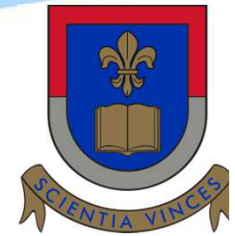




2nd International Aquaculture ConferenCe

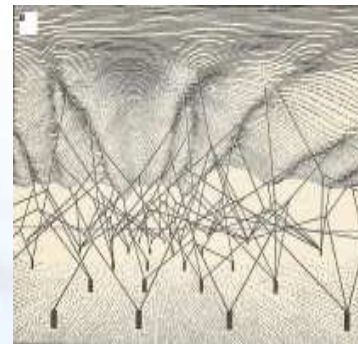
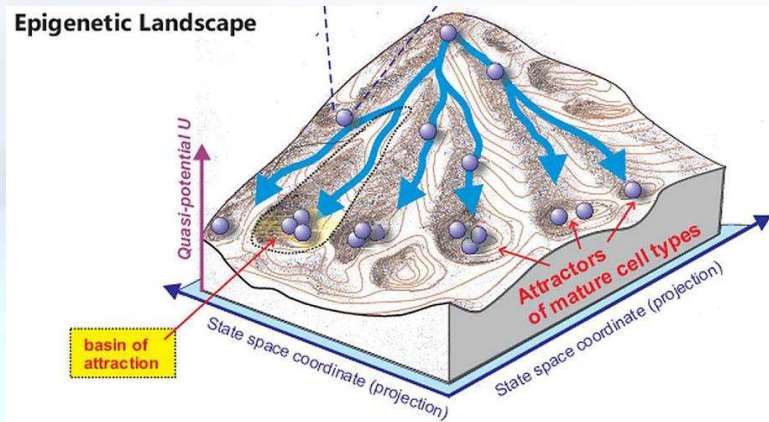


Role of epigenetics in aquaculture

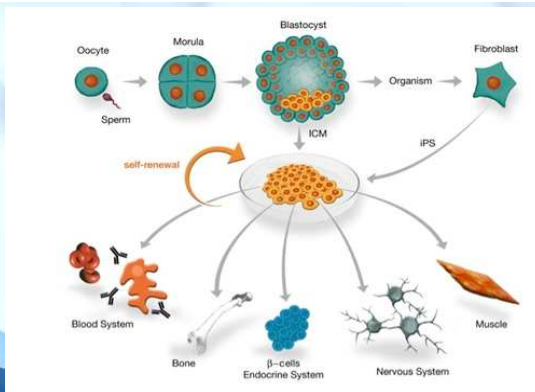
Škute Nataļja
Daugavpils Universitāte,
natalja.skute@du.lv

Epigenetic changes

stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence



Conrad Hall Waddington (1957)
The strategy of the genes, London, Allen and Unwin



Why do cells differ among tissue types since all cells contain the same genes?

The answer to this question is lies in how gene expression is regulated: not all genes are expressed in all tissues at all times

General concepts

genetics



genes and gene function

How *DNA sequences* lead to changes in the cell/host

epigenetics



gene regulation

How DNA is *regulated* to achieve those changes.

genetics

genomics ("what is a gene?", "how is information encoded in DNA?"),

transcriptomics ("how are genes expressed?" / "how is information encoded in RNA?"),

proteomics ("how do proteins interact?" / "how is information encoded in proteins?"),

heredity ("how are traits passed on between generations?" / "how is information passed on through time?"),

evolutionary genetics ("how does DNA change through time?" / "how much genetic information do different organisms share

genetic disease ("how do mutations lead to disruptions in the host?").

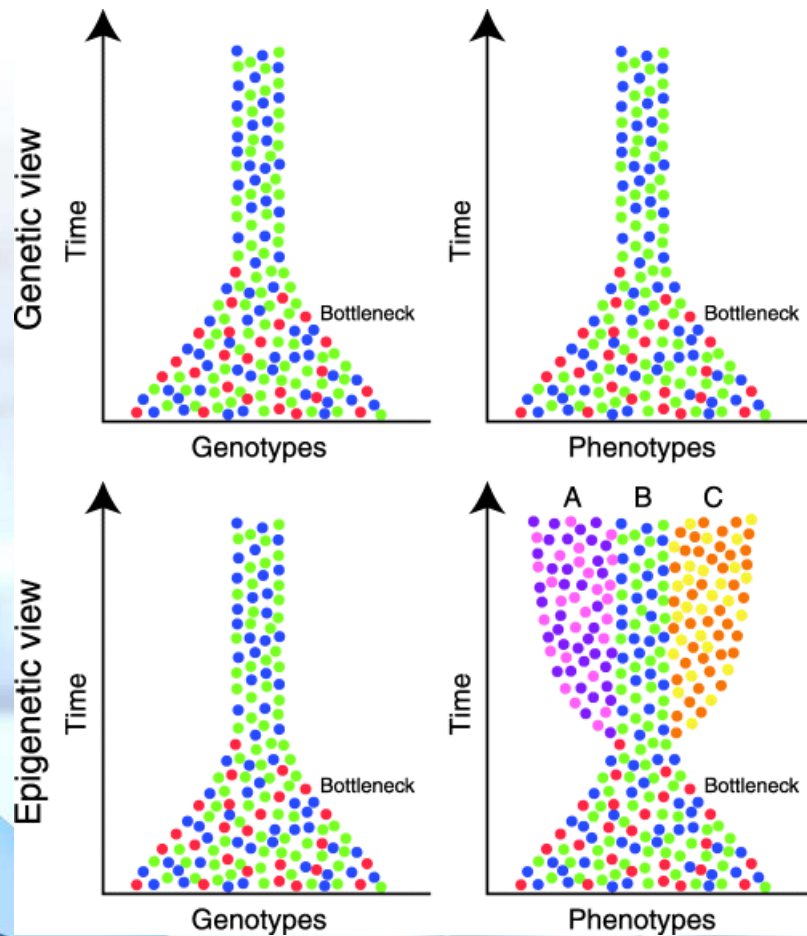
EPIGENETICS

gene regulation ("how does the cell turn genes on and off?" / "how is information 'compiled' to produce a meaningful output?"),

gene-environment interactions ("how does gene expression change in response to environment?" / "how does context affect the way information is compiled?"), and

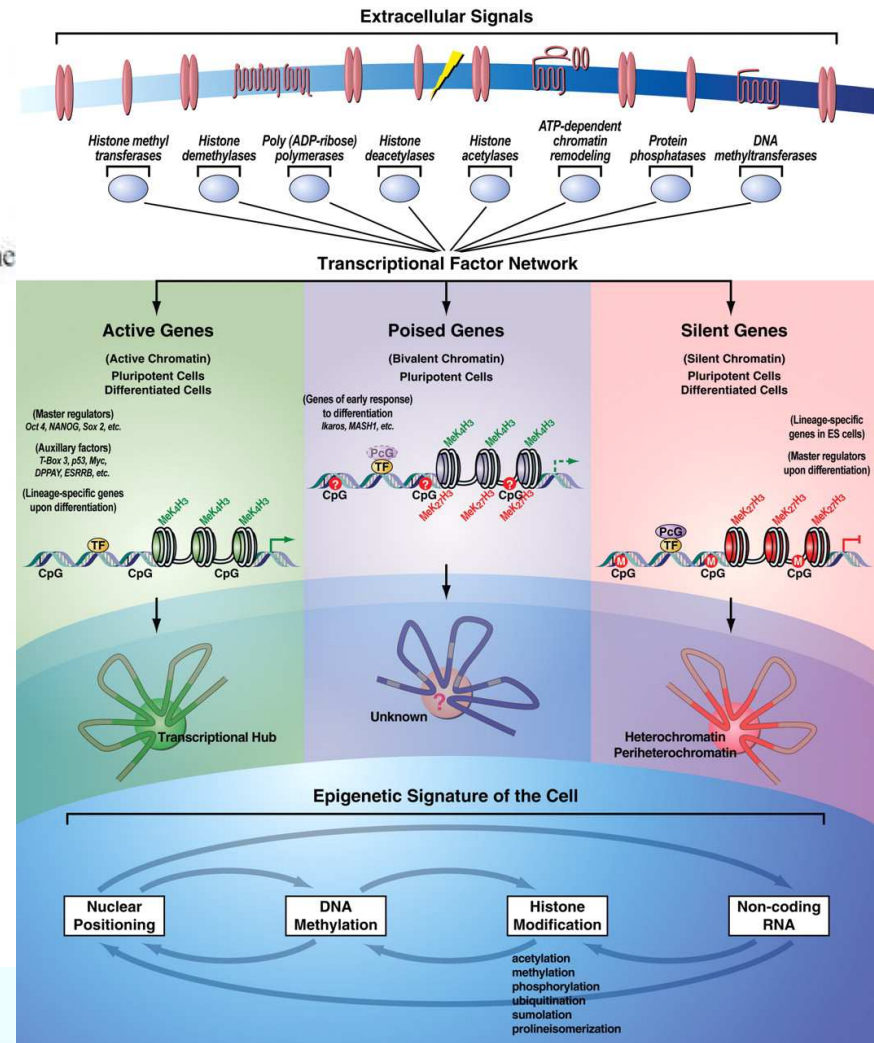
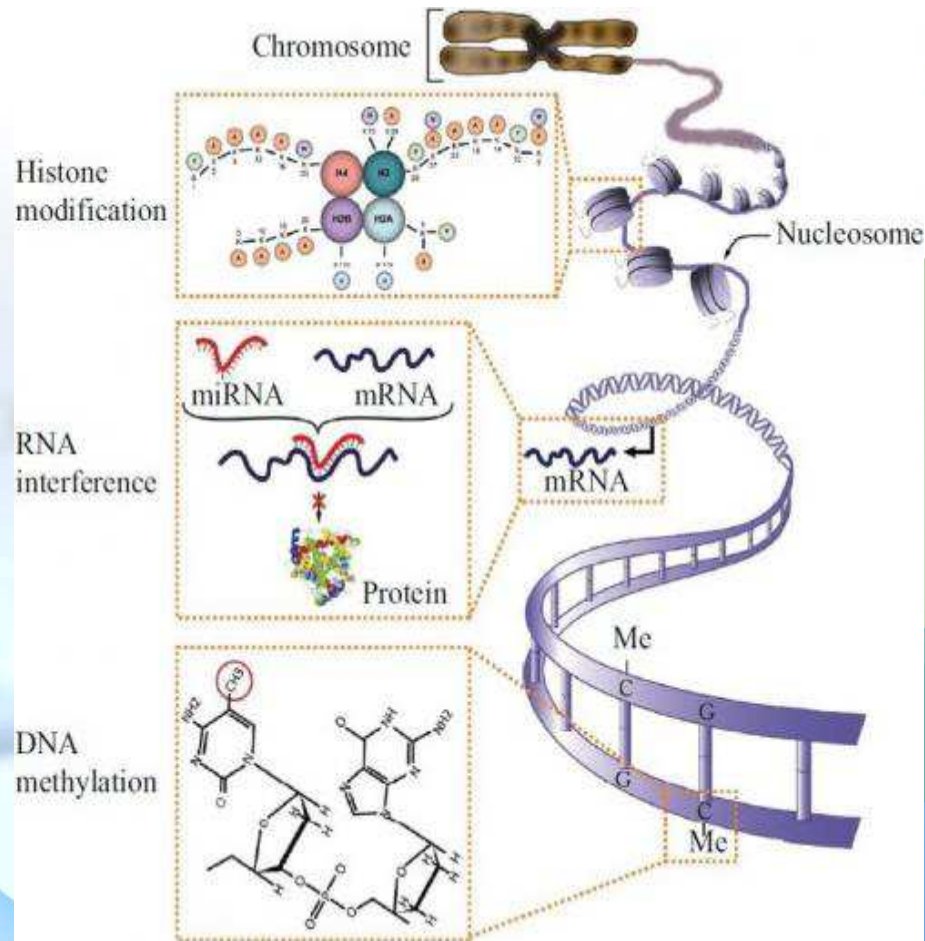
protein-environment interactions ("how does the activity of proteins change in response to the environment?")

