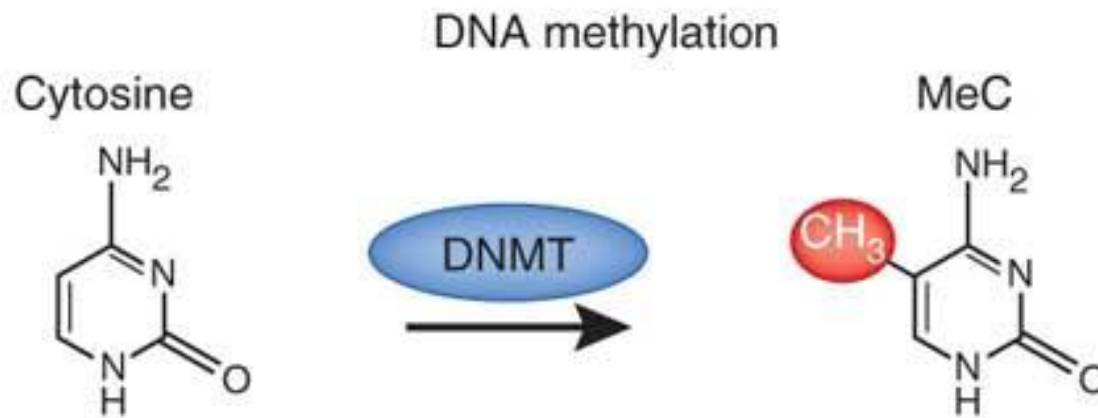
EPIGENETICS



- The Greek prefix epi- (ἐπι- "over, outside of, around") in epigenetics implies features that are "on top of" or "in addition to" the traditional genetic basis for inheritance
 - Phenomena and mechanisms that cause chromosome bound, heritable changes to gene expression that are not dependent on changes to DNA sequence
- | | | |
|--|--|------------------------------------|
| <ul style="list-style-type: none">• <u>DNA methylation</u>• Modifications of histones• Small non-coding RNA's | | Interplay between epigenetic marks |
|--|--|------------------------------------|

DNA methylation

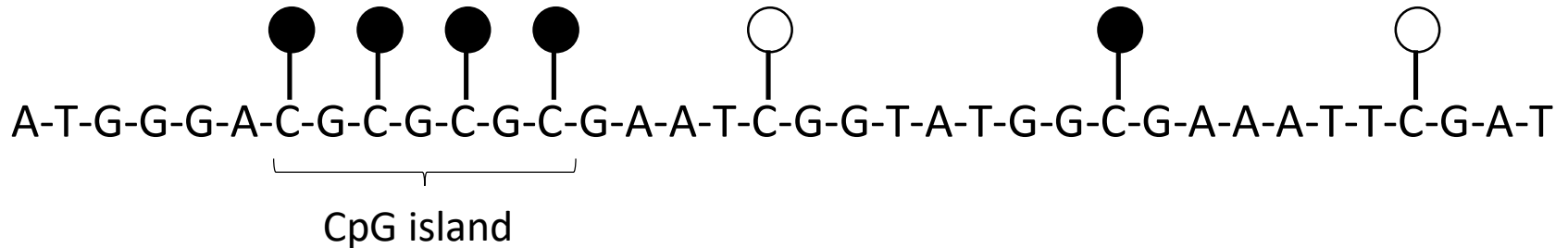
DNA methylation - addition of methyl group (CH_3) to the pyrimidine ring of cytosine



DNA methylation – affects the coiling of DNA around histones and in this way affects the accessibility of transcription factors to bind to the DNA

DNA methylation

DNA methylation – in animals occurs (mostly) within CG dinucleotides (**CpG islands**)

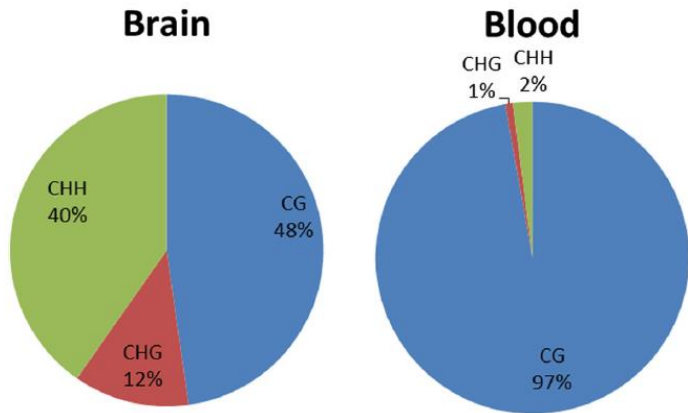
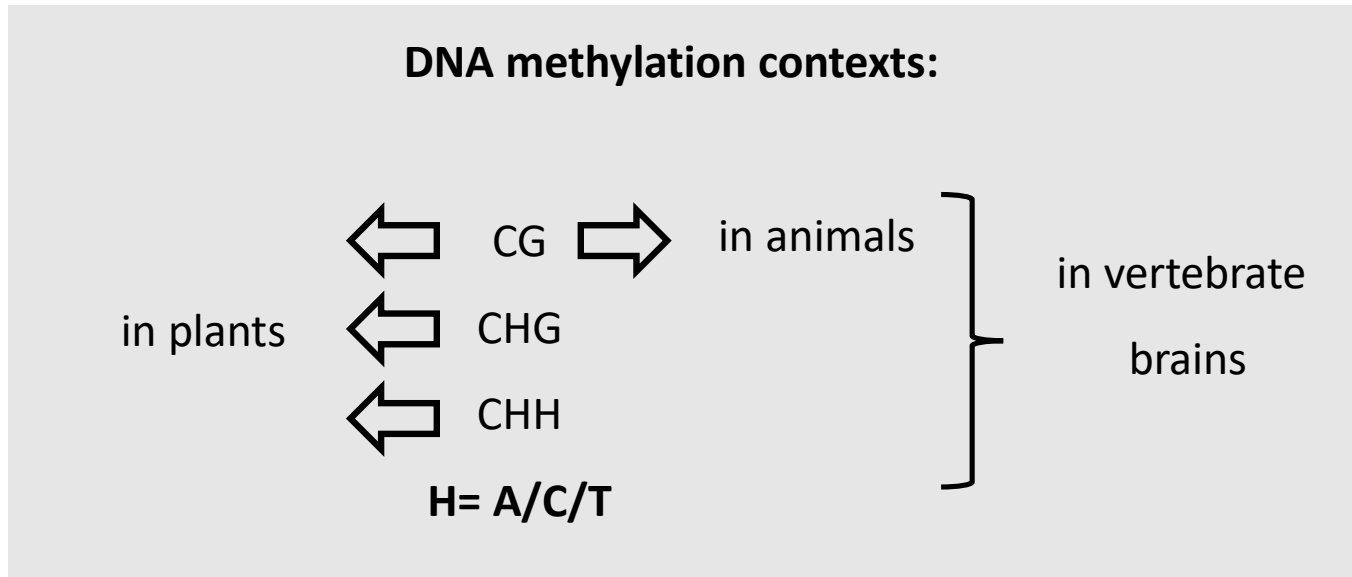


unmethylated C



methylated C

DNA methylation



- **non-CpG methylation is involved in TE silencing and perhaps is the dominant regulatory form of methylation in the brain**

DNA methylation

Vertebrates

- **global pattern of DNA methylation**
- **60-70%** of CpG's are methylated – promoters, gene bodies
- transcriptional regulation (silencing of gene expression by promotor methylation)

Invertebrates

- **mosaic pattern of DNA methylation** – gene body methylation (introns and exons)
- no methylation to intermediate methylation levels (**~15%**)
- regulation of transcriptional activity, alternative exon splicing, reduction of the transcriptional elongation efficiency

DNA methylation – regulation

Genes involved in DNA methylation/demethylation

DNA methyltransferase – DNA methylation

Dnmt1 – maintenance methyltransferase, recognizes hemimethylated DNA and completes the symmetrical methylation on replicated DNA

Dnmt3 – de-novo methylation, establishes new methylation marks on unmethylated DNA

Ten-eleven Translocation – DNA de-methylation

TET 1-5

TET enzymes mediate DNA demethylation by oxidizing 5-methylcytosine (5mC) in DNA to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC).

