Uloga epigenetičkih fenomena u ekologiji i evoluciji biljaka; studija slučaja endemičnih hrvatskih kadulja

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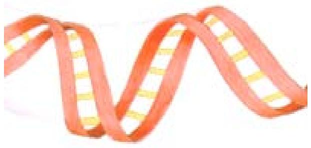
Biološki odsjek, PMF

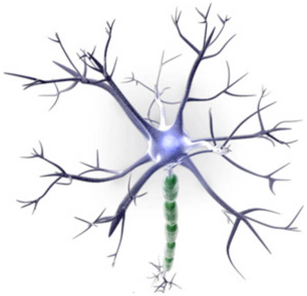
Matt Ray/EHP

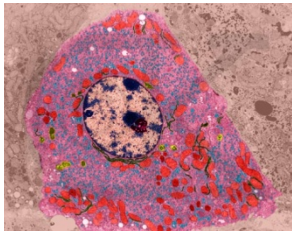
Epigenetics defines cellular identity

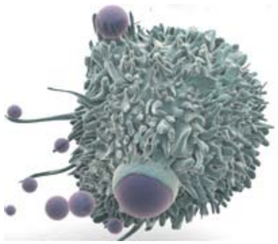
Each cell contains the same DNA



Transcription factors, **Epigenetic information**





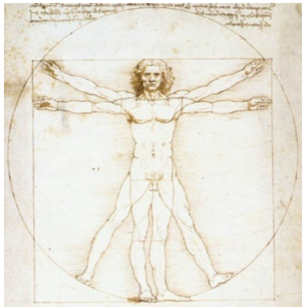


Type of memory necessary to preserve genome function

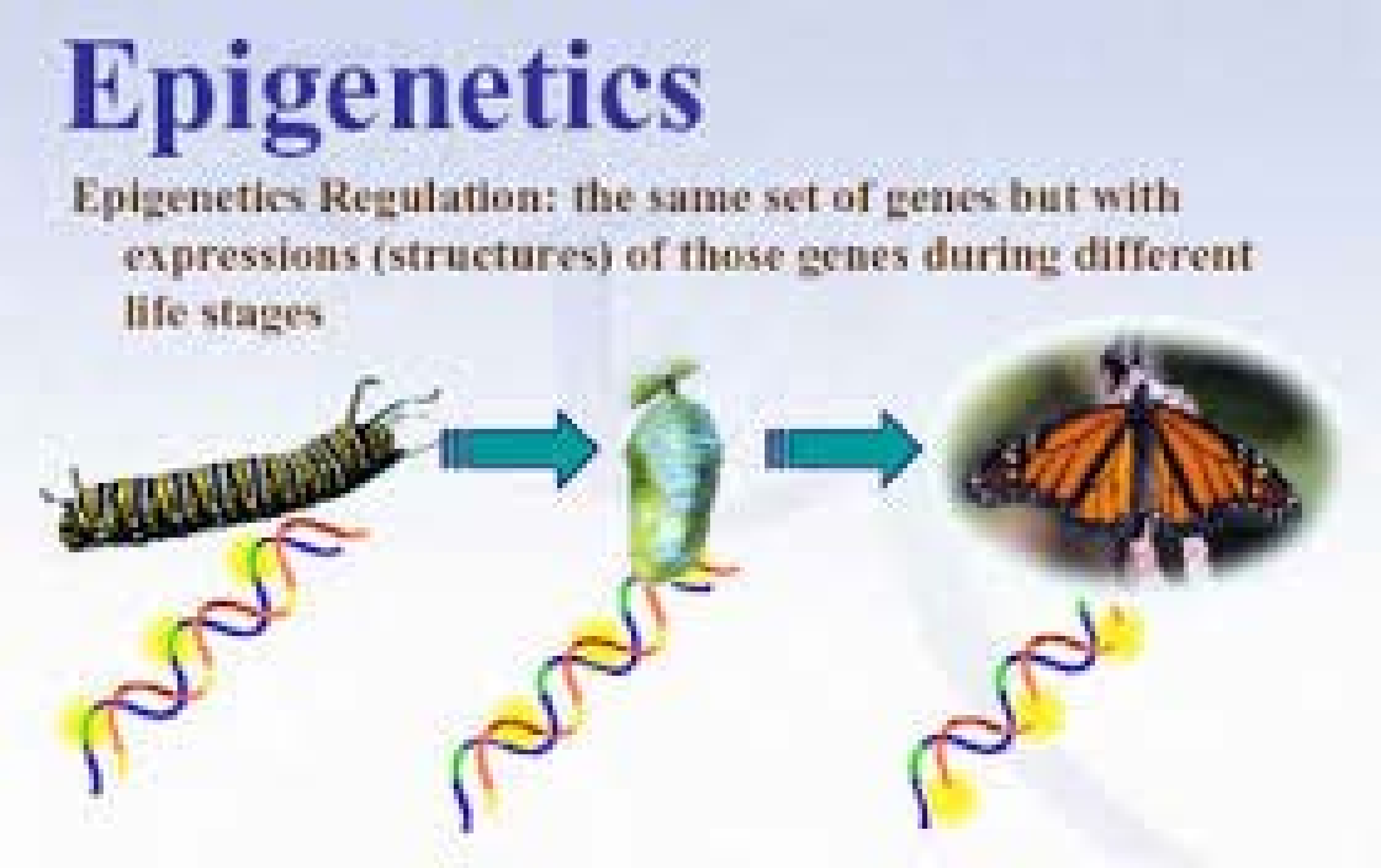
zygote

DEVELOPMENT

Each cell type is characterized by its gene expression profile

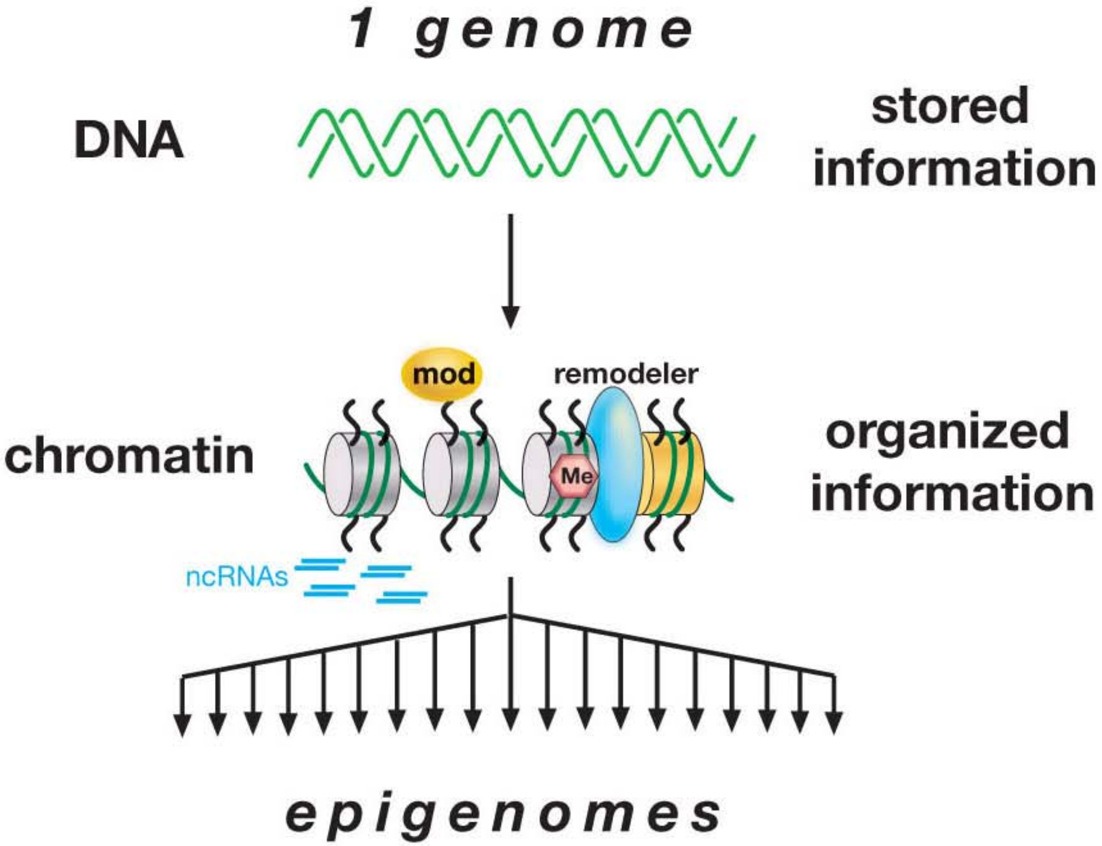


Different patterns of gene expression determine extremly different structures



Epigenetics

Epigenetics Regulation: the same set of genes but with expressions (structures) of those genes during different life stages