Epigenetic changes in evolution

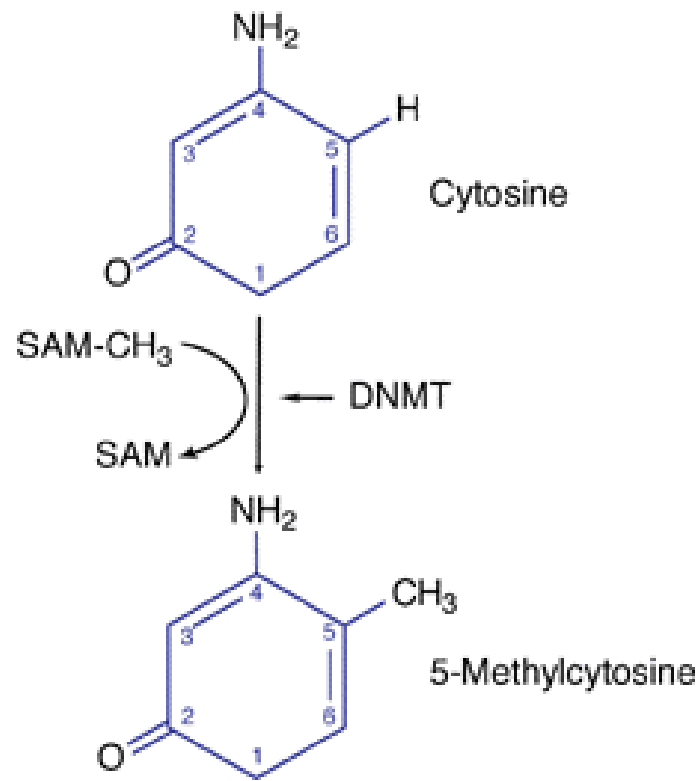


The environmentally-induced DNA methylation causes phenotypic heterogeneity. The role of DNA methylation in adaptive evolution is therefore unclear.

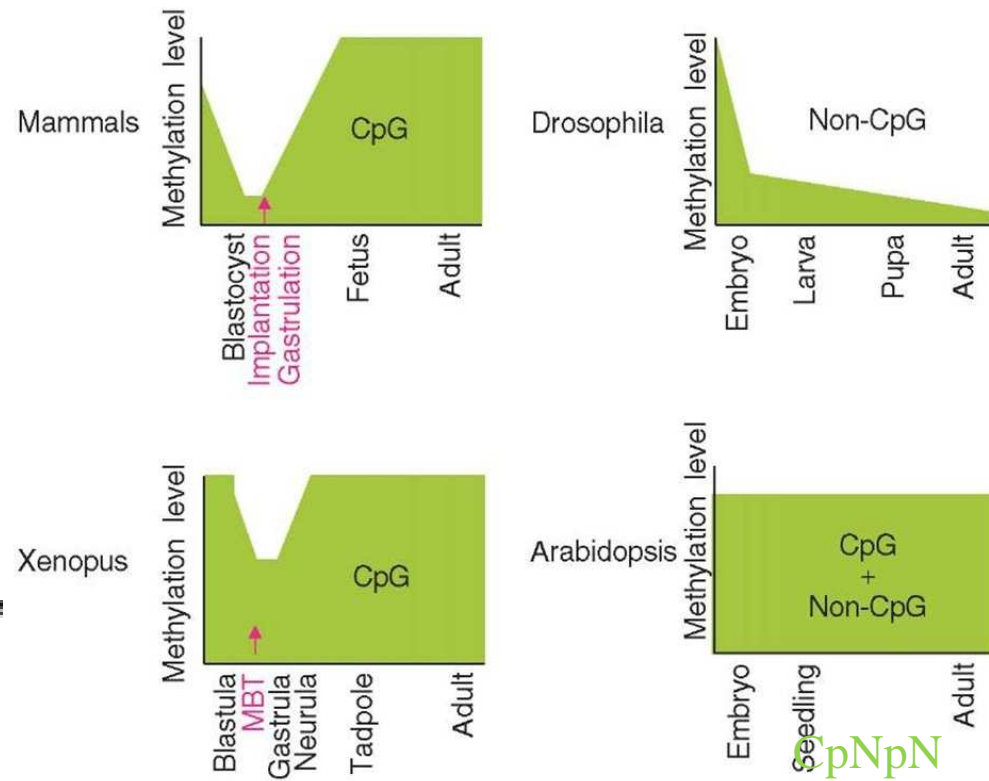
Epigenetic changes



Methylation of nuclear DNA



Mechanism of DNA methylation



- «resetting» of epigenetic status occurs *in each generation* by extensive demethylation and subsequent *de novo* DNA methylation during gametogenesis and early development
- mostly symmetric CpG sequences

animals



- the epigenetic states of plant genes are often stably inherited through generations.
- extensively methylated at two types of symmetric sequences, namely CpG and CpNpG, as well as at asymmetric ones

plants



Maintenance of Cytosine Methylation

Establishment and maintenance

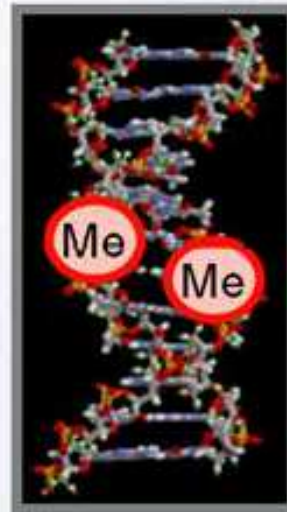


Replication
→
←
Maintenance methylation
Dnmt1



Passive Demethylation of 5-MethylCytosine

Establishment and maintenance



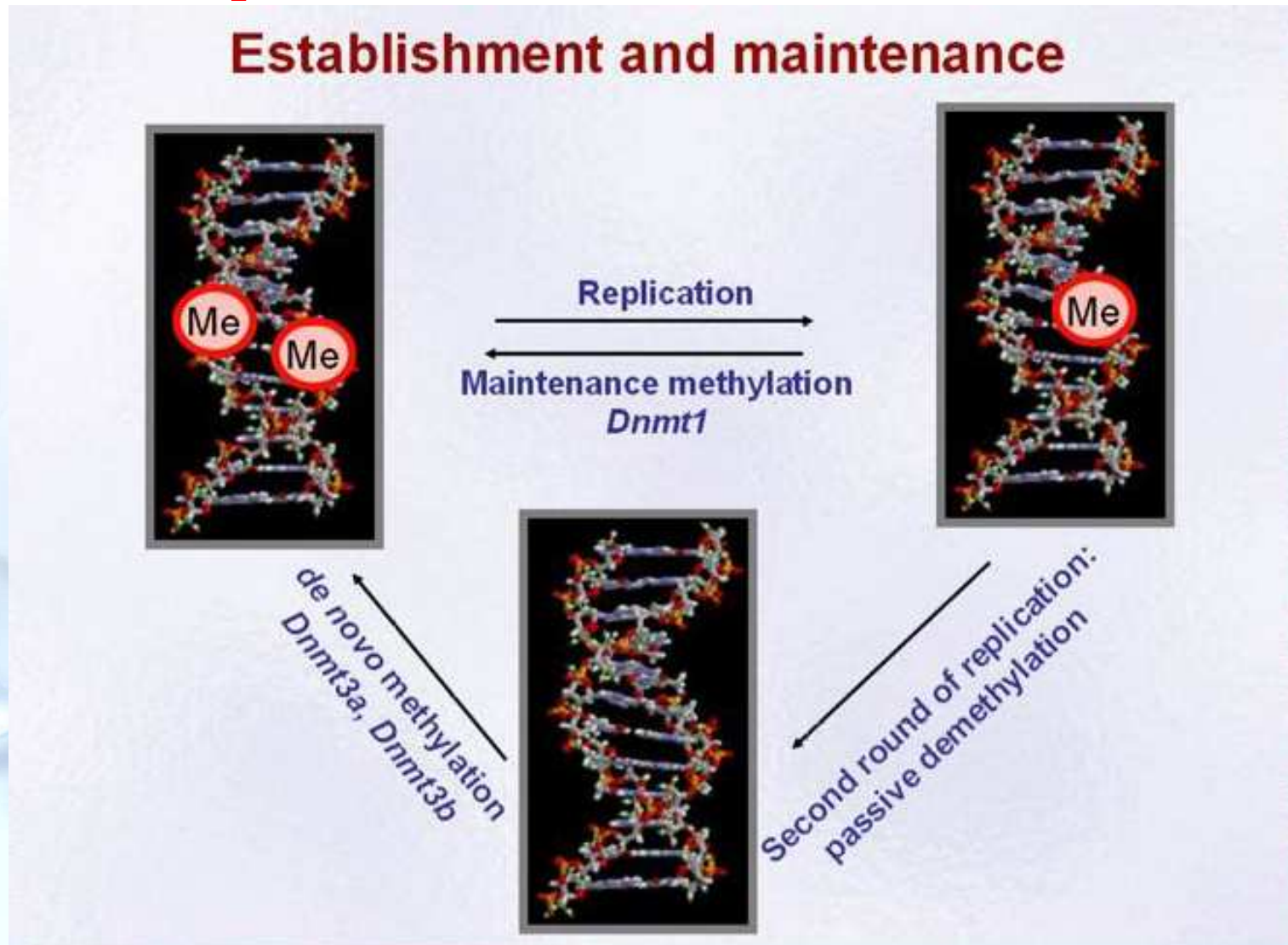
Replication
Maintenance methylation
Dnmt1