DNA methylation – detection methods

Global DNA methylation:

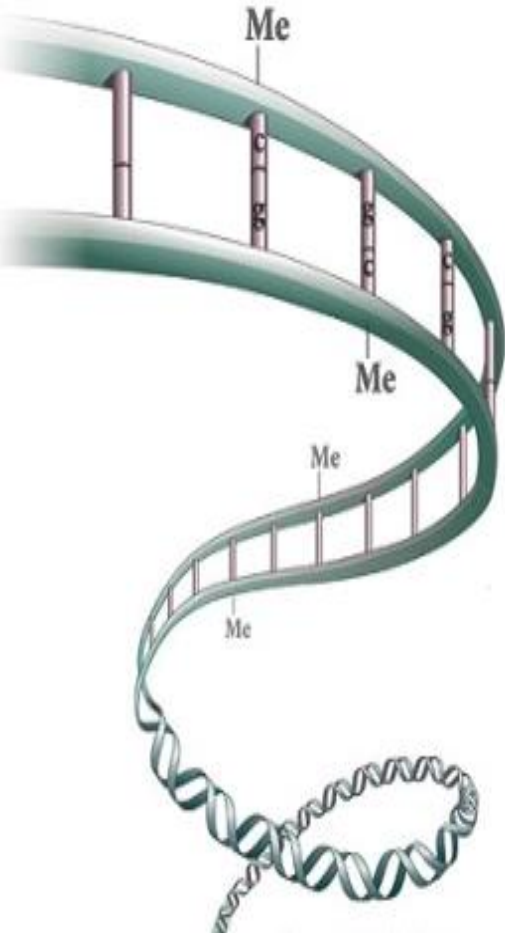
- HPLC-MS
- ELISA like assays
- Immuno-dot blot

Genome wide methylation:

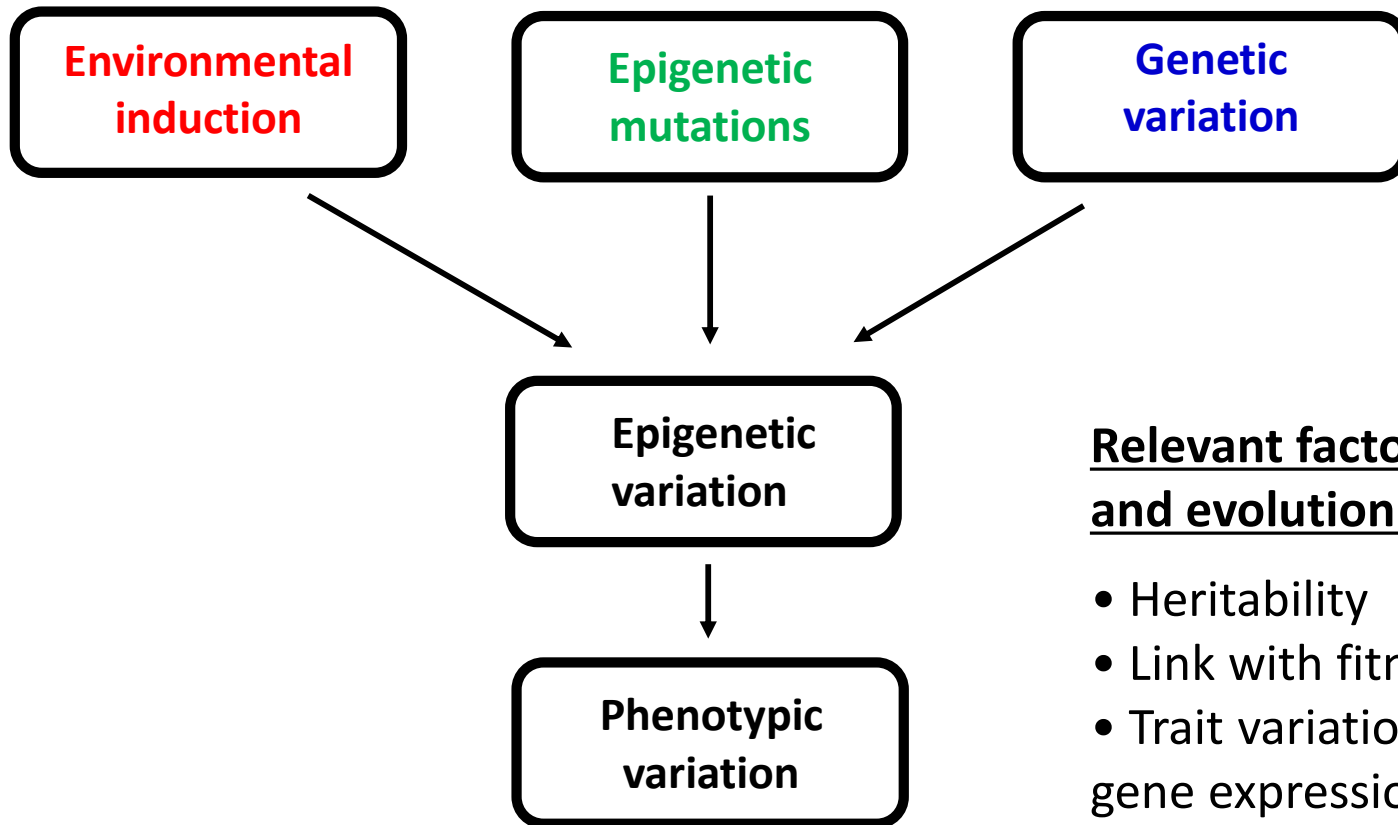
- MSAP
- Bisulfite (or enzymatically) converted DNA coupled with NGS (whole genome or reduced representation sequencing)
- Long read sequencing (PacBio or Nanopore)

Gene specific DNA methylation:

- Digestion based assays (methylation specific enzymes) followed by PCR or qPCR
- Bisulfite (or enzymatically) converted DNA coupled with PCR and sequencing, high resolution melting or methylation specific PCR



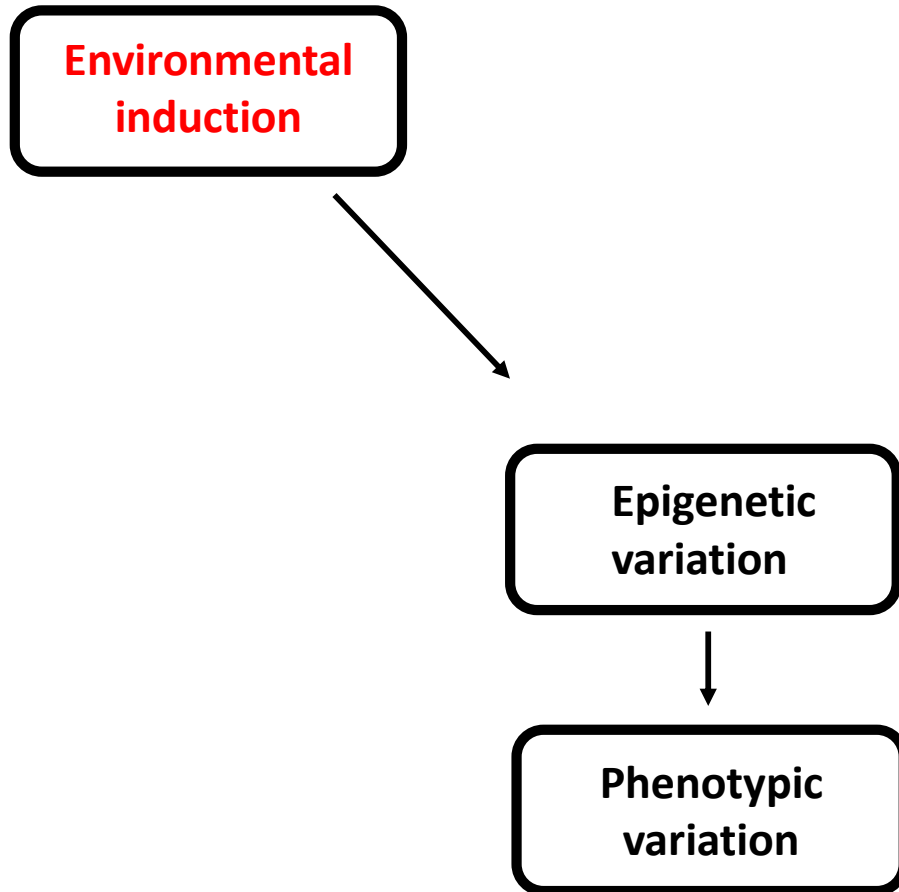
DNA methylation in ecology and evolution