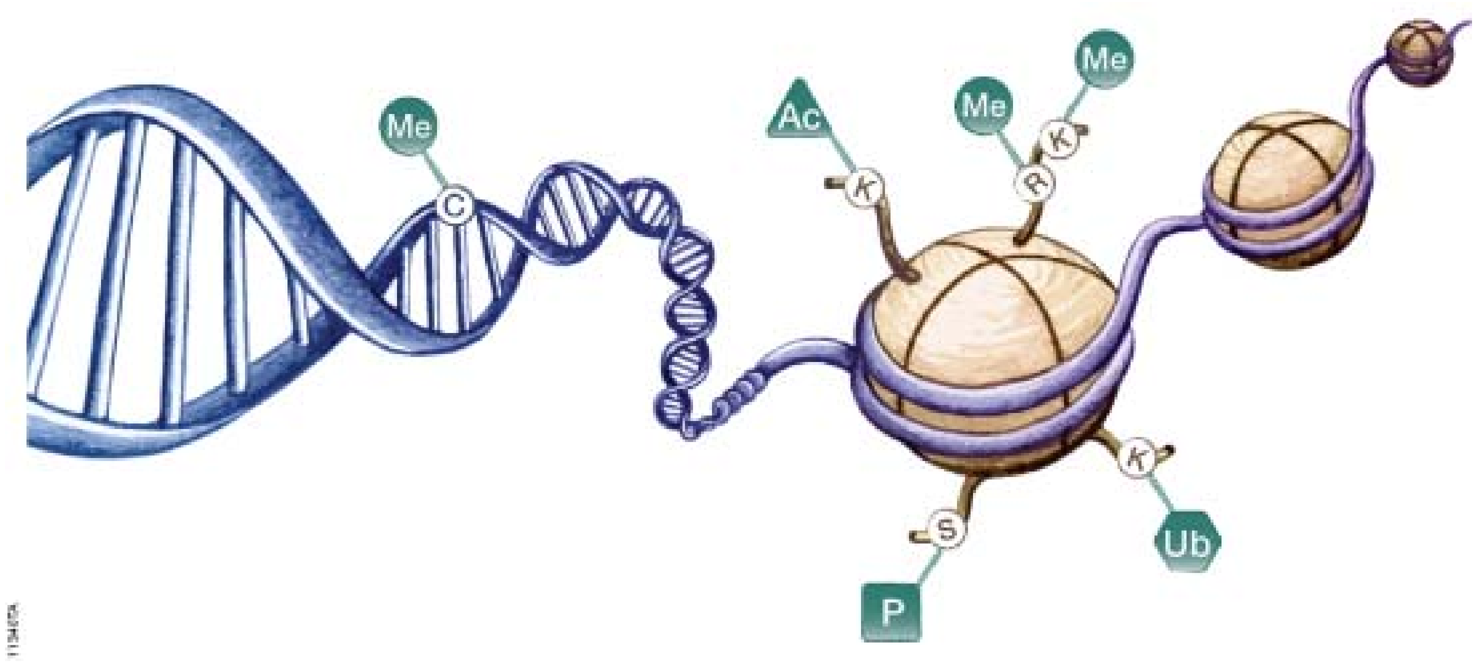


DNA versus CHROMATIN

EPIGENOME – the overal chromatin composition that indexes the entire genome in any given cell.

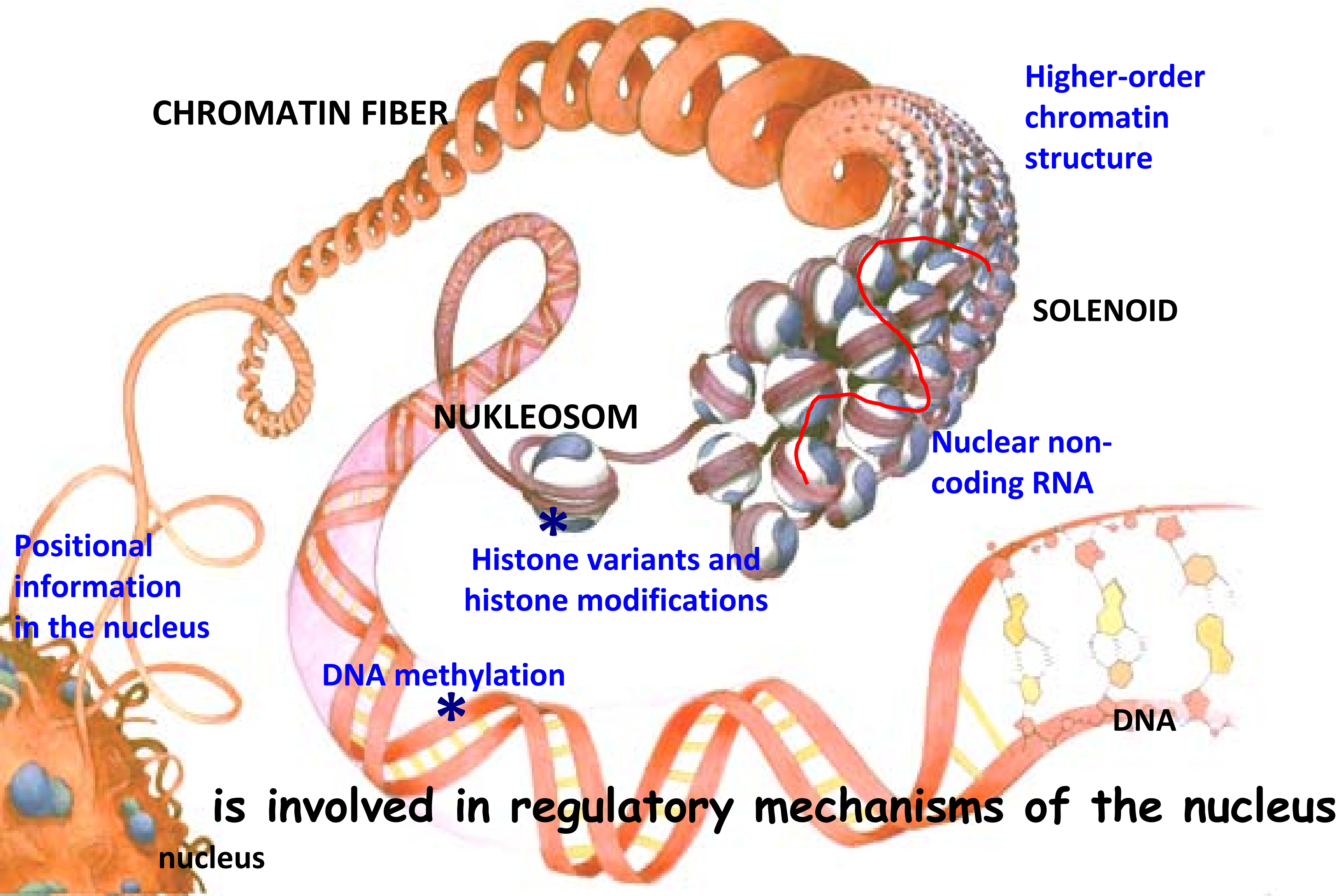
Epigenetic mechanisms

Modifications of DNA molecule and histone tails (Arg, Lys):
by acetylation, methylation, phosphorylation, ubiquitination,
sumoylation, addition o f GlcNac



Histone code - defines open or repressed chromatin state

Epigenetic information at the chromatin level….