Second round of replication:
passive demethylation



Establishment and Maintenance of Cytosine Methylation



Some DNA Methyl Transferases are Essential

Mammalian Dnmts are essential

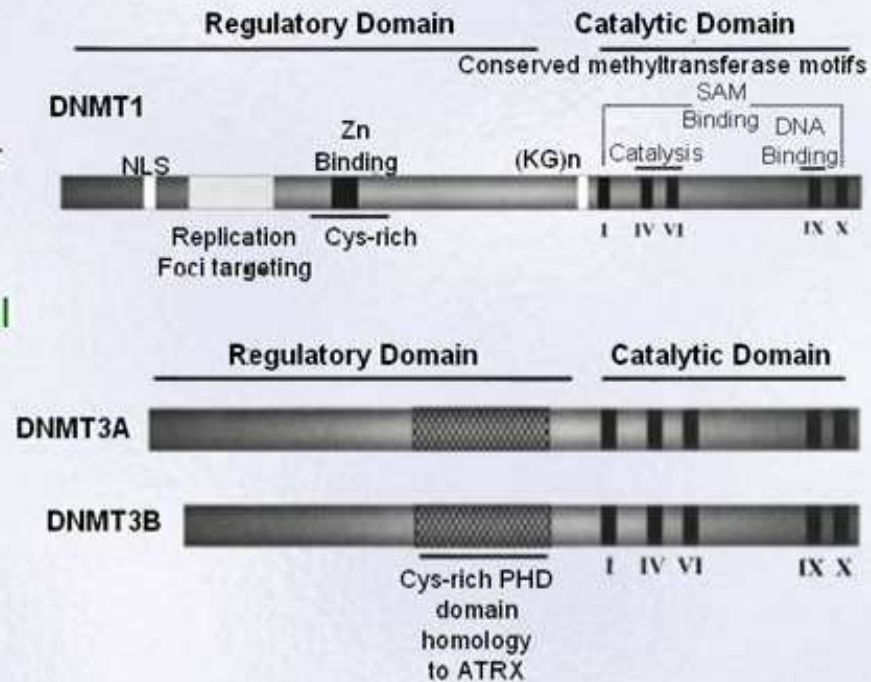
Dnmt1: embryonic lethal

Dnmt2: no obvious effect

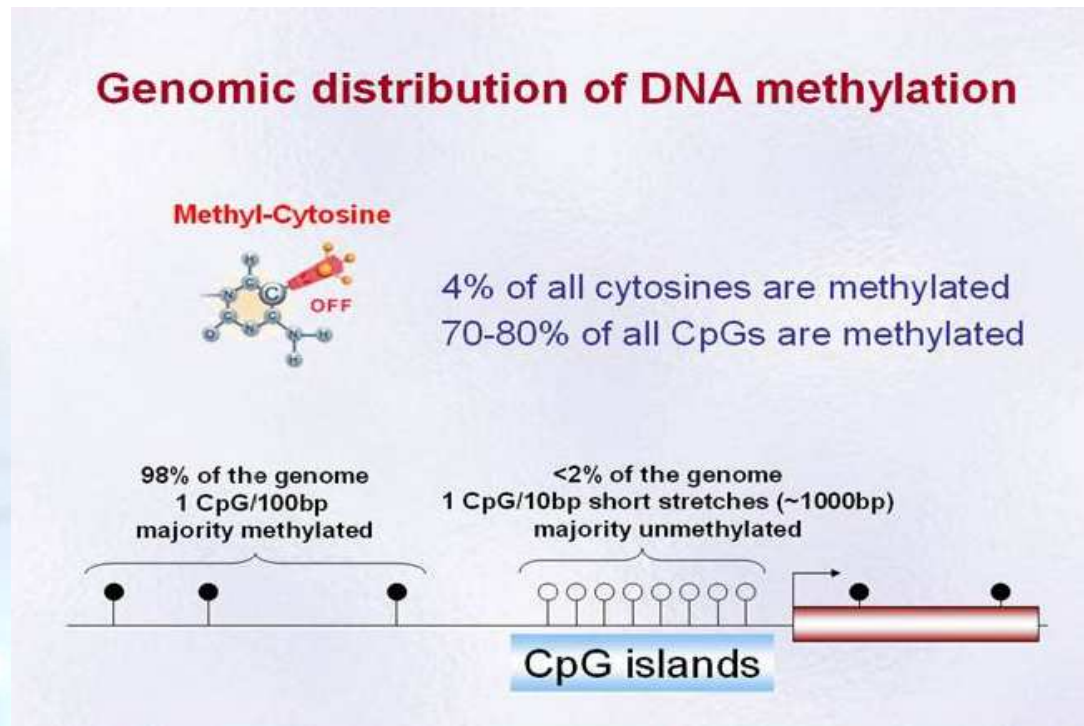
Dnmt3a: perinatal death

Dnmt3b: embryonic lethal

Dnmt3l: no imprints



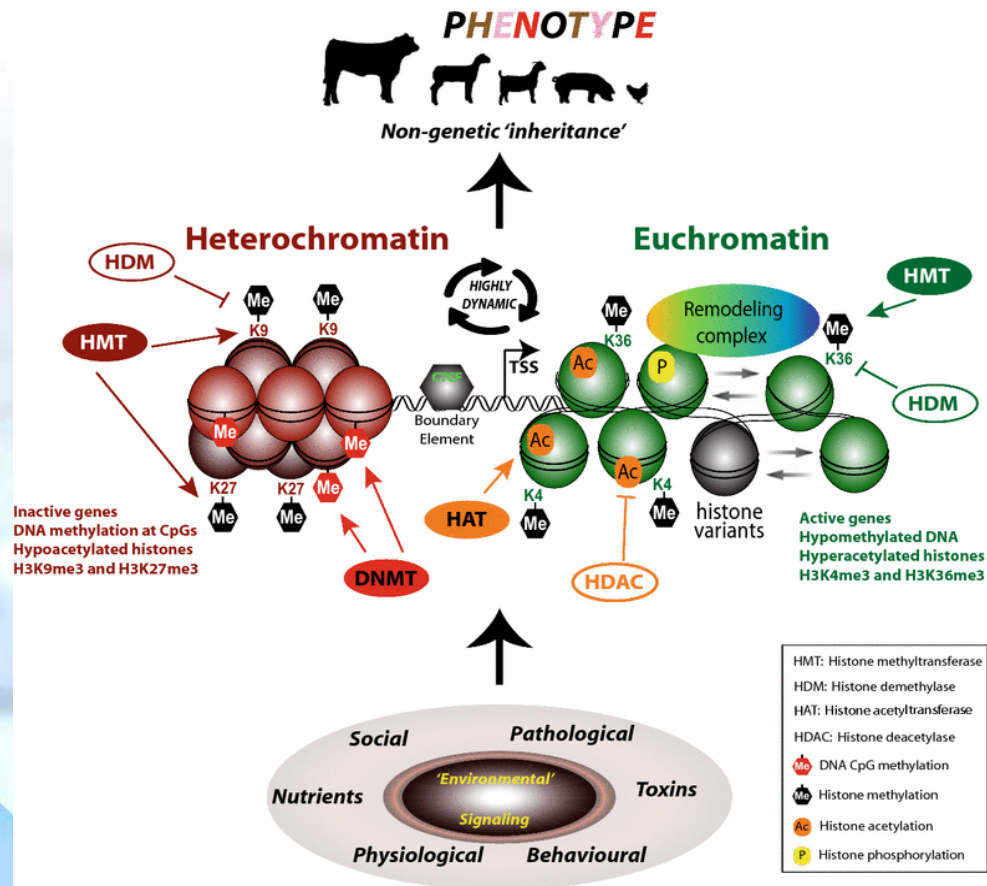
Critical CpG Sequences in CpG Islands Near Promoters



Pyrosequencing technology represents a tool to determine methylation levels of multiple CpG sites in specific genes of interest and this study shows its applicability to investigate pollutant-induced alterations of methylation levels in fish.

Strömqvist M, Tooke N, Brunström B (2010) DNA methylation levels in the 5'flanking region of the vitellogenin I gene in liver and brain of adult zebrafish (Danio rerio)—Sex and tissue differences and effects of 17 α -ethinylestradiol exposure. Aquatic Toxicology 98: 275-281

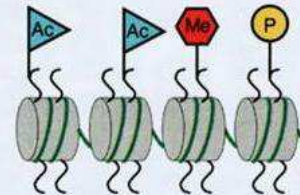
Epigenetics of Euchromatin versus Heterochromatin



HMT: Histone methyltransferase
HDM: Histone demethylase
HAT: Histone acetyltransferase
HDAC: Histone deacetylase
Me: DNA CpG methylation
Me: Histone methylation
Ac: Histone acetylation
P: Histone phosphorylation

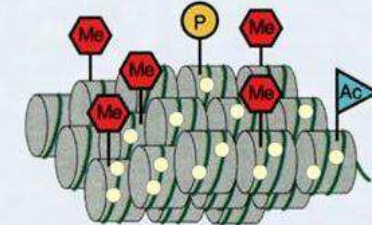
DNA methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials

Euchromatin



- High histone acetylation
- Low DNA methylation
- H3-K4 methylation

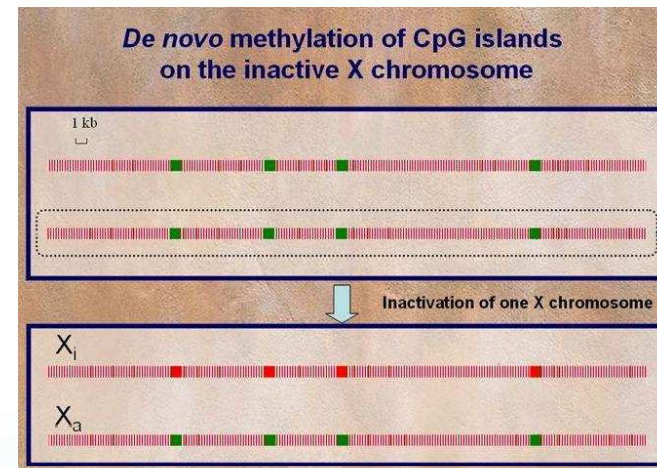
Heterochromatin



- Low histone acetylation
- Dense DNA methylation
- H3-K9 methylation

X Chromosome Inactivation: CG Island Methylation

Genes that are to be silenced from one of the parental allele (i.e. expressed by only one allele) become methylated during the embryonic development in a process called **imprinting**



This reprogramming of DNA methylation found in zebrafish is similar to the reprogramming during mammalian development

FISH and FISHERIES

FISH and FISHERIES, 2013, 14, 528-533

The implications for aquaculture practice of epigenomic programming of components of the endocrine system of teleostean embryos: lessons learned from mammalian studies

Mao Li & John F. Leatherland

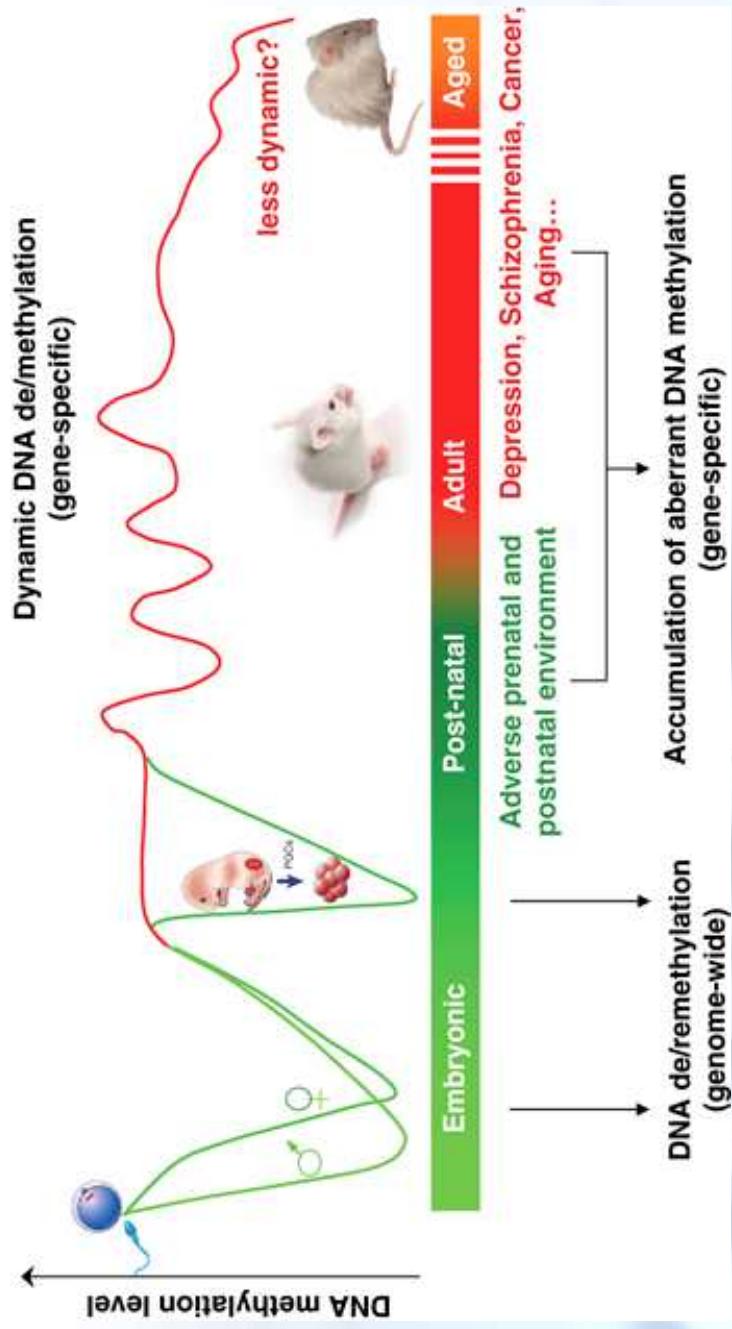
Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, N1G 2W1, Canada

DNA methylation reprogramming was observed during the early embryonic development of **zebrafish** (*Danio rerio*) in a recent study using an anti-5-methylcytosine antibody in immunohistochemistry and southwestern immunoblotting