Relevant factors for ecology and evolution:

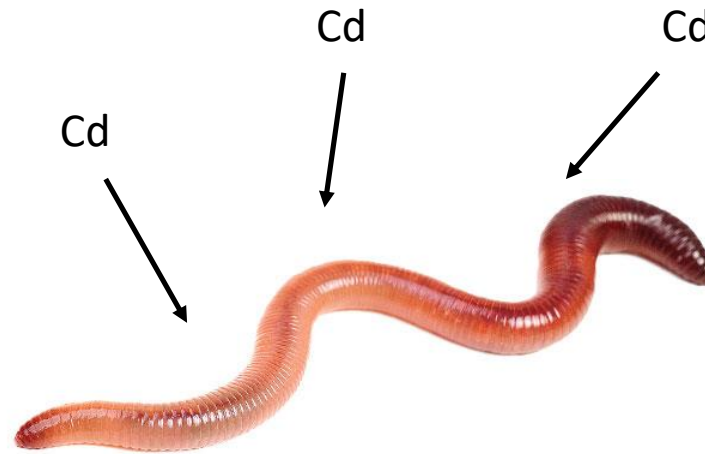
- Heritability
- Link with fitness
- Trait variation (methylation, gene expression and trait variation relation)

Environmentally induced DNA methylation changes



DNA methylation – our research

Earthworm
Lumbricus terrestris



PLOS ONE

RESEARCH ARTICLE

Low levels of Cd induce persisting epigenetic modifications and acclimation mechanisms in the earthworm *Lumbricus terrestris*

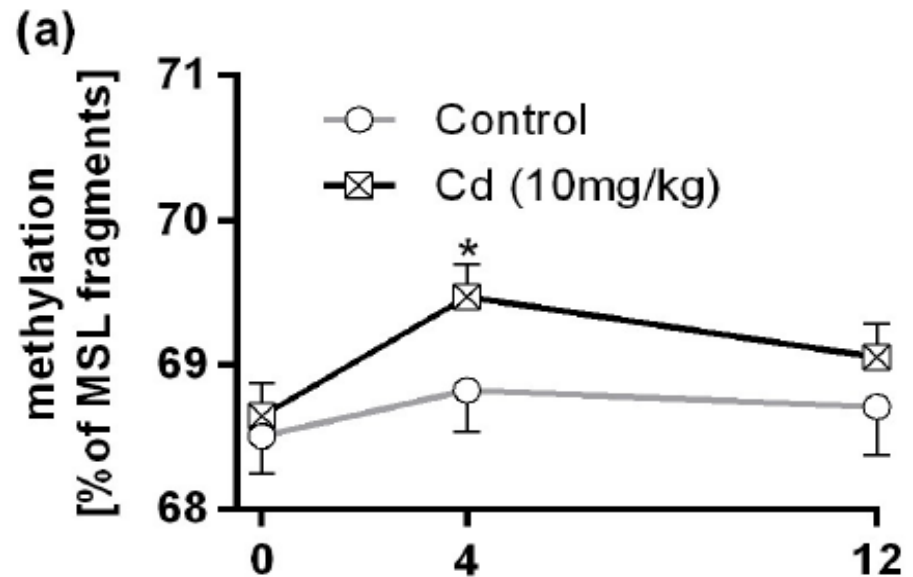
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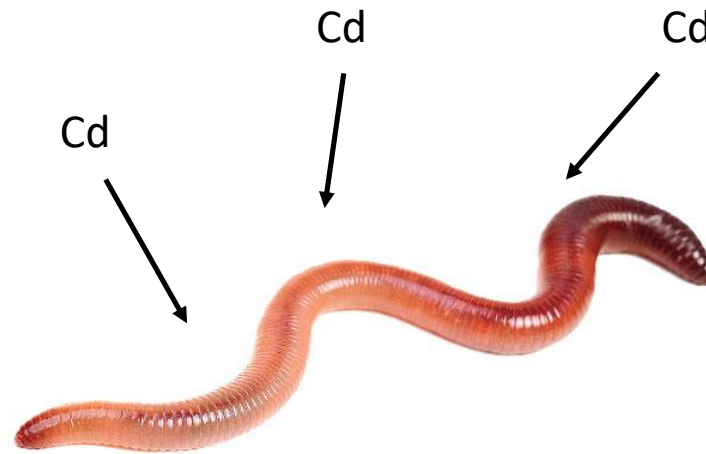
Abstract

Toxic effects of cadmium (Cd), a common soil pollutant, are still not very well understood, particularly in regard to its epigenetic impact. Therefore, the aim of this study was to assess DNA methylation changes and their persistence in the earthworm *Lumbricus terrestris* upon



OPEN ACCESS

DNA methylation – our research



FWF

Der Wissenschaftsfonds.

Genes involved in DNA methylation:

Dnmt1 – maintenance

Dnmt3 – de-novo methylation

TET – DNA

demethylation

Global DNA methylation:

- **Immuno-dot blot** -> association to *Dnmt1*, *Dnmt3* and *TET* gene expression

Genome wide methylation:

- Bisulfite converted DNA coupled with NGS (Whole genome sequencing) -> association to transcriptome

Gene specific DNA methylation – Metallothionein gene

- Bisulfite converted DNA coupled with PCR and sequencing -> association to *MT* gene expression

DNA methylation – our research

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Common mechanisms cannot explain time- and dose-dependent DNA methylation changes in earthworms exposed to cadmium

Gerhard P. Aigner, Verena Pittl, Birgit Fiechtner, Bernhard Egger, Maja Šrut¹, Martina Höckner^{*,1}

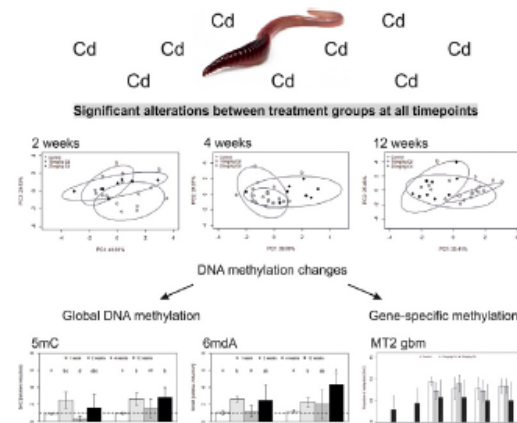
University of Innsbruck, Institute of Zoology, Center for Molecular Biosciences Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria



HIGHLIGHTS

- Cd induces cytosine and adenine hypermethylation.
- Well-known mechanisms of DNA methylation and demethylation (DNMT1, DNMT3 and TET) are present in *L. terrestris* but are not responsible for Cd-induced hypermethylation.
- Changes in MT2 gene body methylation do not affect Cd-induced MT2 gene expression.

GRAPHICAL ABSTRACT




DNA methylation – our research

Marine snail
Hexaplex trunculus



Article

Marine Pollutant Tributyltin Affects DNA Methylation and Fitness of Banded Murex (*Hexaplex trunculus*) Populations

Maja Šrut ¹, Iva Sabolić ², Anita Erdelez ³, Dorotea Grbin ², Martina Furdek Turk ⁴, Robert Bakarić ², Melita Peharda ³  and Anamaria Štambuk ^{2,*}

- ¹ Institute of Zoology, Center for Molecular Biosciences, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria; maja.srut@uibk.ac.at
 - ² Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia; iva.sabolic@gmail.com (I.S.); dorotea.polo@gmail.com (D.G.); rbakari17@gmail.com (R.B.)
 - ³ Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia; aerdelez@gmail.com (A.E.); melita@izor.hr (M.P.)
 - ⁴ Division for Marine and Environmental Research, Ruder Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia; martina.furdek@irb.hr
- * Correspondence: astambuk@biol.pmf.hr