CHROMATIN FIBER

Higher-order
chromatin
structure

SOLENOID

NUKLEOSOM

Nuclear non-
coding RNA

*
Histone variants and
histone modifications

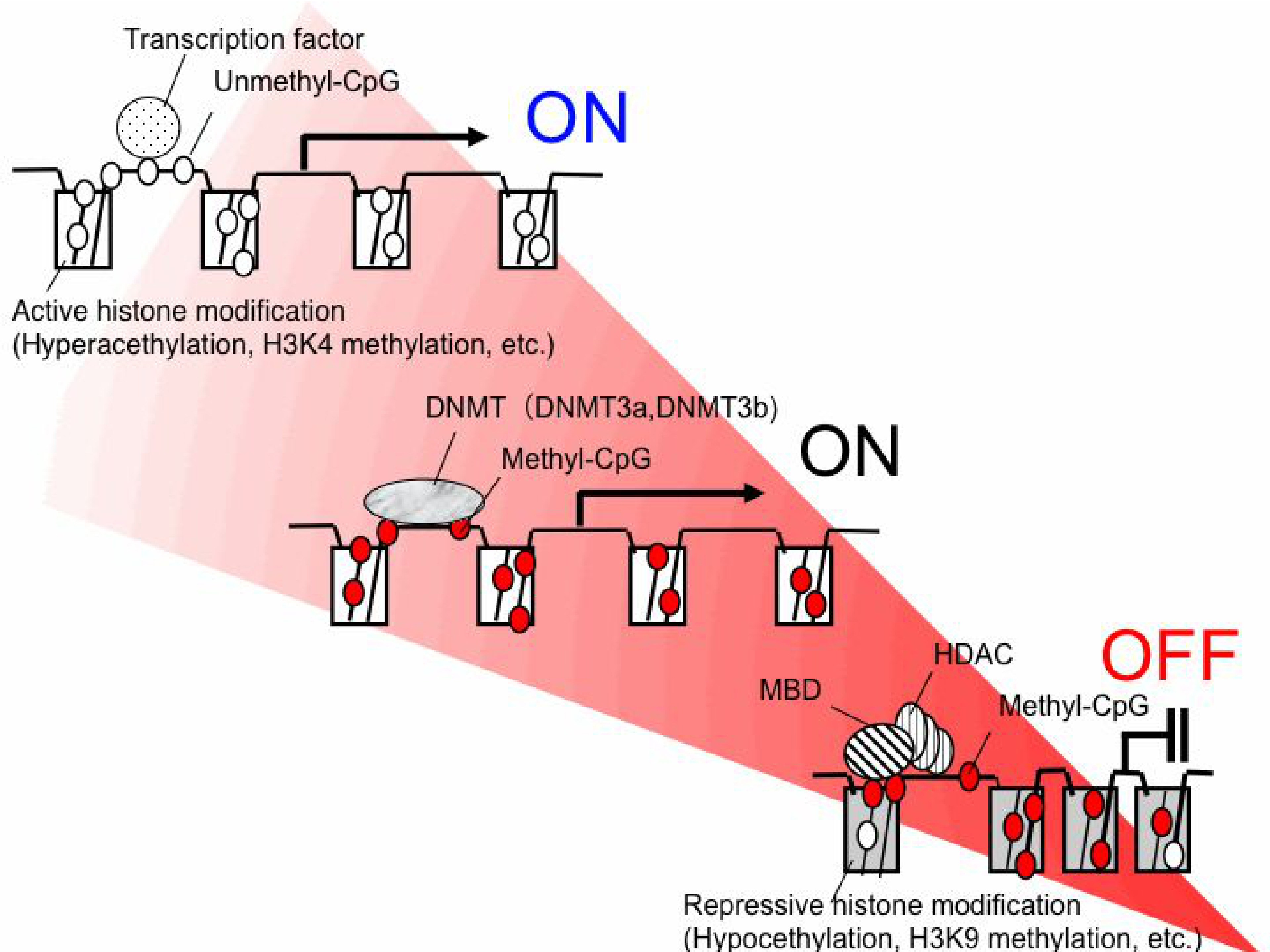
Positional
information
in the nucleus

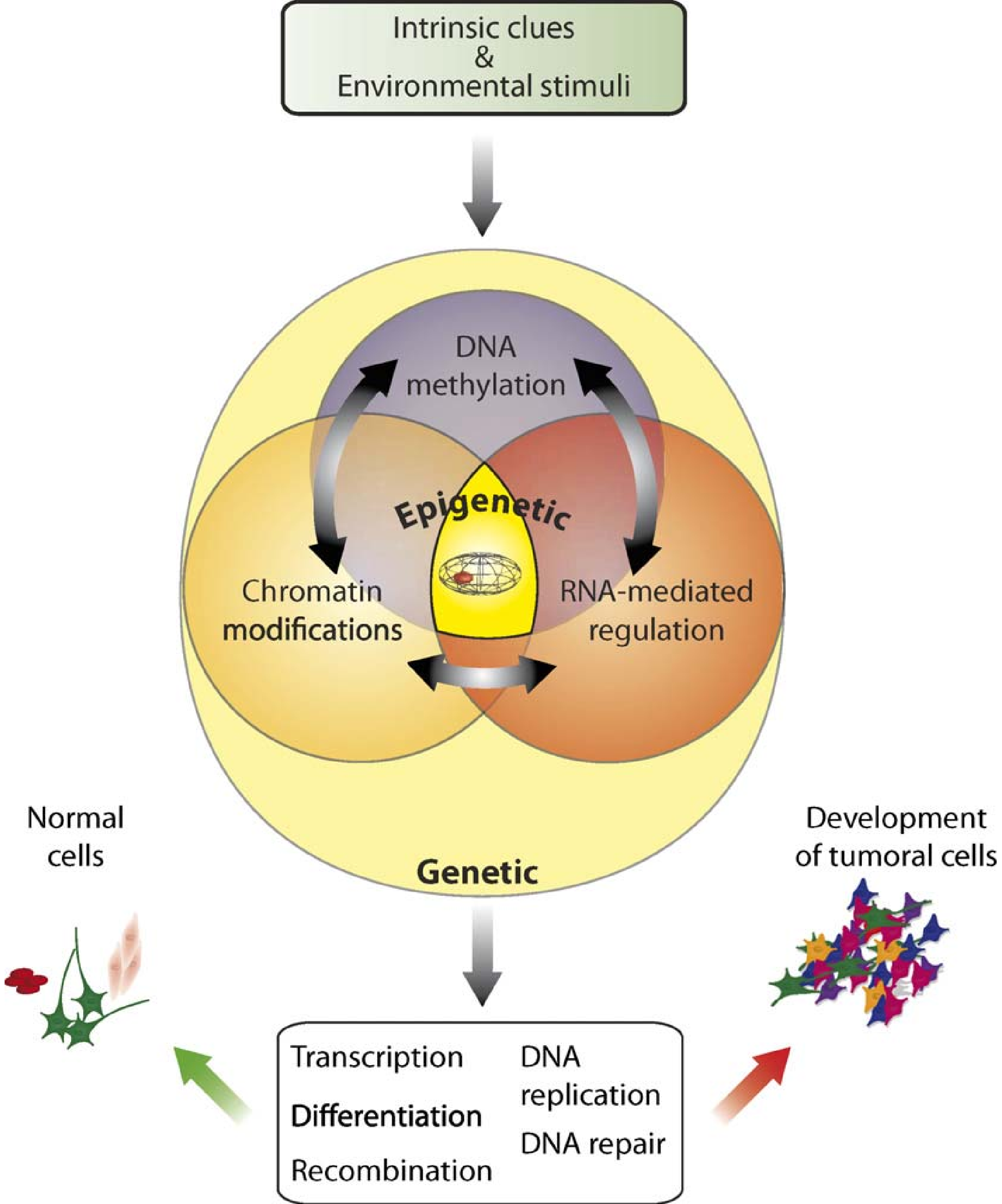
DNA methylation
*

DNA

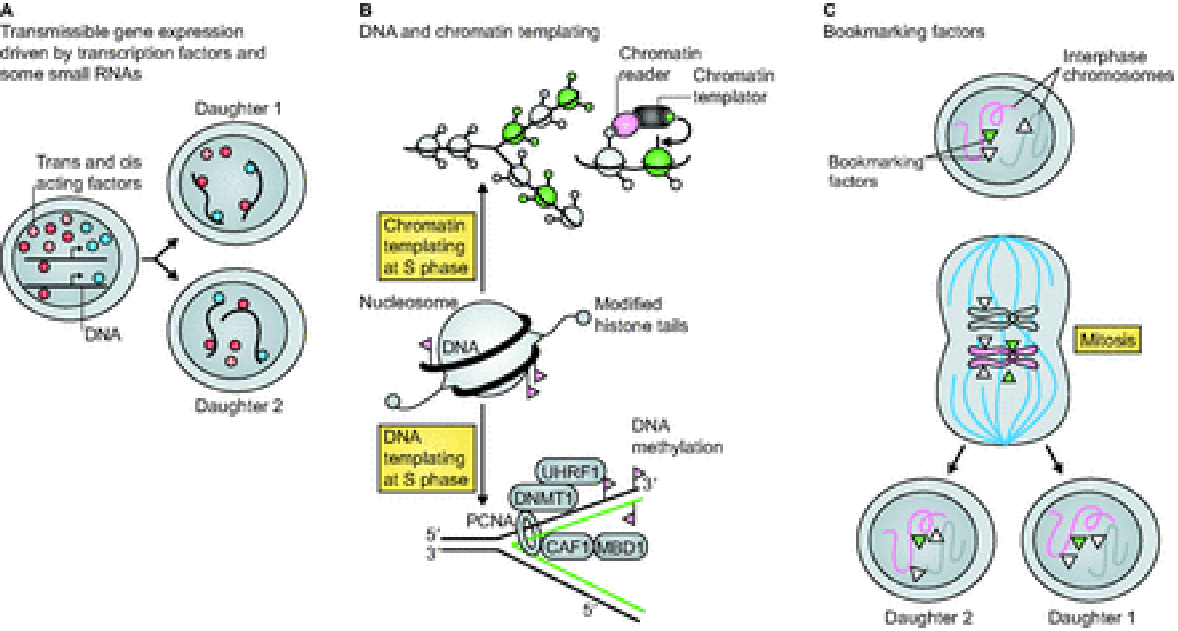
is involved in regulatory mechanisms of the nucleus

nucleus


Transcription factor
Unmethyl-CpG
ON
Active histone modification
(Hyperacethylation, H3K4 methylation, etc.)
DNMT（DNMT3a,DNMT3b）
Methyl-CpG
ON
HDAC
OFF
MBD
Methyl-CpG
Repressive histone modification
(Hypocethylation, H3K9 methylation, etc.)