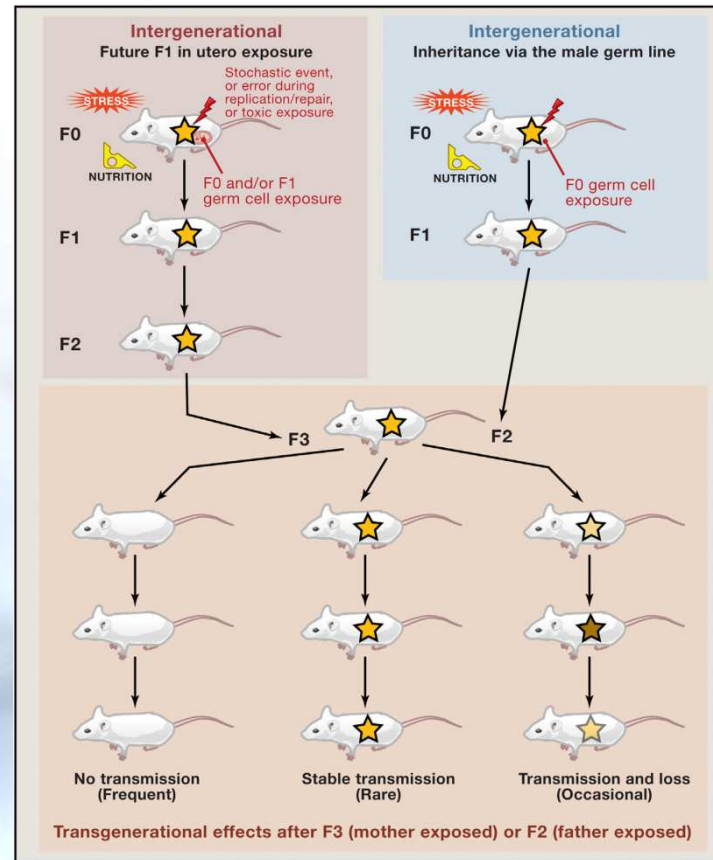
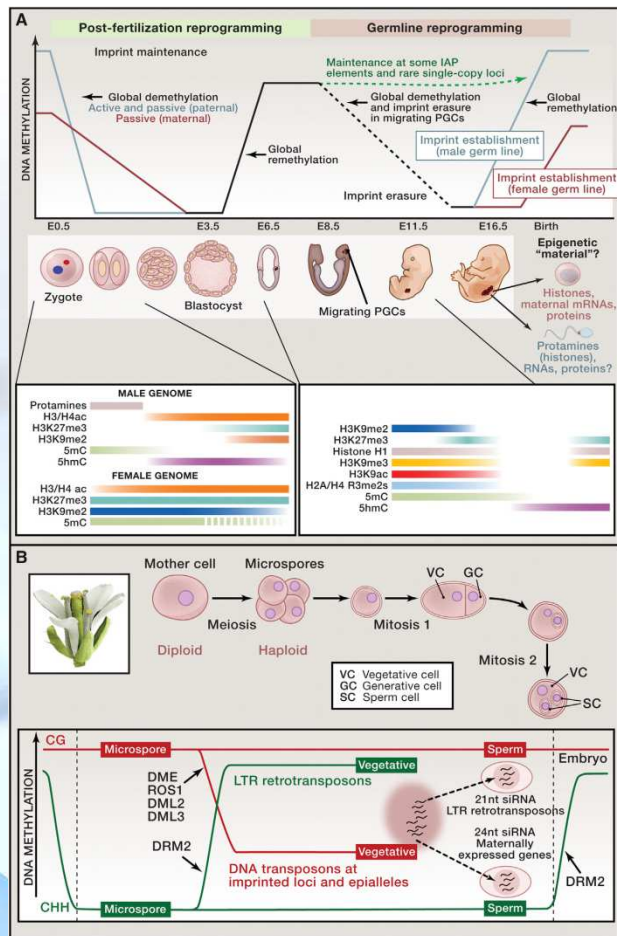


MacKay AB, MhanniAA, McGowanRA, Krone PH (2007) *Immunological detection of changes in genomic DNAmethylation during early zebrafish development. Genome 50: 778-785.*

Santos F, DeanW (2004) *Epigenetic reprogramming during early development in mammals. Reproduction 127: 643-651.*



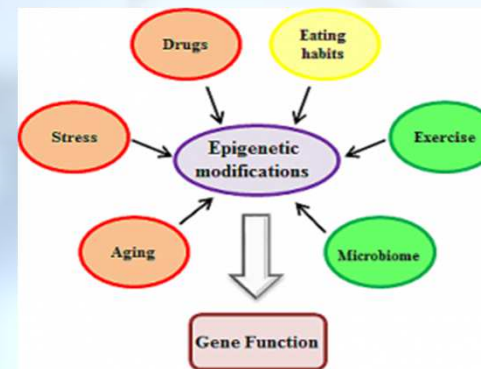
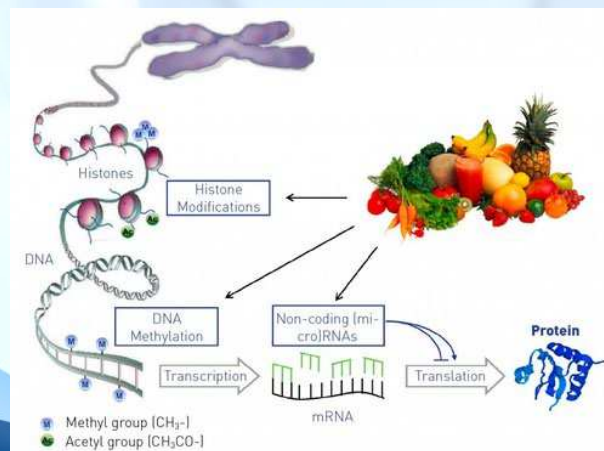
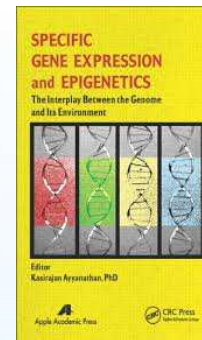
Epigenetic changes can inhere the next generation



Edith Heard and Robert A. Martienssen
<http://dx.doi.org/10.1016/j.cell.2014.02>

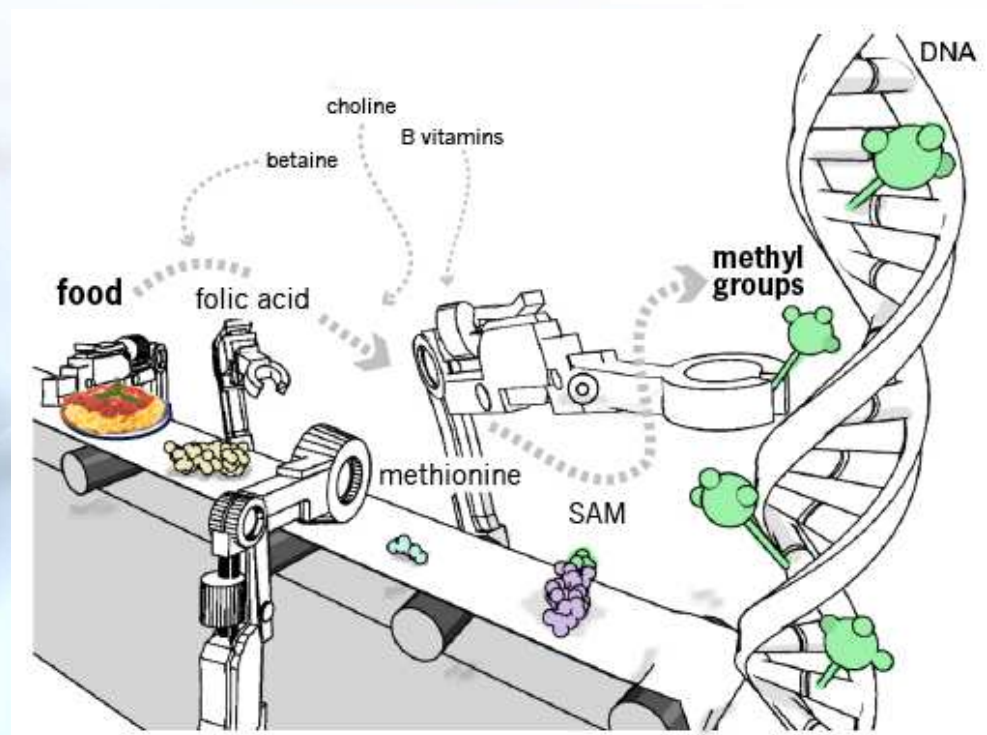
Environmental factors can influence on epigenetic changes

There are epigenetic disease



Familiar nutrients like folic acid, B vitamins, SAM-e (S-Adenosyl methionine, a popular over-the-counter supplement) are key components of this methyl-making pathway.

Diets high in these methyl-donating nutrients can rapidly alter gene expression, especially during early development when the epigenome is first being established

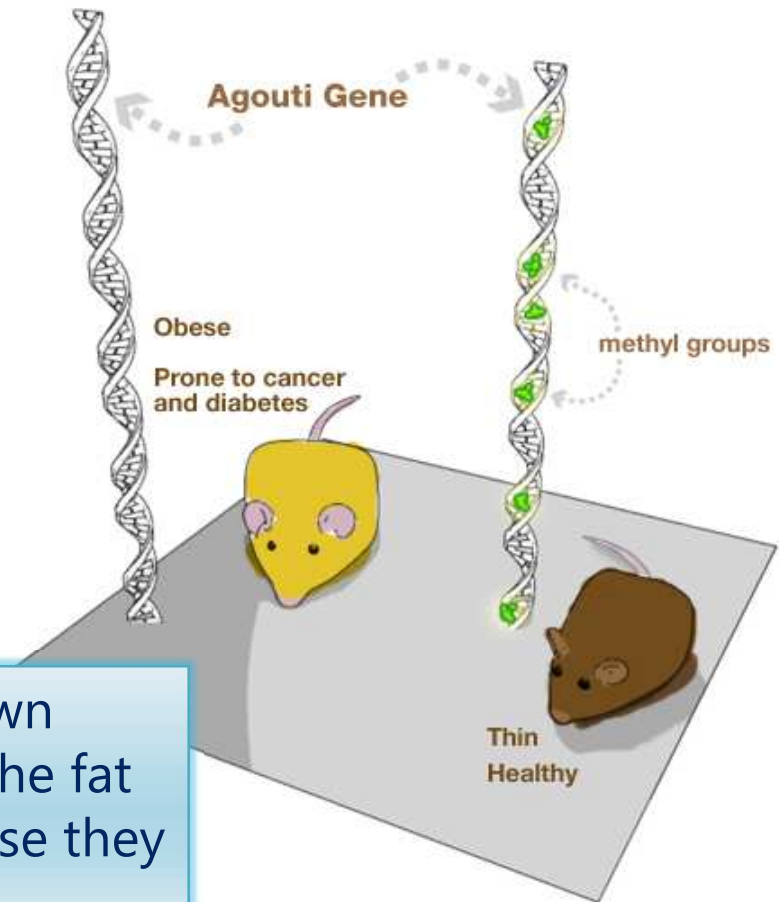


How important a mother's diet is in shaping the epigenome of her offspring

When a mouse's *agouti* gene is completely unmethylated, its coat is yellow and it is obese and prone diabetes and cancer.

When the *agouti* gene is methylated (as it is in normal mice), the coat color is brown and the mouse has a low disease risk.

Fat yellow mice and skinny brown mice are genetically identical. The fat yellow mice are different because they have an epigenetic "mutation."

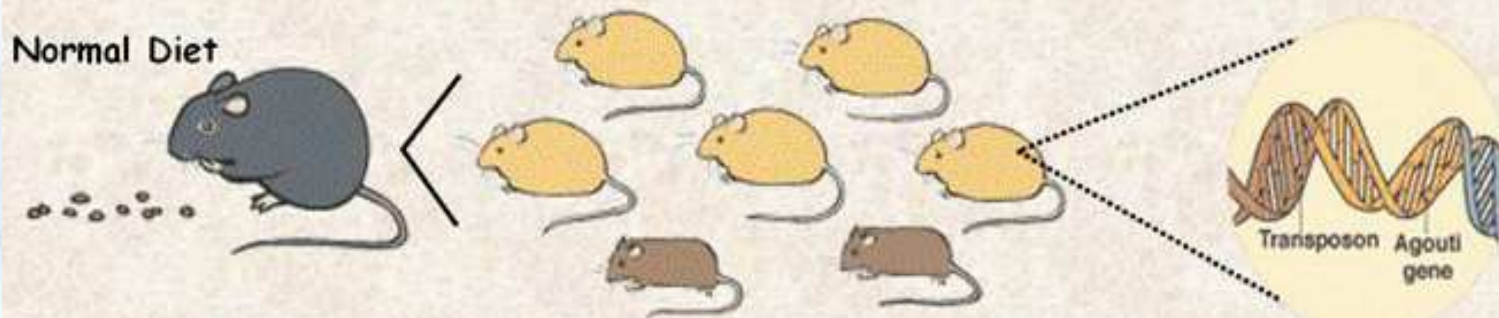


Environment can Influence on Epigenetic Changes

Can environment influence these processes?