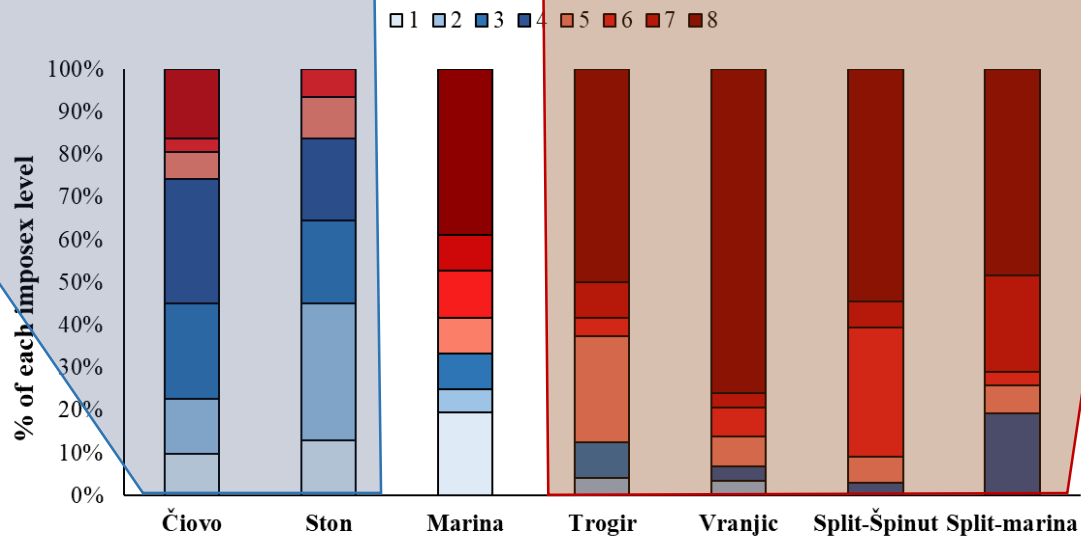
The aim: unravel the interactions between tributyl tin (TBT) bioaccumulation, phenotypic responses and epigenetic and genetic endpoints in native populations of murex snail *H. trunculus*.



Tributyl tin (TBT) – banned
in 2008

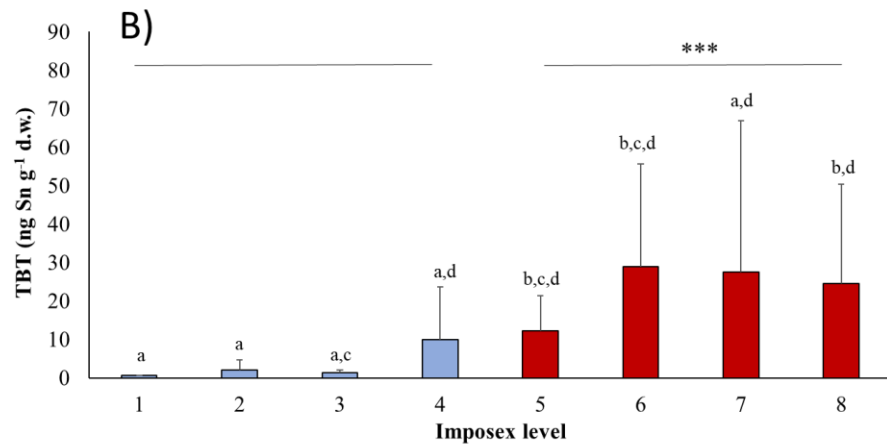
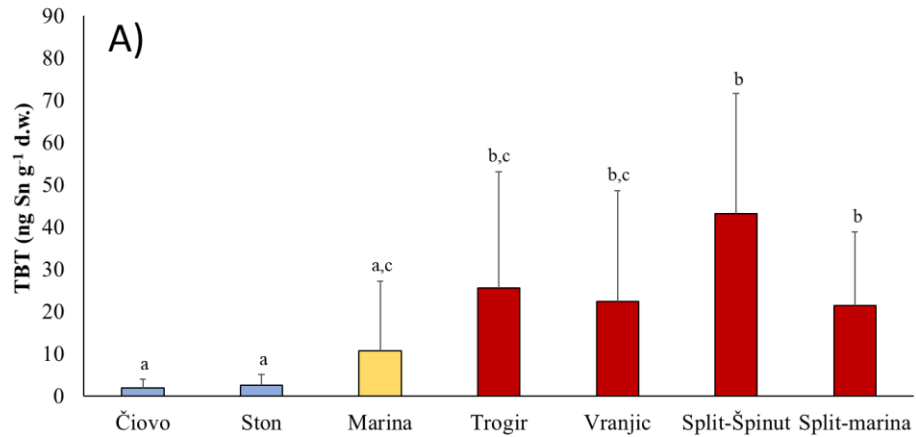
Development of imposex (stages 1-8)

DNA methylation – our research



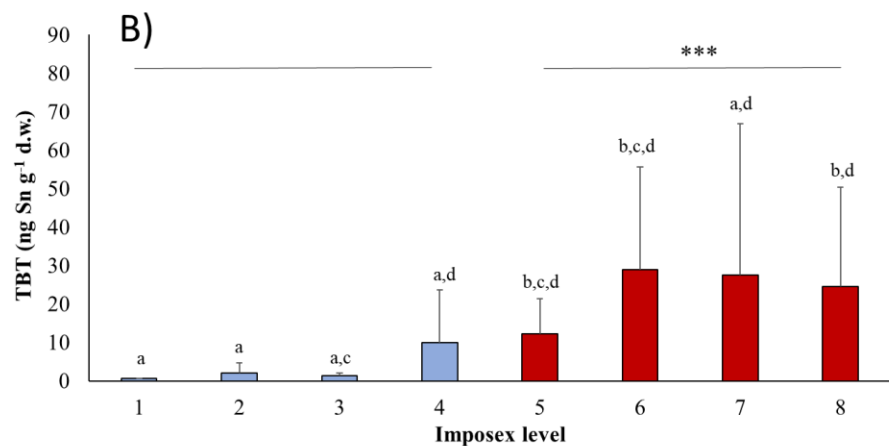
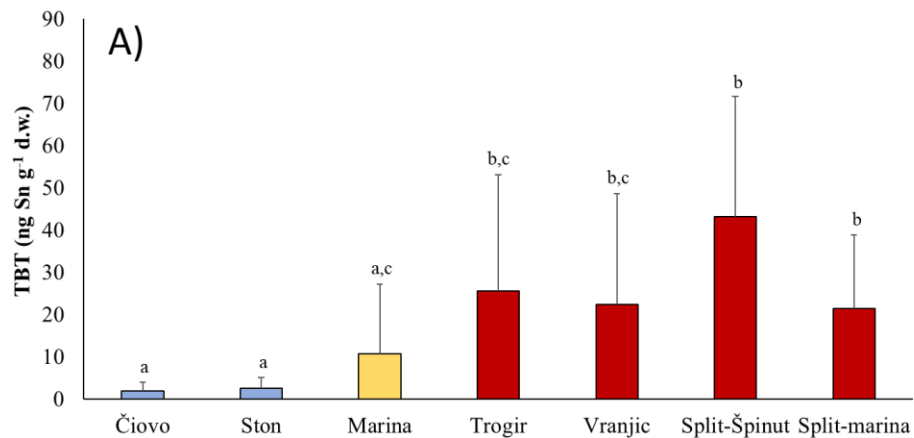
DNA methylation – our research

TBT accumulation

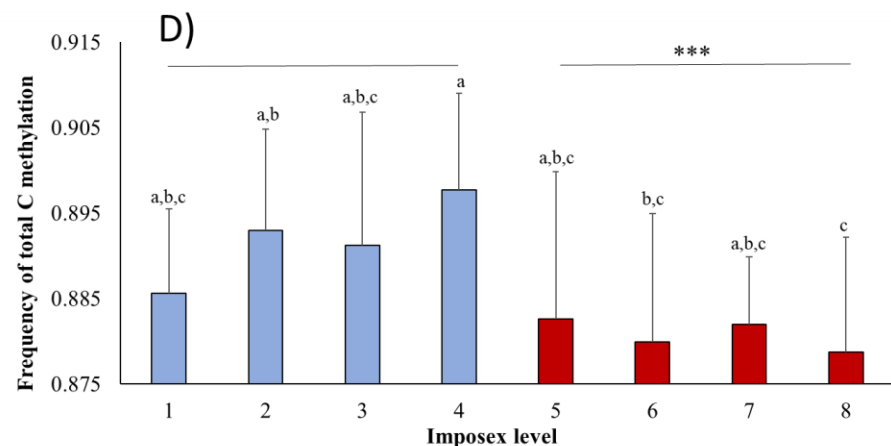
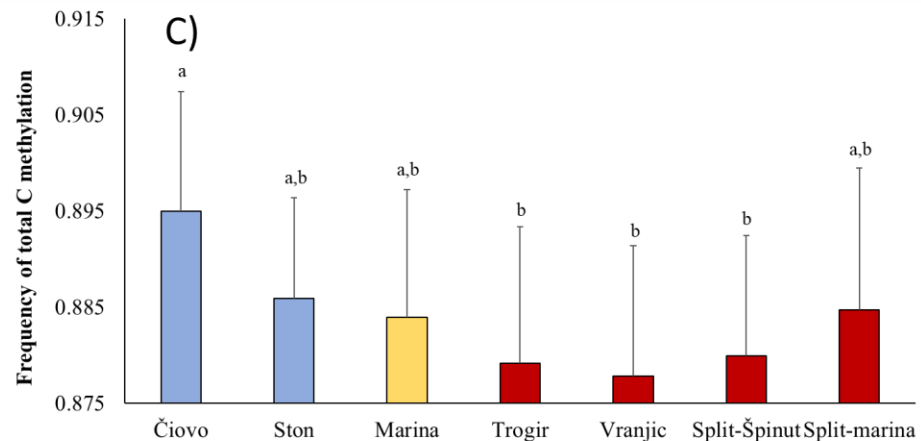