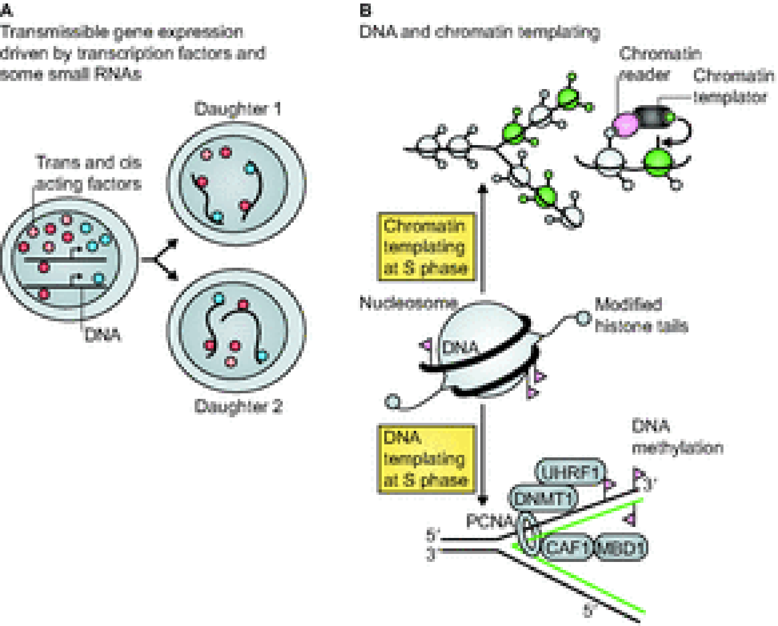

Intrinsic clues
&
Environmental stimuli
DNA methylation
Epigenetic
Chromatin modifications
RNA-mediated regulation
Genetic
Normal cells
Development of tumoral cells
Transcription
Differentiation
Recombination
DNA replication
DNA repair

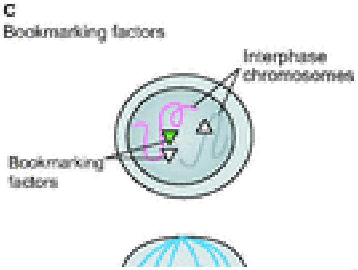
Epigenetic cell memory



- Inheritance of the epigenetic information during mitosis (DNA replication)
- Inheritance of epigenetic information through gametes during meiosis – TRANSGENERATIONAL EPIGENETIC INHERITANCE