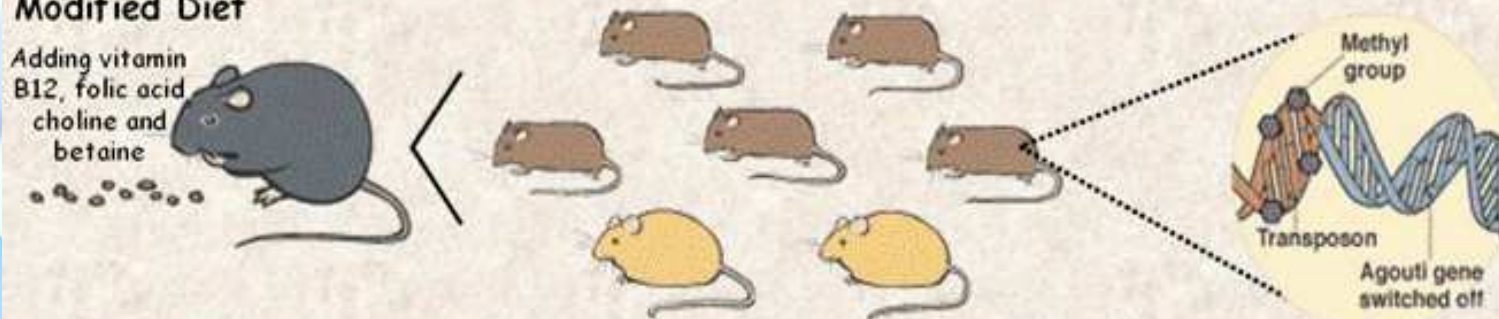
They are what she ate...

Normal Diet



Modified Diet

Adding vitamin B12, folic acid, choline and betaine

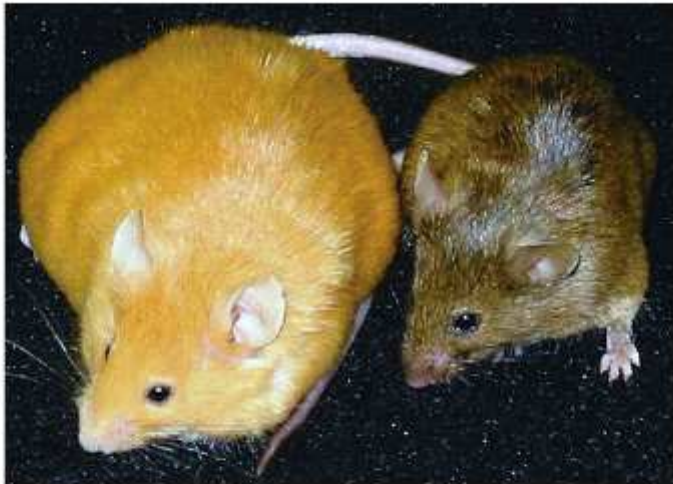


Source: Waterland & Jirtle, Mol Cell Biol (2003)
Also Wolff & Cooney, FASEB J (1998)

Our health is not only determined by what we eat, but also what our parents ate

pregnant mother's diet can affect the child's epigenetic outcome,

These Two Mice are Genetically Identical and the Same Age



While pregnant, both of their mothers were fed Bisphenol A (BPA) but **DIFFERENT DIETS**:

The mother of this mouse received a **normal mouse diet**

The mother of this mouse received a diet **supplemented** with choline, folic acid, betaine and vitamin B12

BPA appears to reduced methylation of the agouti gene. In the strain of mice that was studied, yellow mothers give birth to pups with a range of coat colors from yellow to brown.

When mothers were fed BPA, their babies were more likely to be yellow and obese—like the one shown on the left.

However, when mothers were fed BPA along with methyl-rich foods, the offspring were more likely to be brown and healthy—like the one on the right.

The maternal nutrient supplementation had counteracted the negative effects of exposure

Our health is not only determined by what we eat, but also what our parents ate

dad's diet can affect the child's epigenetic outcome,

food availability between the ages of nine and twelve for the paternal grandfather affected the lifespan of his grandchildren. But not in the way you might think.



Shortage of food for the grandfather was associated with extended lifespan of his grandchildren.

Food abundance, on the other hand, was associated with a greatly shortened lifespan of the grandchildren.

Early death was the result of either diabetes or heart disease

Нехватка продовольствия для дедушки была связана с увеличением продолжительности жизни его внуков.

С другой стороны, изобилие пищи было связано с значительно сокращенной продолжительностью жизни внуков.

Ранняя смерть была результатом либо диабета, либо болезни сердца

The larvae that develop into workers and queens are genetically identical. But because of her royal jelly diet, the queen will develop ovaries and a larger abdomen for egg laying, while the worker will be sterile.

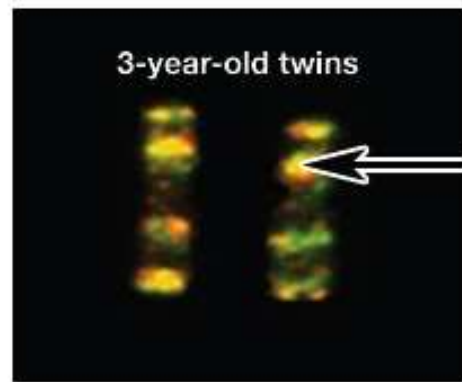


Royal jelly silences a key gene (*Dnmt3*), which codes for an enzyme that silences a group of queen genes.

When *Dnmt3* is turned "on," the queen genes are epigenetically silenced, and the larvae develop into the default "worker" variety. But when royal jelly turns *Dnmt3* "off," the queen genes jump into action, turning the larvae into queens

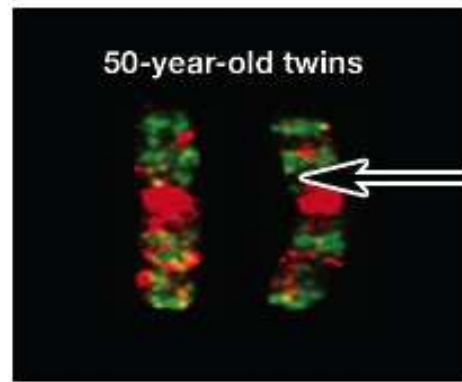
Twins share the same genes but their environments become more different as they age.

Chromosome 3 Pairs
3-year old twins vs. 50-year-old twins



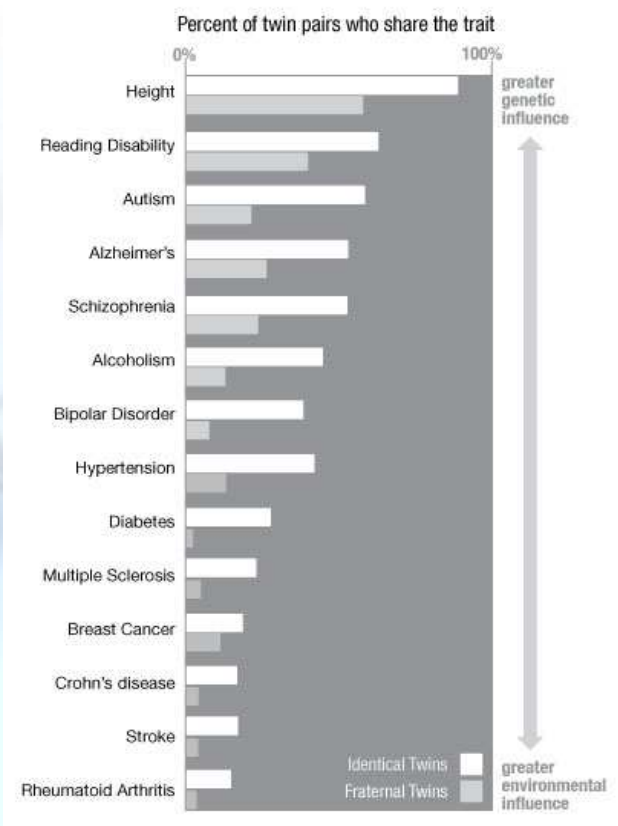
3-year-old twins

Yellow shows where the twins have epigenetic tags in the same place.



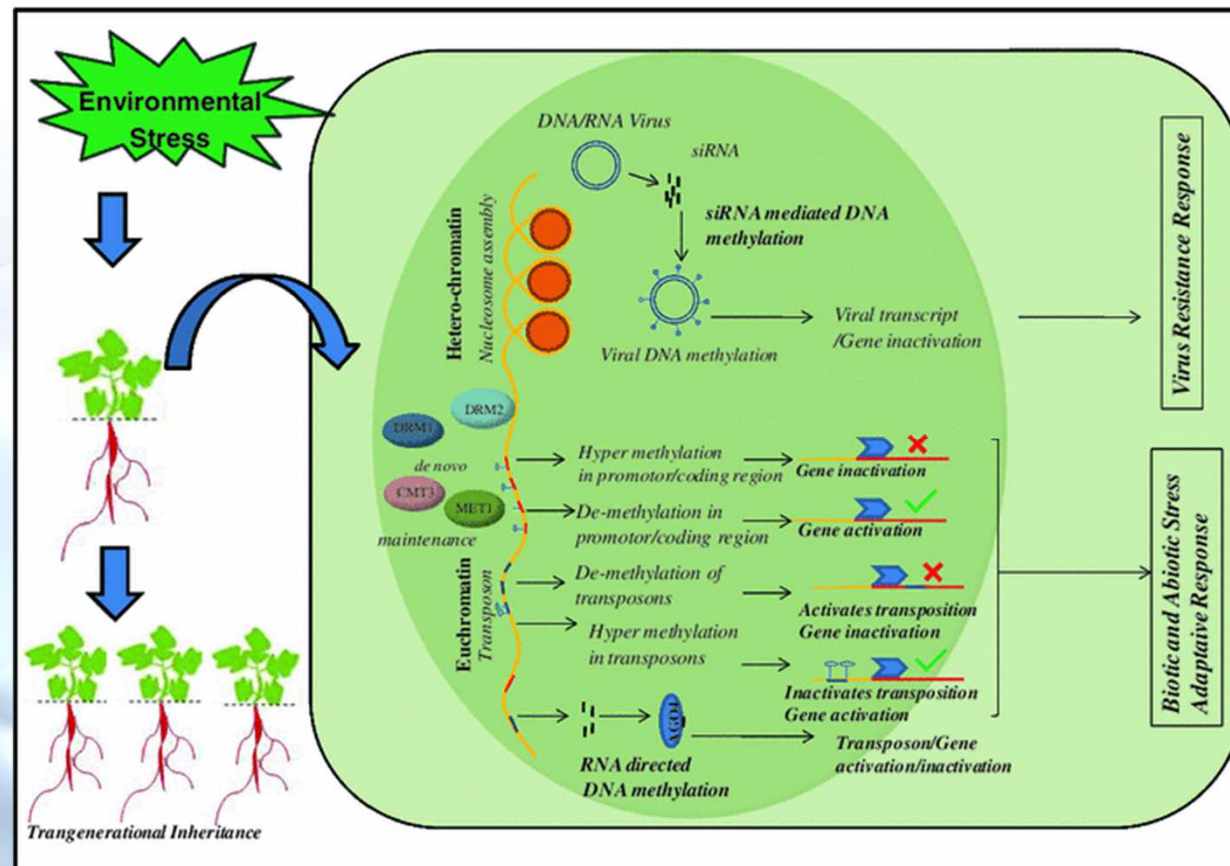
50-year-old twins

Red and green show where the twins have epigenetic tags in different places.



Environment can Influence on Epigenetic changes

The effect of environmental factors on epigenetic inheritance is equally important to the ecological sciences.