DNA methylation – our research

TBT accumulation

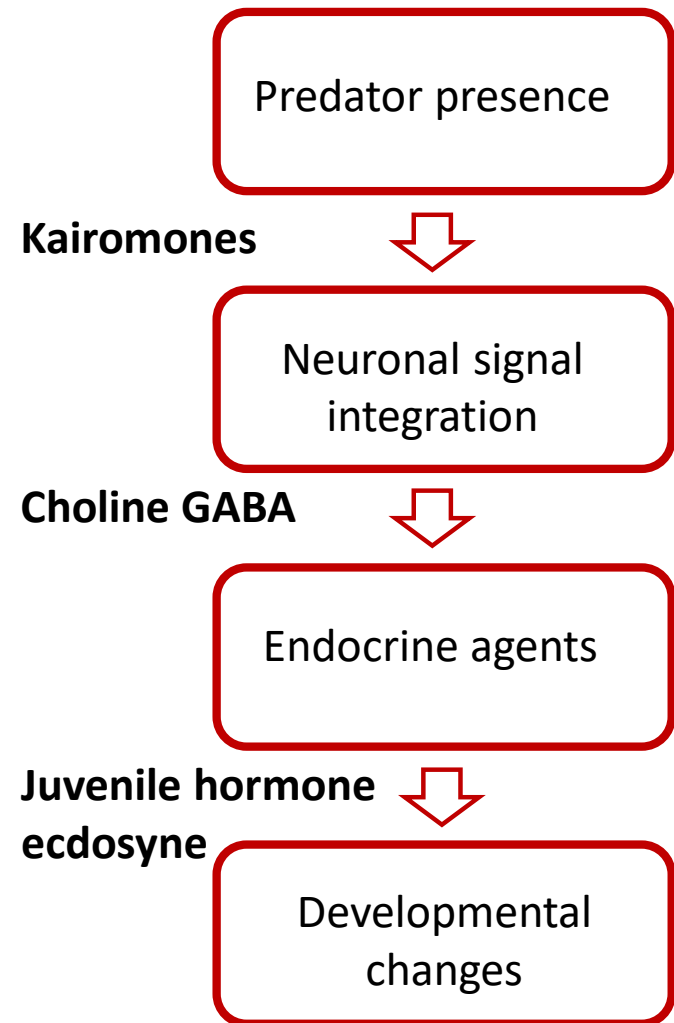
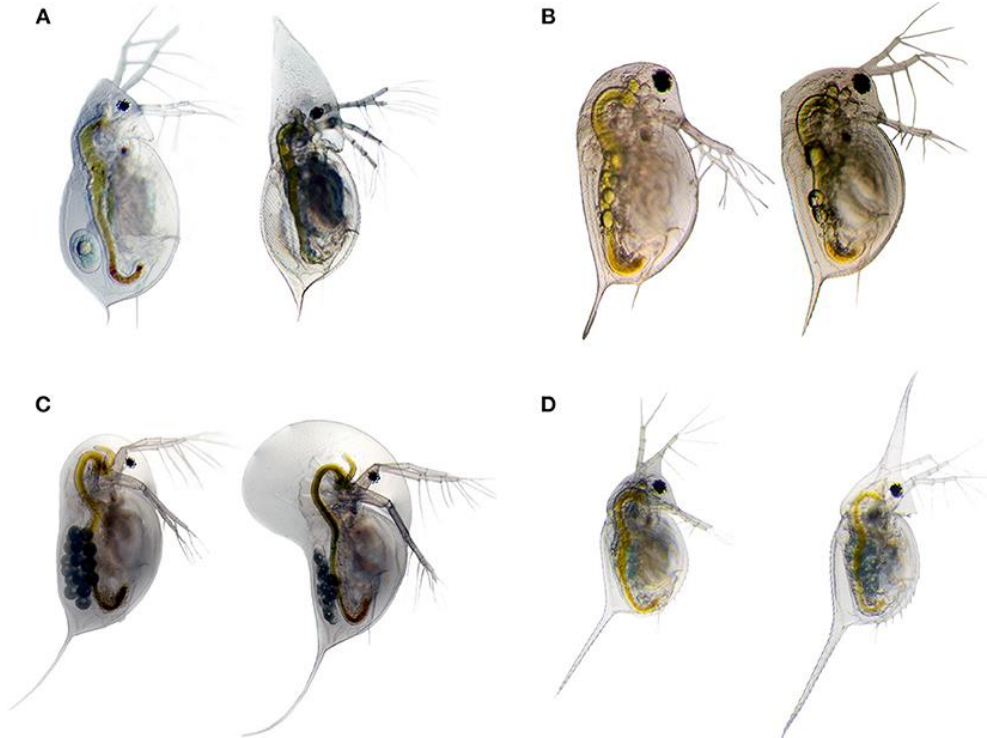


DNA methylation



Environmentally induced DNA methylation changes

Inducible morphological defences in Daphnia



Weiss 2019. Front Behav Neurosci

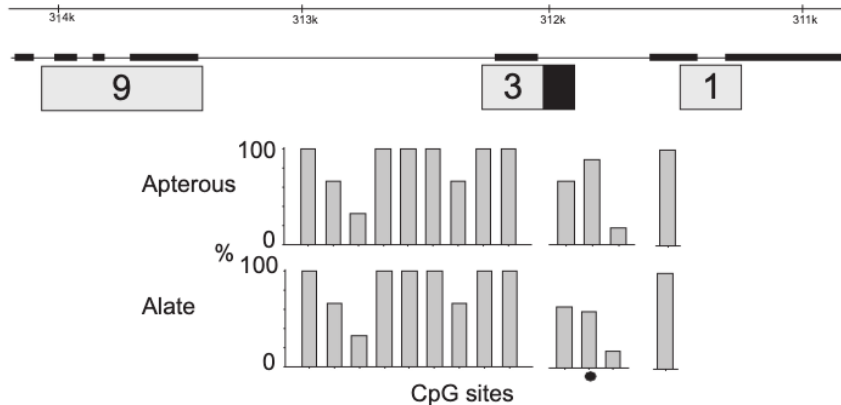
Environmentally induced DNA methylation changes