Epigenetic cell memory





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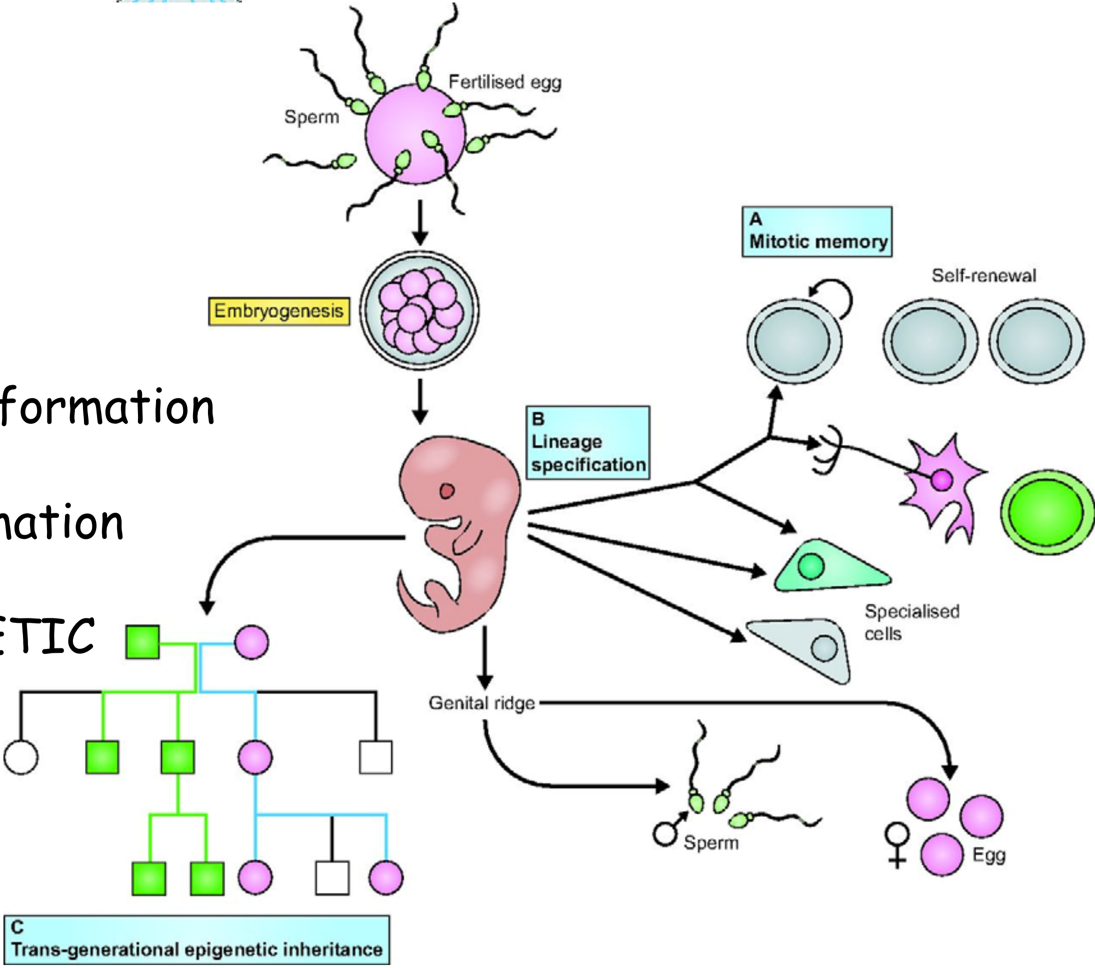