A Review of Epigenetic Imprints in Aquatic Animals

Hamed Daneshfozouna^{1*}, Faraz Panjvinib², Fatemeh Ghorbani³ and Hamid Farahmandd⁴

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³Department of Genetic, The University of Isfahan, Isfahan, Iran

⁴Department of Aquaculture, University of Tehran, Karaj, Iran



Gene

Volume 385, 30 December 2006, Pages 111–121



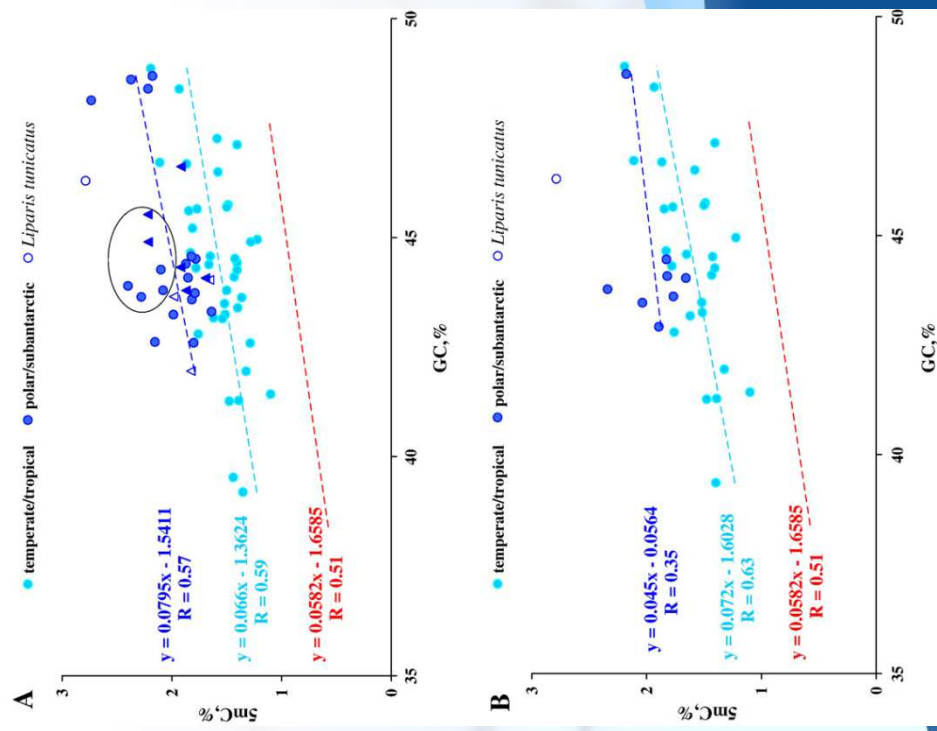
DNA methylation and body temperature in fishes

Annalisa Varriale, Giorgio Bernardi [Show more](#)

10.1016/j.ygeno.2006.10.003

0969-5962/\$ - see front matter © 2006 Elsevier B.V. All rights reserved.

Order	Family	Species	Source	evaluated	Species	GC	5mC	R ²	Families/ genera	
									GC	5mC
Osteoglossiformes	Pomacentridae	<i>Pomacentrus biwaensis</i>		0.77	45.66	1.77	3.88			
	Notopetridae	<i>Notopetris notopetris</i>			44.96	1.22	2.72			
	Cyprinidae	<i>Caridina pulchellus</i>	a	1.60	39.12	1.40	2.98			
	Cyprinidae	<i>Brevoortia tyrone</i>	b	1.68	39.19	1.34	3.45			
	Serranidae	<i>Oreochromis mossambicus</i>	c	2.49	45.75	1.49	3.25			
	Gobiidae	<i>Micropogonias undulatus</i>	d	0.84	46.09	2.18	4.47			
	Gobiidae	<i>Boreogadus saida</i>	d	0.88	46.40	2.22	4.58			
	Gobiidae	<i>Stenogobius genivittatus</i>	d	0.88	46.40	2.22	4.58			
	Gobiidae	<i>Stenogobius genivittatus</i>	d	0.40	46.13	2.17	4.68			
	Gobiidae	<i>Notobrama flava</i>	e	1.20	45.99	1.52	3.49			
Cyprinodontiformes	Apocheilichthys	<i>Apocheilichthys elegans</i>			45.70	1.50	3.28			
	Poeciliidae	<i>Poecilia reticulata</i>	e	1.36	42.80	1.76	4.11			
	Poeciliidae	<i>Gambusia affinis holbrooki</i>	e	0.76	41.45	1.10	2.66			
	Poeciliidae	<i>Artemia salina</i>			43.17	1.62	3.75			
	Poeciliidae	<i>Aphanipterus fuscatus</i>	e		40.59	1.87	3.90			
	Cyprinodontidae	<i>Sicyopterus japonicus</i>	b	1.40	41.27	1.48	3.58			
	Cyprinodontidae	<i>Scorpaenopsis brevipinnis</i>	b		44.31	1.78	4.02			
	Cyprinodontidae	<i>Leuciscus leuciscus</i>	b		44.39	1.66	3.75			
	Cyprinodontidae	<i>Lepomis gibbosus</i>	d	0.88	43.40	1.40	3.23			
	Cyprinodontidae	<i>Lepomis microlophus</i>	d		43.25	1.51	3.59			
Poeyiformes	Apoecyprididae	<i>Apoecypridina</i>		0.94	44.58	1.65	3.70			
	Carangidae	<i>Trachurus affinis</i>	a	0.90	44.65	1.83	4.10			
	Sparidae	<i>Brevoortia tyrone</i>	c	0.52	44.10	1.43	3.25			
	Mullidae	<i>Merluccius merluccius</i>	f		41.26	1.41	3.18			
	Phycitidae	<i>Herichthys minckleyi</i>	b	1.10	43.15	1.54	3.57			
	Pomacentridae	<i>Microgobius gulosus</i>	b	1.21	44.90	1.28	2.85			
	Labridae	<i>Cirrhilabrus</i>	b	0.80	44.43	1.41	3.16			
	Labridae	<i>Thalassoma grammacium</i>	b	0.73	45.22	1.81	4.01			
	Labridae	<i>Stomobatus</i>	a	1.25	41.95	1.32	3.13			
	Labridae	<i>Scorpaenopsis</i>	b	1.07	46.62	1.92	4.11			
Serranidae	Zoaridae	<i>Illicorynus phalarinus</i>	b*	43.65	1.98	4.54				
	Brevoortidae	<i>Centropristis striata</i>	b*	44.08	1.66	3.76				
	Brevoortidae	<i>Eleutheronotus temminckii</i>	b*	44.40	1.87	4.22				
	Notembranchiidae	<i>Notembranchia caesiops</i>	b	44.52	1.78	4.00				
	Notembranchiidae	<i>Notembranchia rostrata</i>	b	42.62	2.15	5.05				
	Gobiomorphidae	<i>Gobiomorus dormitor</i>	b*	44.32	1.92	4.33				
	Gobiomorphidae	<i>Gobiomorus dormitor</i>	b*	43.47	2.04					

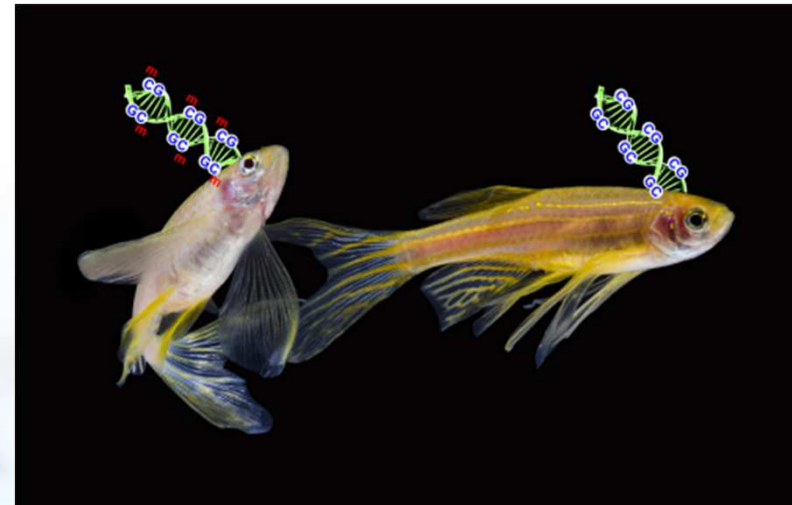


Research

Epigenetic modification and inheritance in sexual reversal of fish

Changwei Shao,^{1,8} Qiye Li,^{2,3,4,5,8} Songlin Chen,^{1,8,9} Pei Zhang,² Jinmin Lian,² Qiaomu Hu,¹ Bing Sun,¹ Lijun Jin,² Shanshan Liu,¹ Zongji Wang,^{2,3} Hongmei Zhao,² Zonghui Jin,² Zhuo Liang,¹ Yangzhen Li,¹ Qiumei Zheng,² Yong Zhang,² Jun Wang,^{2,6,7} and Guojie Zhang^{2,4,9}

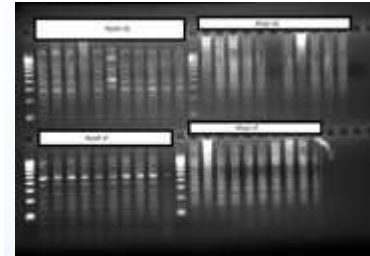
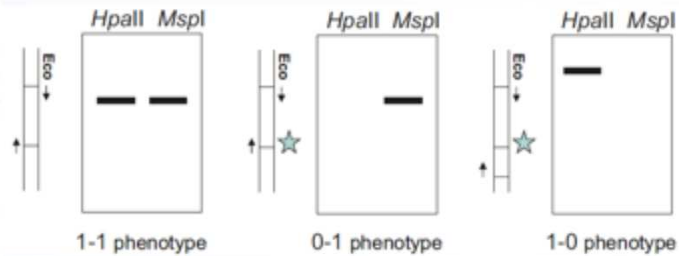
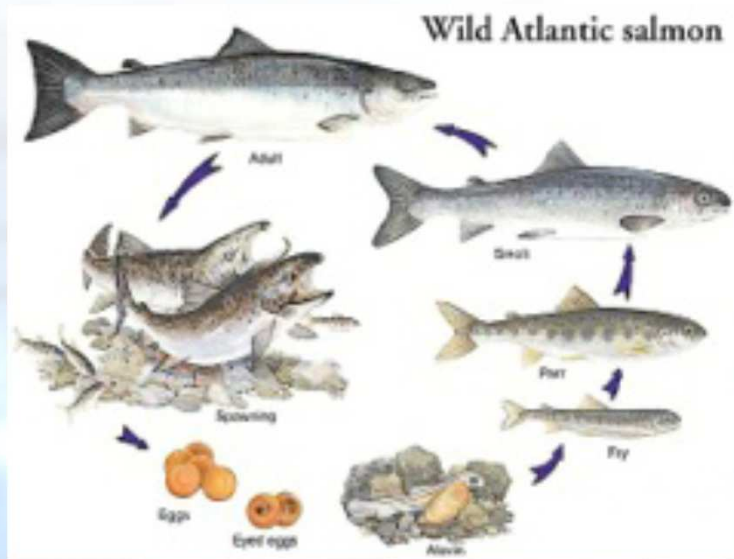
¹Yellow Sea Fisheries Research Institute, CAFS, Key Lab for Sustainable Development of Marine Fisheries, Ministry of Agriculture,



Epigenetic mechanisms (i.e., DNA methylation) are involved in determining sexual traits and sex specific gene expression

To look at brain because of its role in behavioural differences between sexes, as well as it is the second most sexually dimorphic organ (after the gonads) and there is a wealth of evidence supporting sex specific gene expression other organisms

Epigenetics can explain the phenotypic variance that cannot be explained by genetic variance

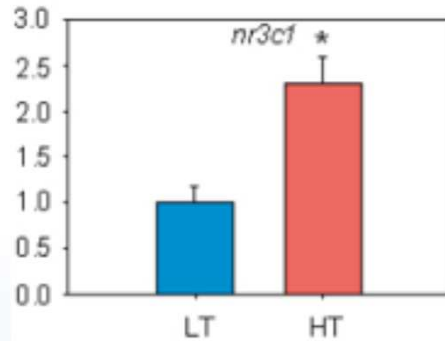


GeneHc. AFLP: EcoRI + MseI

EpigeneHc. MS-AFLP: EcoRI + HpaII or MspI

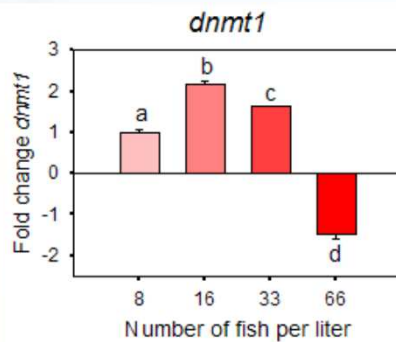
Epigenetics and Stress

Sea bass
Thermal stress

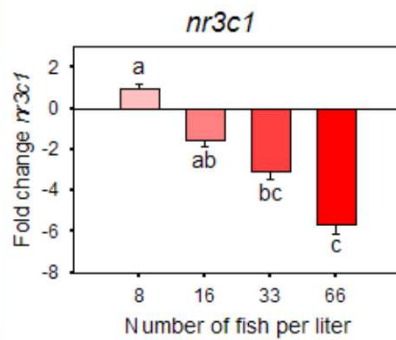


Díaz & Piferrer (2015).
BMC Genomics

Zebrafish
Confinement
stress



Valdivieso et al. (Unpubl. Obs.)