Apterous

Alate



ApJHBP



Walsh et al. 2010. Insect Mol Biol

Population density



DNMT2
expression

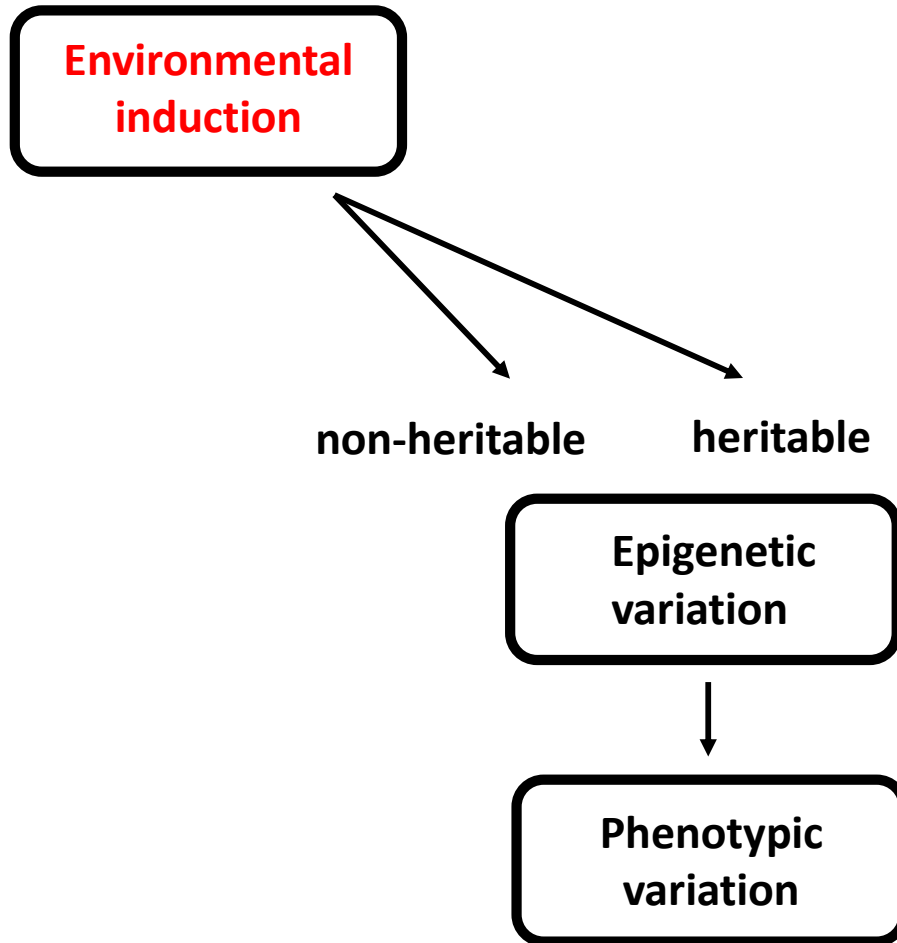


Methylation of
JHBP

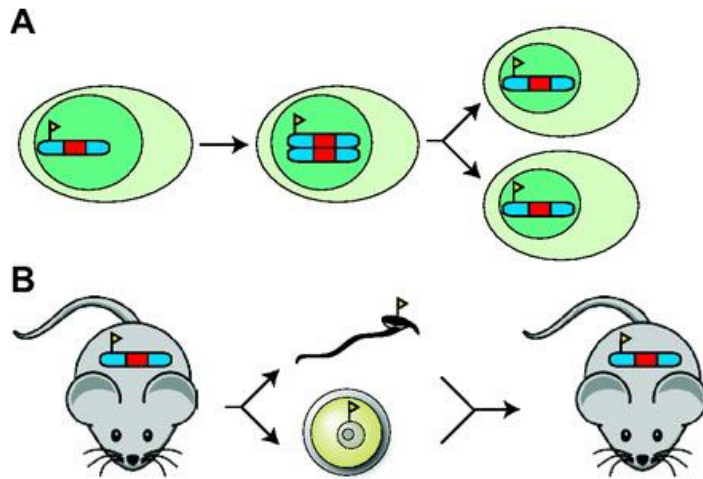


Production of
wings

Environmentally induced DNA methylation changes



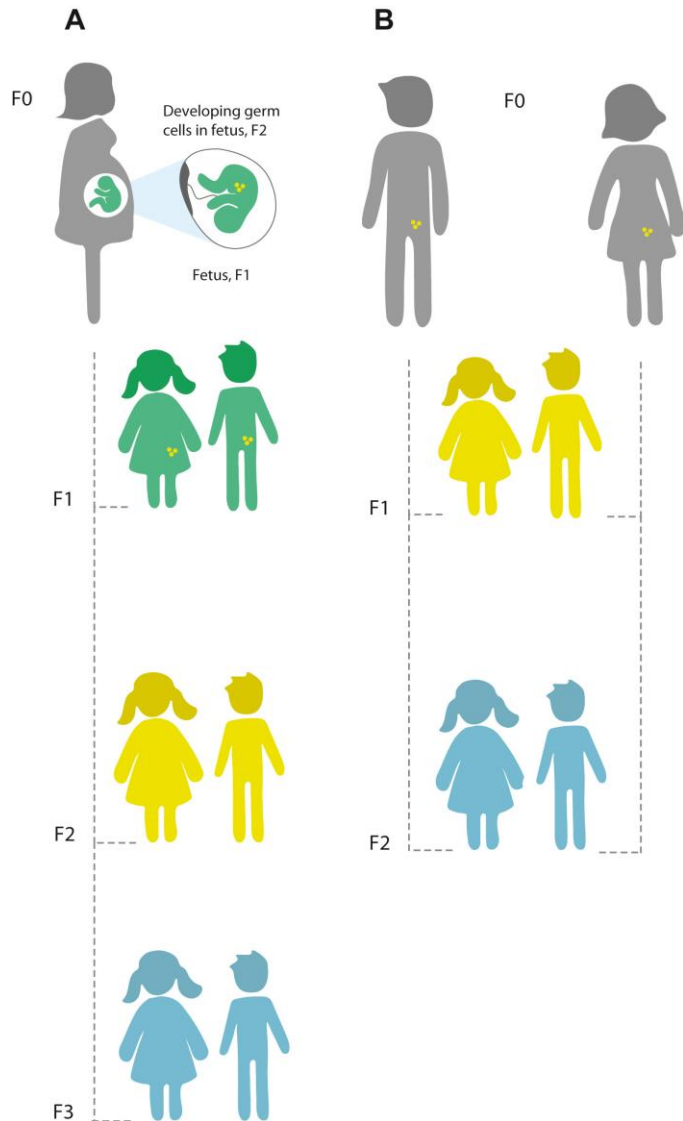
DNA methylation inheritance



Mitotic epigenetic inheritance: replication of epigenetic marks throughout DNA replication.

Meiotic epigenetic inheritance: controls transgenerational transmission of information that does not reside in the DNA sequence

DNA methylation inheritance



Intergenerational epigenetic inheritance versus Transgenerational epigenetic inheritance

- (A) If a pregnant woman (F0) is exposed to an environmental stressor, her son/daughter (F1, green) and his/her germ cells that will form F2 (yellow) are also directly exposed and this may result in intergenerational effects. The third generation (F3, blue) is the 1st generation that could represent transgenerational epigenetic inheritance.
- (B) If a man or a woman (F0) and their germ cells to F1 (yellow) is directly exposed to an environmental stressor, the F2 offspring (blue) is the 1st generation that could represent transgenerational epigenetic inheritance.

DNA methylation inheritance-mammals

The Dutch famine of 1944–45, Hunger winter

The Dutch Birth Cohort study



Extreme famine during fetal development

F1



- Smaller babies at birth
- Diabetes, obesity, cardiovascular disease at adult age