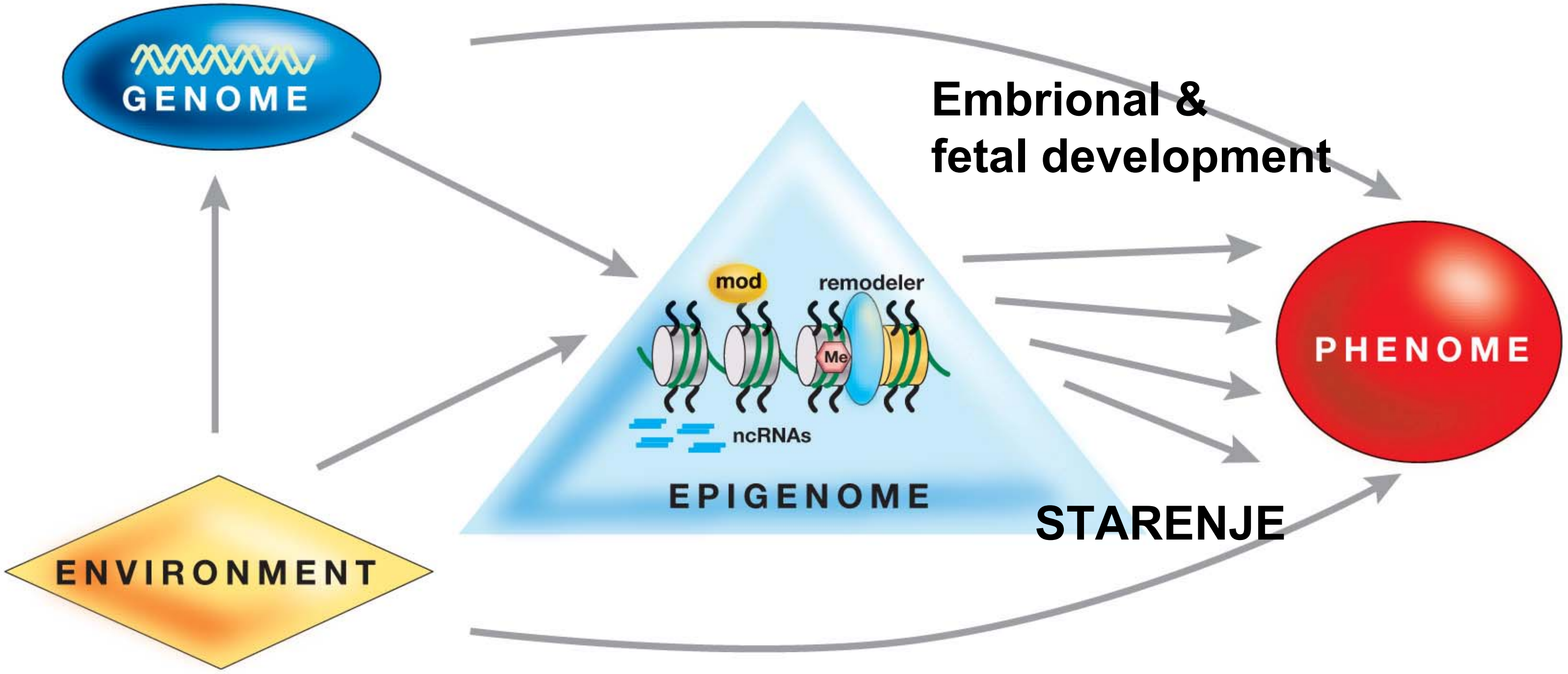
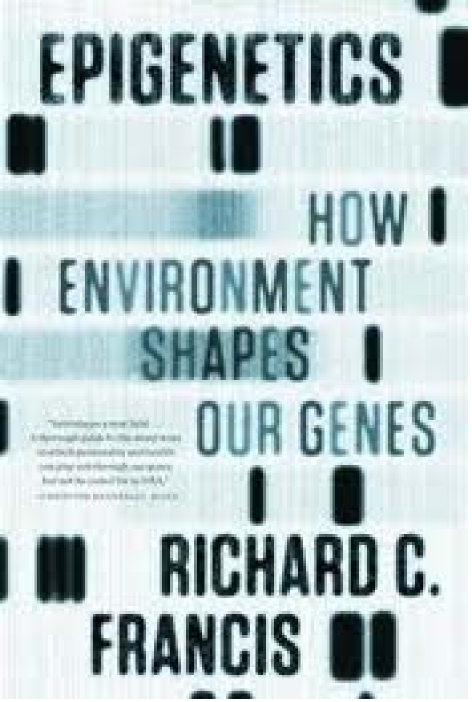
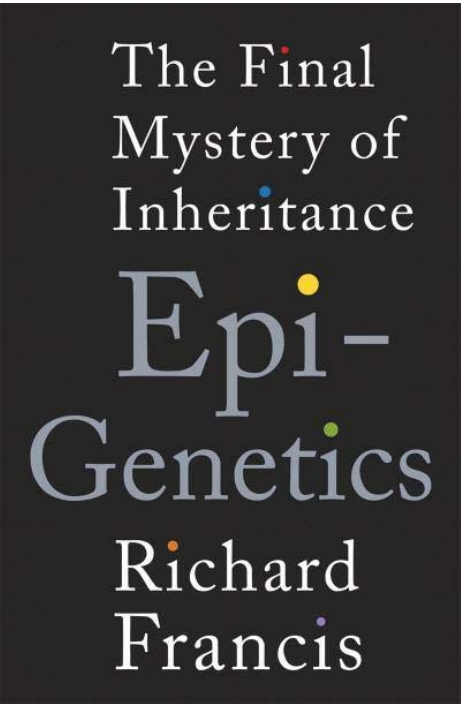