DNA methylation is unique from other epigenetic mechanisms increased mutability

For example, the rate of C-to-T mutations is 10-fold to 50-fold higher in humans' methylated cytosines

(Duncan and Miller 1980; Bulmer 1986; Britten et al. 1988; Sved and Bird 1990).

patterns of depletion of CpG dinucleotides include adaptive mutations or reflect neutral and tolerated genomic changes

(Flores and Amdam 2011)

DNA methylation and polyploids

Aquaculture

August 2009, Volume 293, Issues 3-4, Pages 125-156
<http://dx.doi.org/10.1016/j.aquaculture.2009.04.036>
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Archimer
Archive Institutionnelle de l'Ifremer
<http://www.ifremer.fr/doc/elec>

<http://dx.doi.org/10.1016/j.aquaculture.2009.04.036>

Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment

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Spontaneous polyploids have been observed in several phylogenetically distant orders, including both wild and farmed fish species. In the vertebrates, polyploid species are not exclusive to fish, since they have been reported in different groups, from amphibians to occasionally even in mammals. Polyploids can originate either from alterations of meiotic or mitotic processes in specimens within a species (autopolyploidy) or by reproductive contact among species (allopolyploidy).

Schulz R.J. (1967) Gynogenesis and triploidy in the viviparous fish *Poeciliopsis*. *Science* 157:1564-1567.

Thorgaard G.H, Gall G.AE (1979) Adult triploids in a rainbow trout family. *Genetics* 93: 961-973.

Piferrer F., Beaumont A., Falguière J., Flajšhans M, Haffray P. (2009) Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293:125-156.

Conclusion

-Epigenetics can explain the phenotypic variance that cannot be explained by genetic variance

-Epigenetic mechanisms provide organisms with the ability to integrate genomic and environmental information to produce a given phenotype

-The effect of the environment can be passed down to the following generations even if the original stimulus is no longer present. This opens the possibility of epigenetic programming in animal husbandry, which is a non-pharmacological approach to production improvement

Conclusion

-Timing is everything. Effects are stronger if the stimulus occurs during early development. Epigenetic modifications can be permanent and are involved in the process of domestication

-Transgenerational epigenetic inheritance is a type of non-Mendelian inheritance that involves retention vs. reprogramming of epigenetic marks and is dependent on DNA methylation

The regulatory effects of DNA methylation could be divided in two broad categories:

Specific
a particular
gene activity is
directly
influenced by
methylation of
its regulatory
regions,

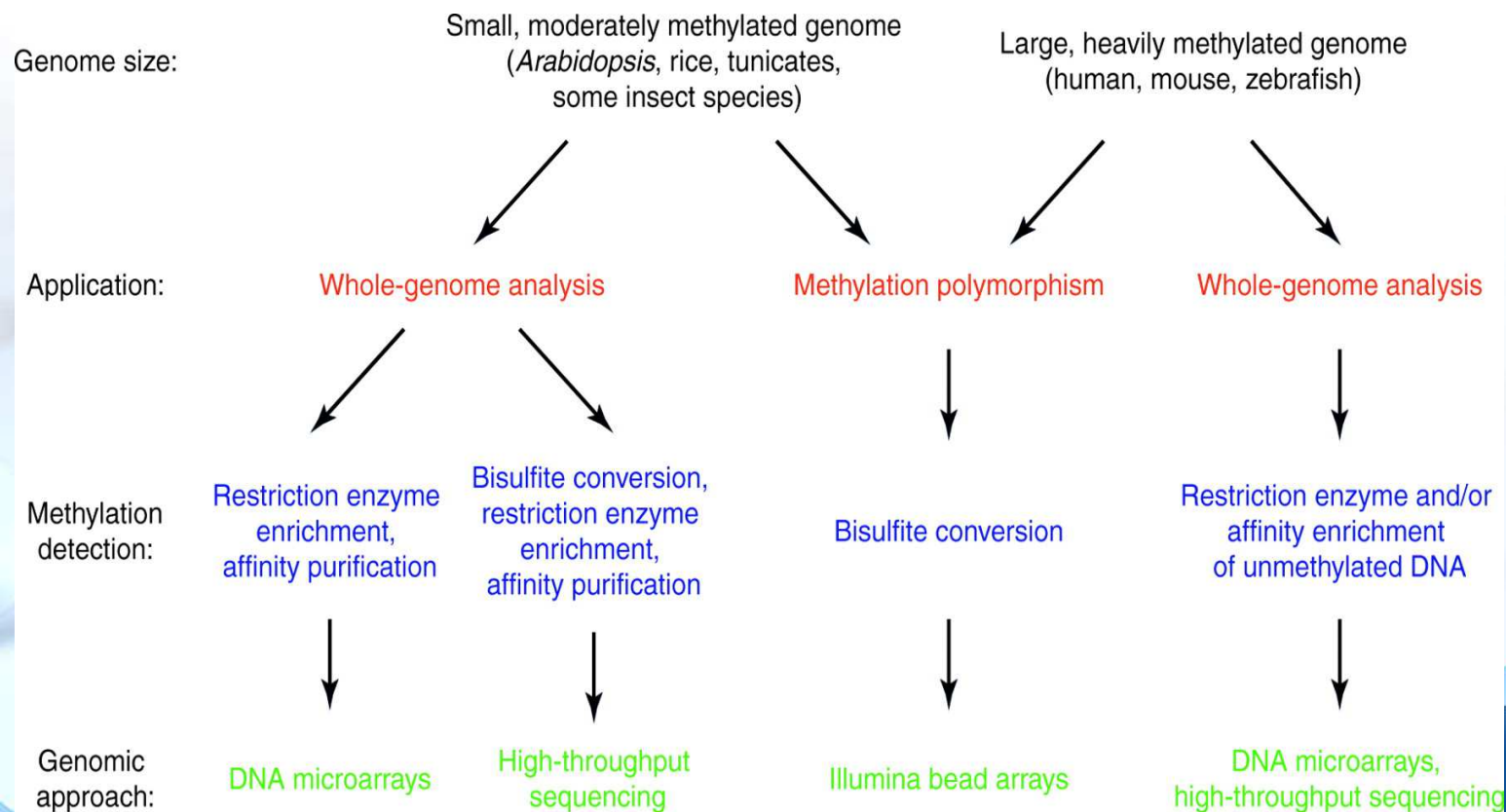
General
methylation
causes changes
in the chromatin
structure

Specific methylation

Table 1 Summary of gene, transposons, and promoter fragments of genome differentially regulated through DNA methylation as an epigenetic reprogramming

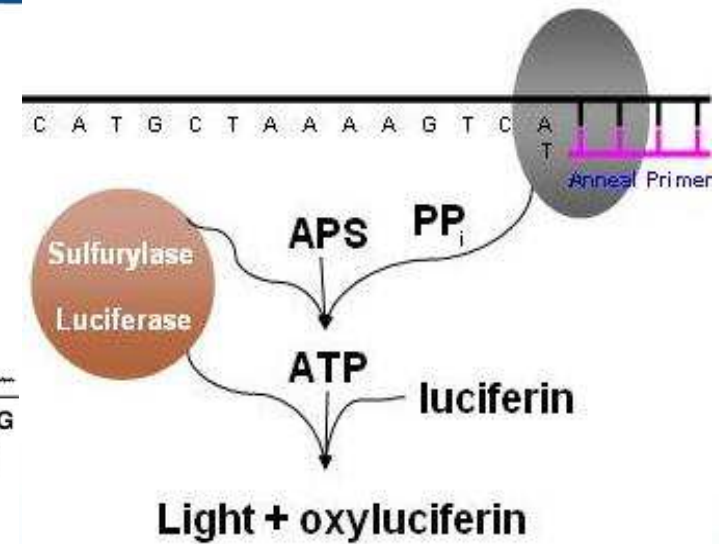
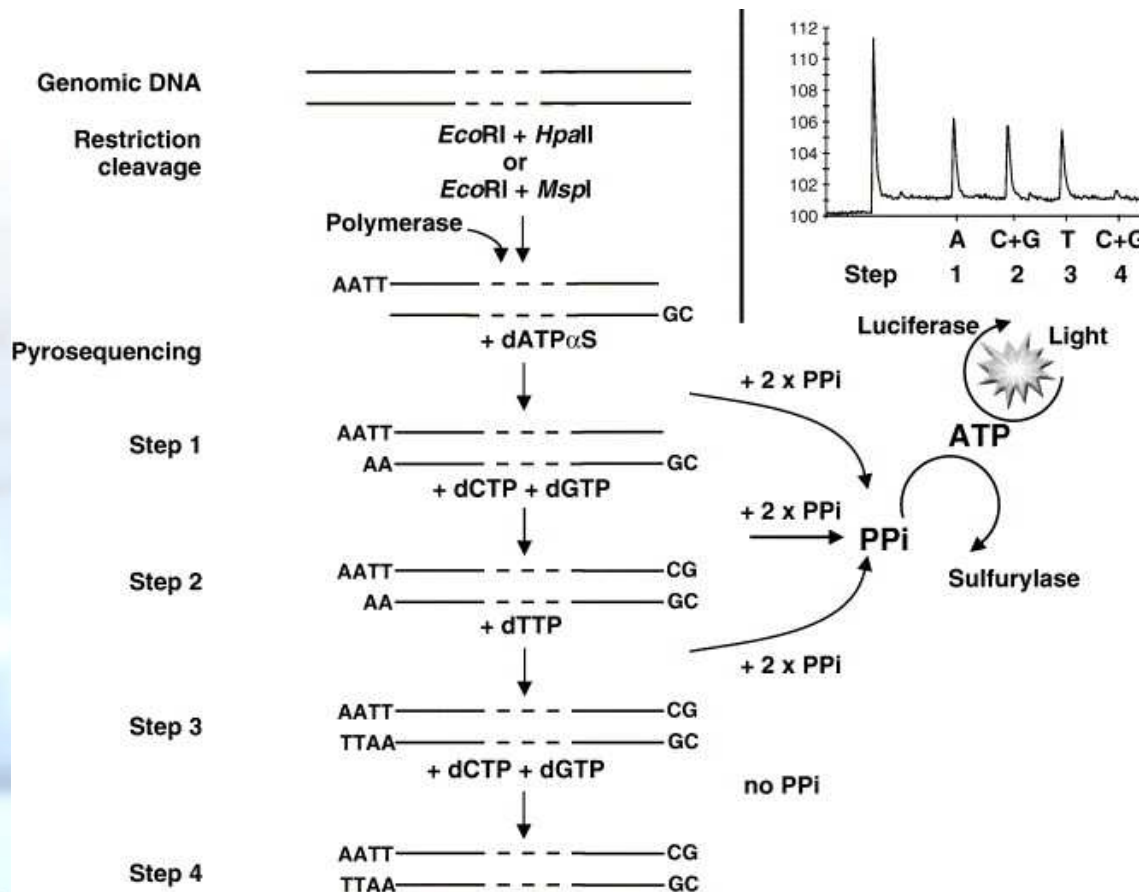
S. No.	Genome region	Plant	Methylation status	Stress	Mode of action	References
Transposons						
1	TAM3	<i>Antirrhinum majus</i>	Hypomethylation	Low temperatures stress	Methylation at CHH motifs	Hashida et al. (2006)
2	<i>MuDR</i>	Maize	Hypomethylation	N+ implantation	Increases the expression of <i>mudrA</i> and <i>mudrB</i>	Hashida et al. (2006)
3	<i>Ac/Ds</i> transposon	Maize	Demethylation	Cold stress	Cold-induced root-specific demethylation	Steward et al. (2000)
Gene/coding segment						
1	<i>ZmM11</i>	Maize	Demethylation	Cold stress	Cold-induced root-specific demethylation	Steward et al. (2000)
2	Nuclear genome	<i>Mesembryanthemum crystallinum</i>	Hypermethylation	High salinity	CpNpG methylation	Dyachenko et al. (2006)
3	Sodium transporter gene (<i>AtHKT1</i>)	<i>Arabidopsis</i>	Hypomethylation	Salt tolerance	Loss in cytosine methylation in a putative small RNA target region	Baek et al. (2011)
4	Non-transposon <i>Asr1</i>	Tomato	Asymmetric CNN methylation	Water stress	Drought conditions brought about higher CG methylation levels in the first exon	González et al. (2011)
5	<i>NtAlxl</i>	Tobacco	Hypomethylation	<i>Tobacco mosaic virus</i>	Altered DNA methylation	Wada et al. (2004)
Promotor						
1	<i>Glyma1g02400</i>	Soybean	Hypomethylation	Salinity stress	-518 to -274, most of the cytosines were demethylated following exposure to salinity stress for 1-24 h	Song et al. (2012)
2	<i>Glyma16g27950</i>	Soybean	Hypomethylation	Salinity stress	Hypomethylation at transcription start codon (+24 to +233)	Song et al. (2012)
3	<i>Glyma20g30840</i>	Soybean	Hypomethylation	Salinity stress	Hypomethylated cytosines at promoter region 1 (-87 to +163)	Song et al. (2012)
4	<i>RMG1 promoter</i>	<i>Arabidopsis</i>	Demethylation	<i>Pseudomonas syringae</i>	RMG1 is targeted by RdDM and ROS1-Dependent DNA demethylation	Yu et al. (2013b)