F2

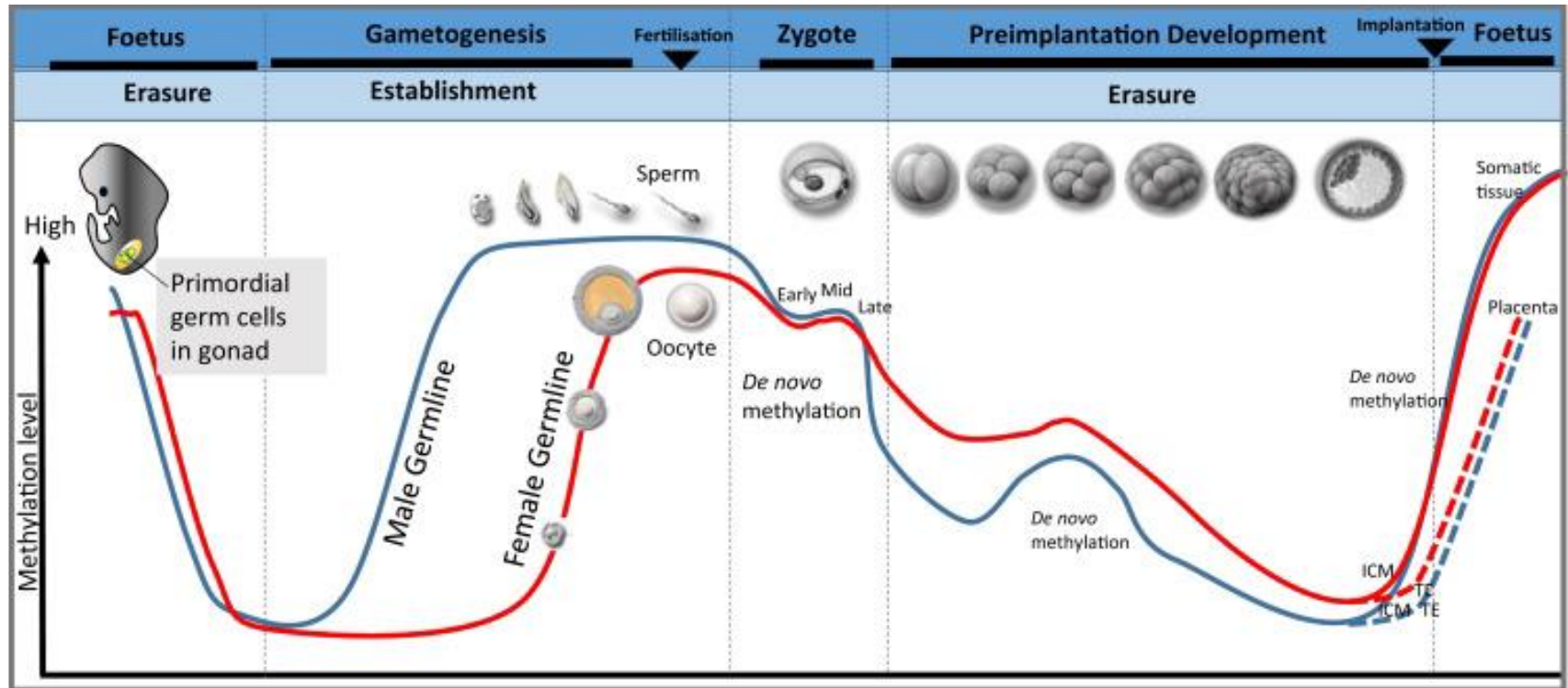


- Smaller babies at birth
- Diabetes, obesity, cardiovascular disease at adult age

**INTERGENERATIONAL
INHERITANCE**

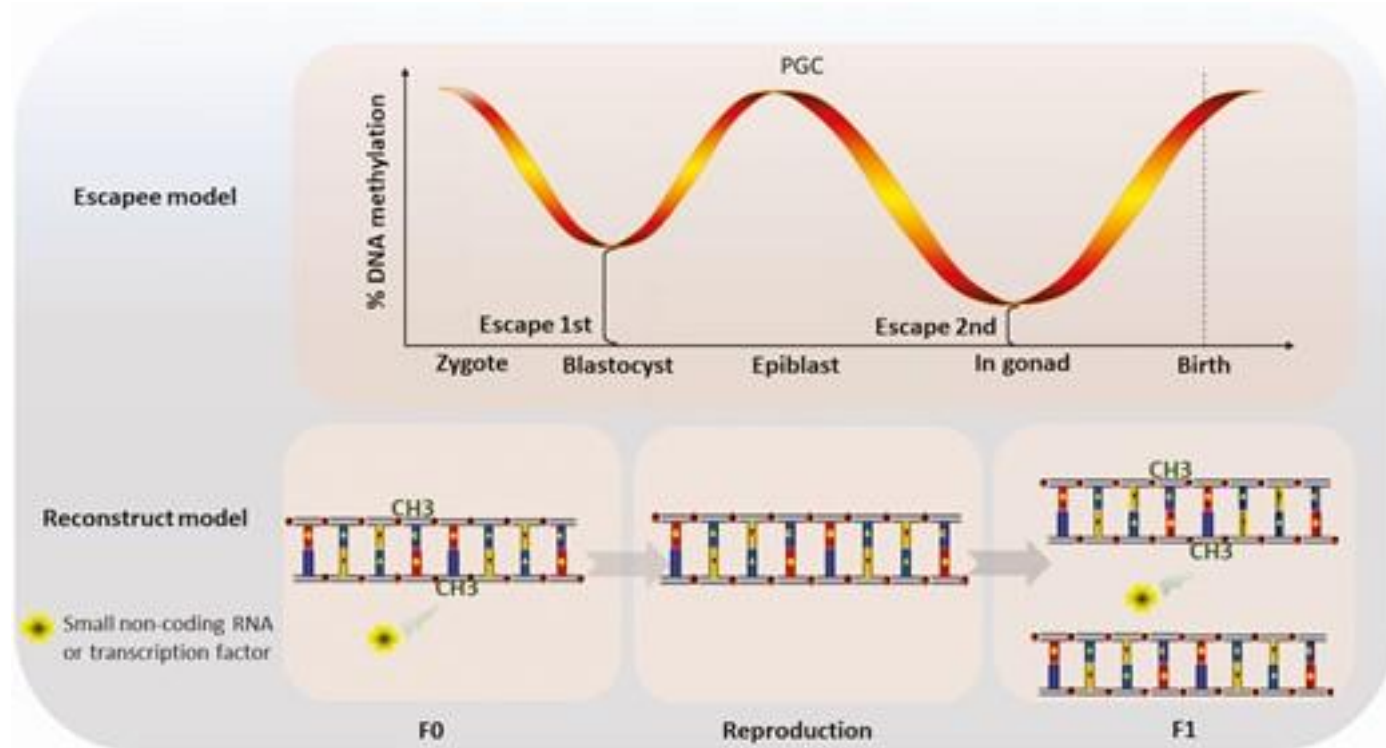
Less methylation of the insulin-like growth factor II (*IGF2*) gene

DNA methylation reprogramming



Huntriss 2021. Epigenetics and Reproductive Health

DNA methylation reprogramming

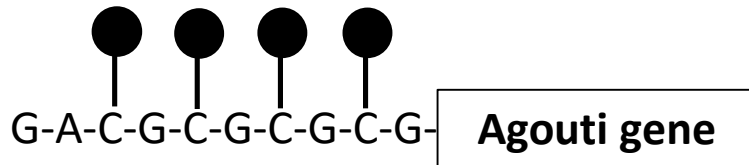


Zhang and Sirard 2021. Animal Frontiers

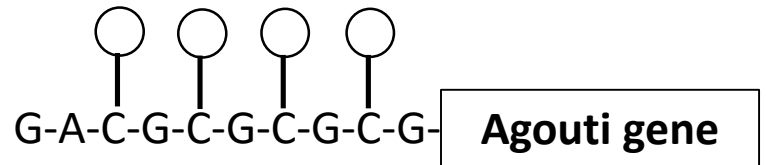
- **Escapee model:** some regions could escape DNA methylation reprogramming after fertilization and the PGC development process
- **Reconstruct model:** after reproduction process, DNA methylation may re-establish with the help of some small non-coding RNAs or transcription factors

DNA methylation inheritance-mammals

Genetically identical mice

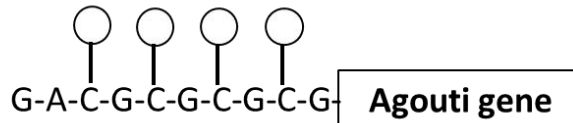


Agouti gene **silenced**
Mouse with **brown fur**
Healthy



Agouti gene **continually active**
Mouse with **yellow fur**
Develops diabetes and obesity