The methods of total DNA methylation analysis



Daniel Zilberman, and Steven Henikoff Development 2007;134:3959-3965

Luminometric methods (LUMA)



Luminometric methods (LUMA)

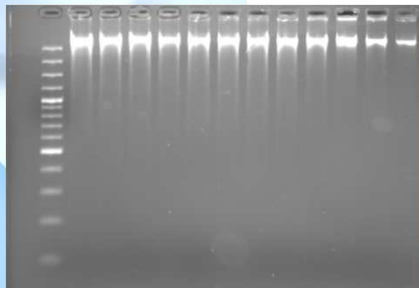
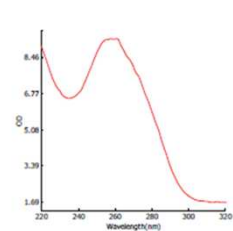
DNA isolation

Tika izmantotas trīs DNS izdalīšanas metodes:
izsālīšanas metode *Aljanabi 1997*
metode ar fenola-hloroforma attīrīšanu
izdalīšana ar DNeasy Plant MiniKit palīdzību *ar ražotāja rekomendācijām.*

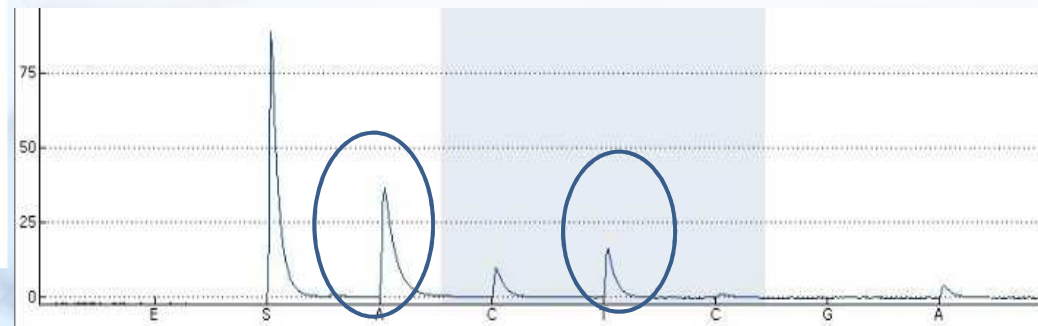
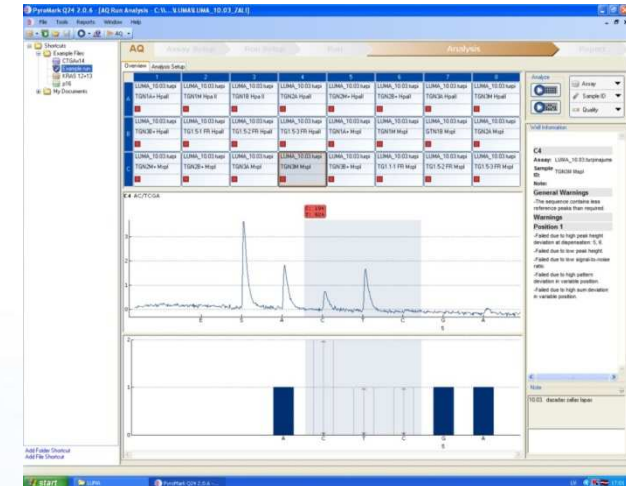


Nucleic Acid Conc : 383.08 ng/ μ L
OD260/280 : 1.80
OD260/230 : 1.46

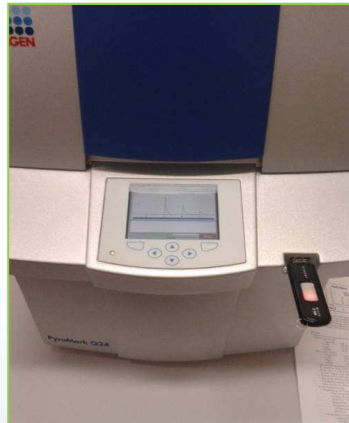
Item	Result
OD260	9.323
OD280	5.920
OD230	6.892
OD320	1.561
Pathlength (mm)	0.672
Dilution	1.000



Our study showed that LUMA is sensitive to poor DNA quality and DNA fragmentation



Luminometric methods (LUMA)



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Plant genetics

EVALUATION OF LUMINOMETRIC METHODS FOR STUDY OF THE GC AND GNC METHYLATION IN NUCLEAR DNA OF SOME PLANTS

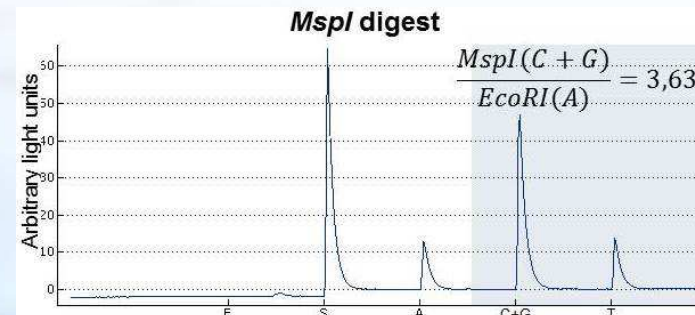
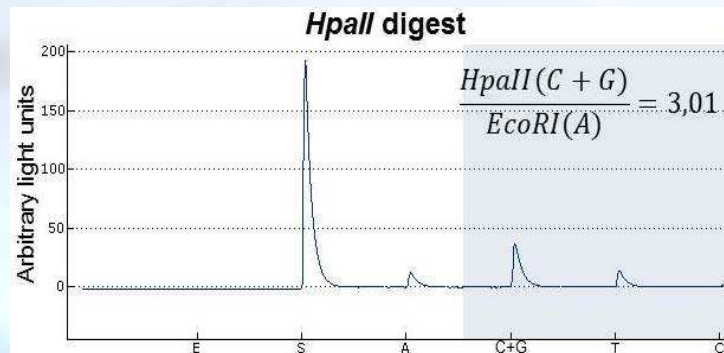
Natalja Shkute*, Dmitrijs Uzhamckis

Ecology Institute, Daugavpils University,
Vienības 13, LV-5400 Daugavpils, Latvia

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Enzymatic methylation of nuclear DNA with creation of 5-methylcytosine (5mC) is one of major mechanisms of epigenetic modification. Change in overall DNA methylation pattern

8.33 x 11.67 in



Per cent methylation is calculated

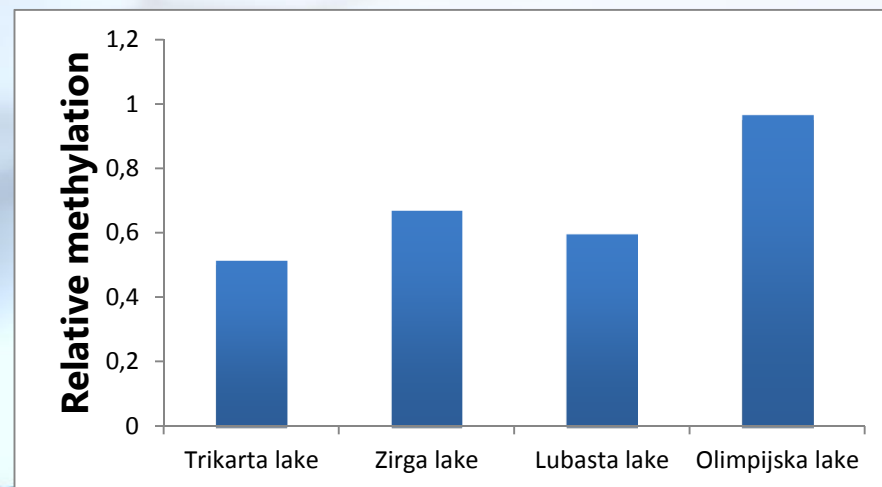
$1 - \frac{HpaII(G)}{EcoRI(T)} / \frac{MspI(G)}{EcoRI(T)} \times 100$, where G and T are the peak heights for HpaII or MspI (methylation) and EcoRI (input DNA), respectively

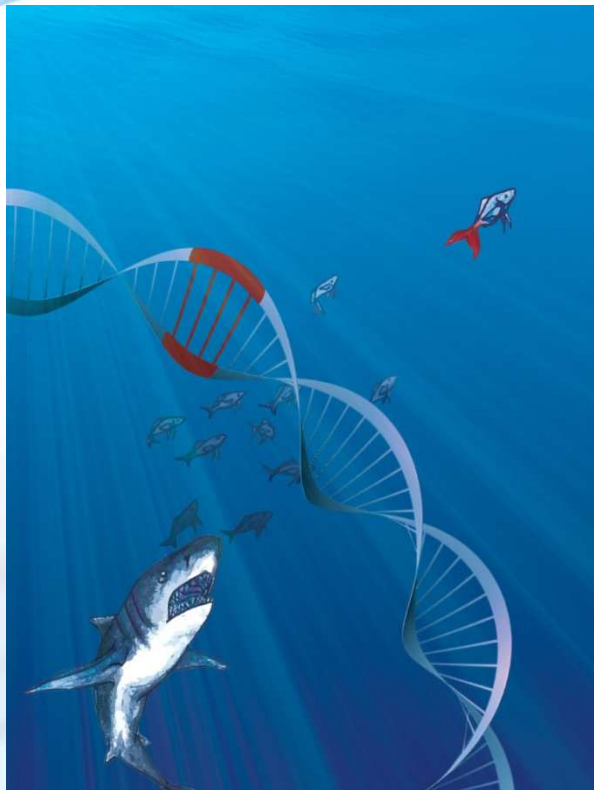
Perccottus glenii



Perccottus glenii, caught in. Lake
Trikartu, Daugavpils A., Pupins M.,
Skute A., Pupina Ag., Karklins A.
2015

The study of invasion success must be considered complete with the evolutionary genetics, as it might be correlated with the genetic polymorphism of populations, which directly influence the invasive species capacity for dissemination (Lee, 2002).





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Thank your for attention