DNA methylation inheritance-mammals

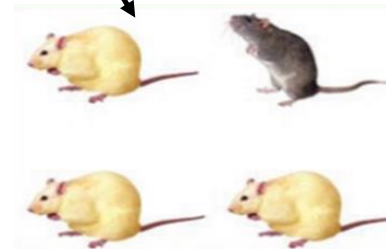


dietary supplementation
during pregnancy with
additional methyl groups



Offspring mostly **brown and healthy**
Agouti gene **methylated and silenced**

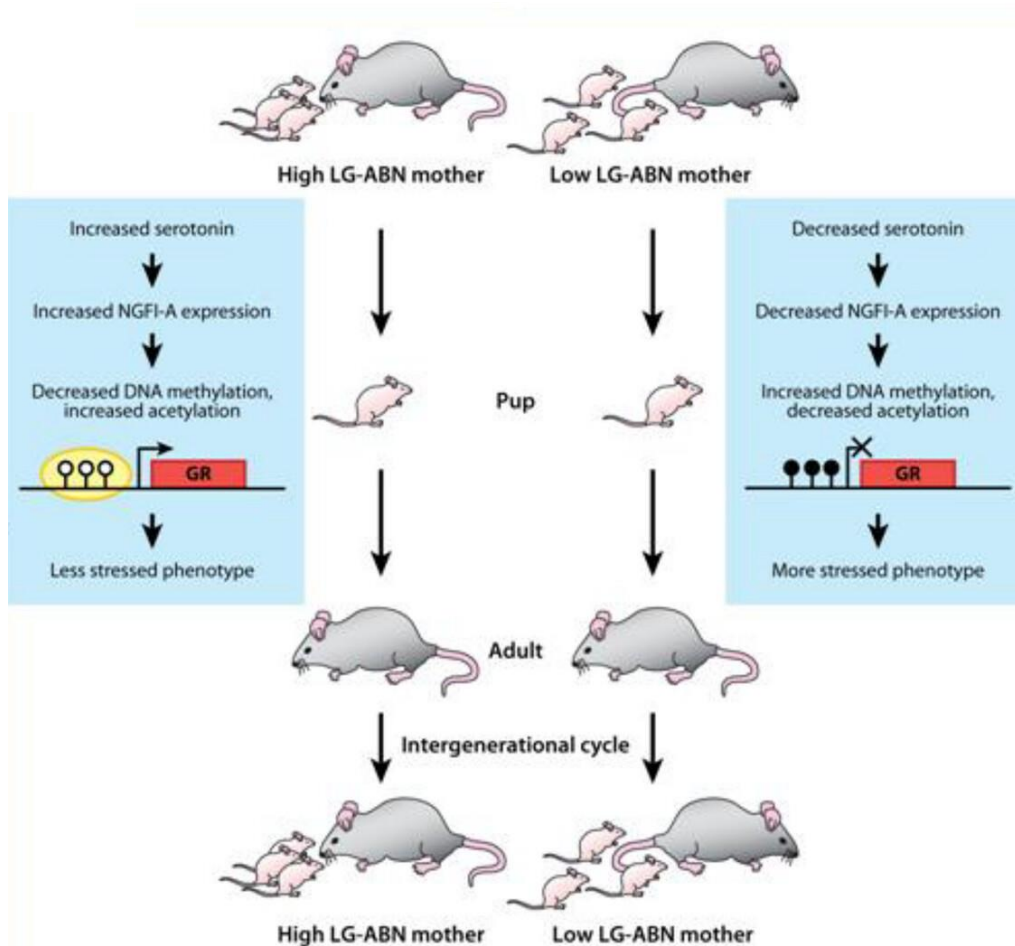
no dietary
supplementation



Offspring mostly **yellow and unhealthy**
Agouti gene **unmethylated and active**

**INTERGENERATIONAL
INHERITANCE**

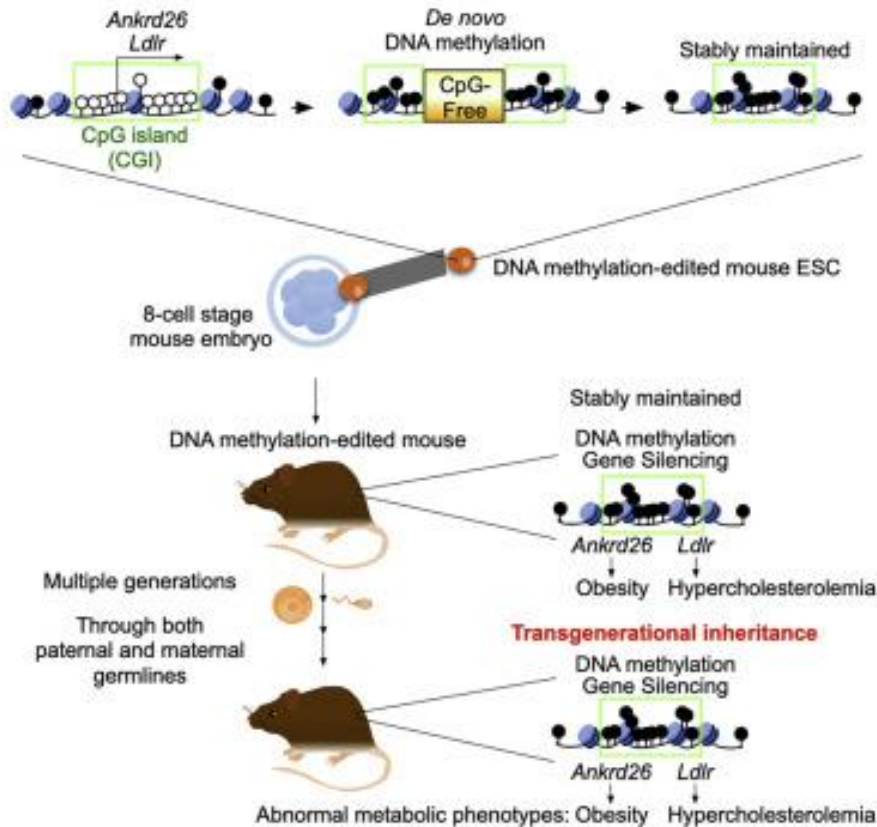
DNA methylation inheritance-mammals



Epigenetic inheritance of mothering style and stress in rats. Mothering style (licking/grooming (LG) and arched-back nursing (ABN)) that results in different DNA methylation and histone acetylation status at the promoter of the glucocorticoid receptor (GR) gene provokes the occurrence of the same epigenetic markers in the offspring.

**INTERGENERATIONAL
INHERITANCE**

DNA methylation inheritance-mammals



DNA methylation-edited mice: the targeted CGI of two different loci are methylated - Ankyrin repeat domain 26 (**Ankrd26**) and low-density lipoprotein receptor (**Ldlr**).

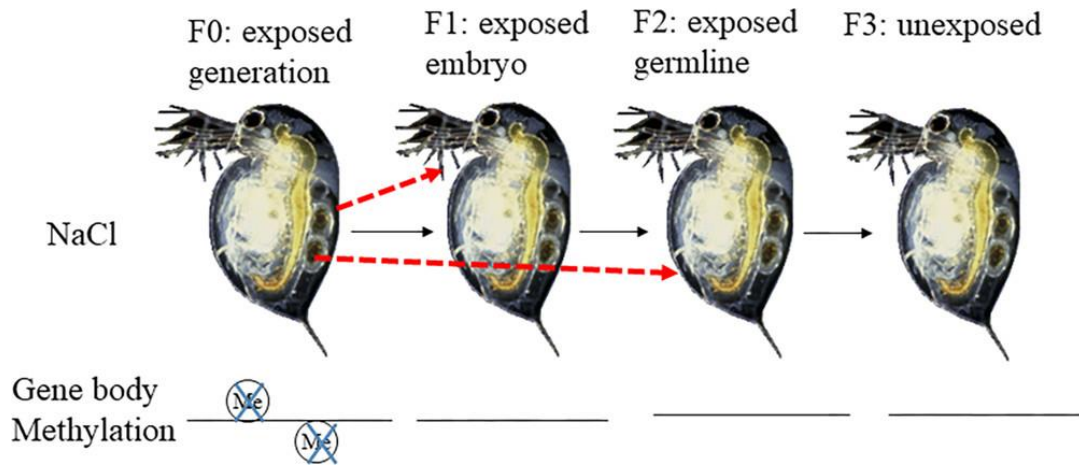
The knockout of Ankrd26 or Ldlr results in obesity or hypercholesterolemia, respectively, but does not affect mouse viability and fertility

Acquired methylation of the targeted CGI and the phenotypic traits were **maintained and transmitted across multiple generations.**

Takahashi et al. 2023. Cell

**TRANSGENERATIONAL
INHERITANCE**

DNA methylation inheritance-invertebrates



Jeremias et al. 2018. Environ Sci Technol

High salinity levels
In F0



Hypomethylation
of genes involved in
cellular stress response



Noticed until F3
generation

**TRANSGENERATIONAL
INHERITANCE**

DNA methylation: current status in ecology and evolution

- DNA methylation has many different aspects: CG, CHG, CHH
- DNA methylation has multiple functions (gene expression regulation, controlling transposable elements...)
- DNA methylation is species specific, tissue specific, developmental stage specific
- DNA methylation is heritable (in mammals mostly intergenerationally)
- Still **many things unclear and unknown – LOT TO INVESTIGATE!**