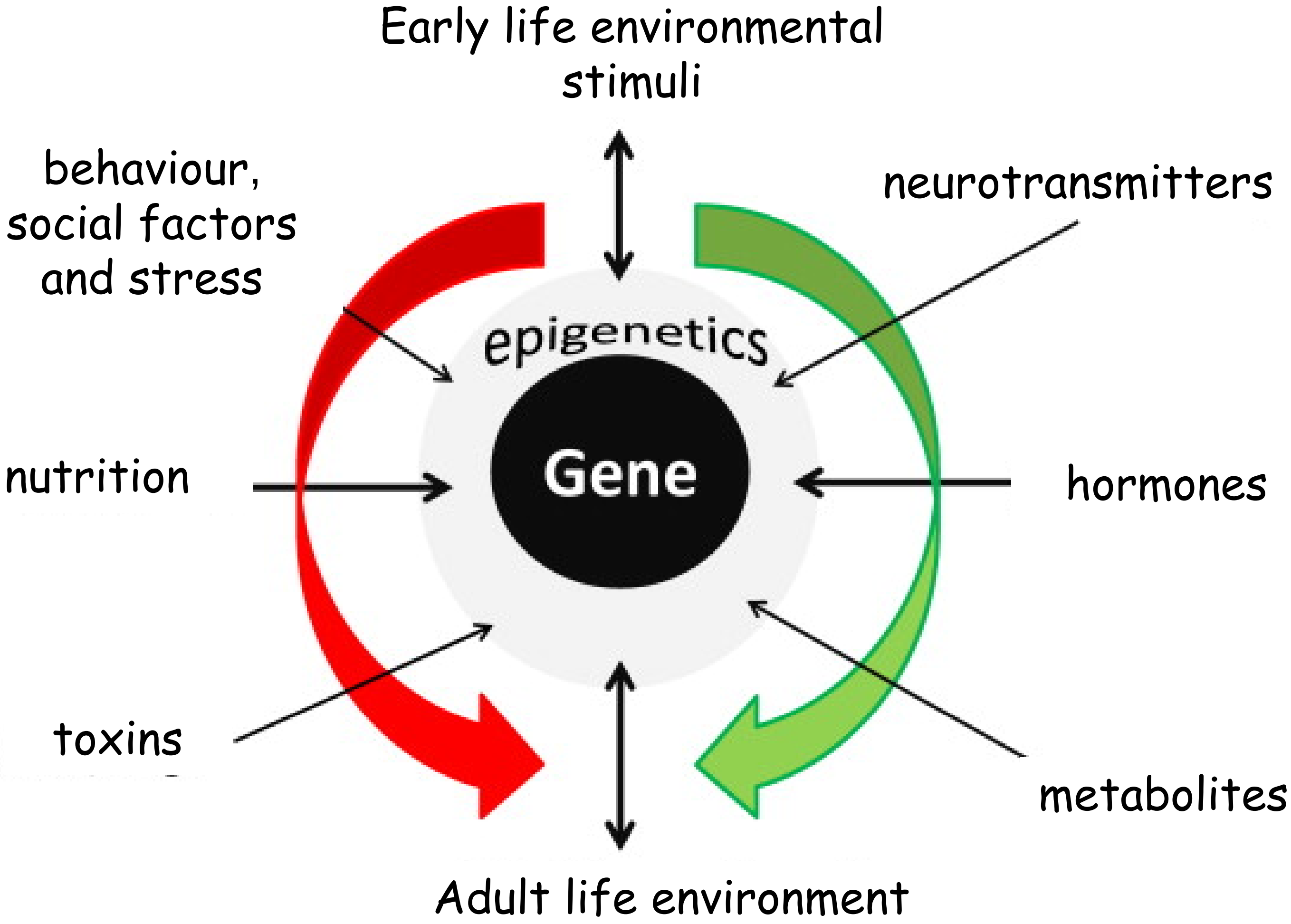
- Epigenome is a mediator between environment and a genome and thus contributes to phenotype (during early development, and also during adult life of an individual through stochastic epigenetic changes provoked by enviromental factors)
- **The capacity for epigenetic plasticity is selected by evolution and contributes to adaptive processes**

Probabilistic epigenetics


EPIGENETICS
HOW ENVIRONMENT SHAPES OUR GENES
RICHARD C. FRANCIS


The Final Mystery of Inheritance
Epi-Genetics
Richard Francis

structure -------- function
gene -------- environment


Early life environmental stimuli

behaviour, social factors and stress

neurotransmitters

nutrition

hormones

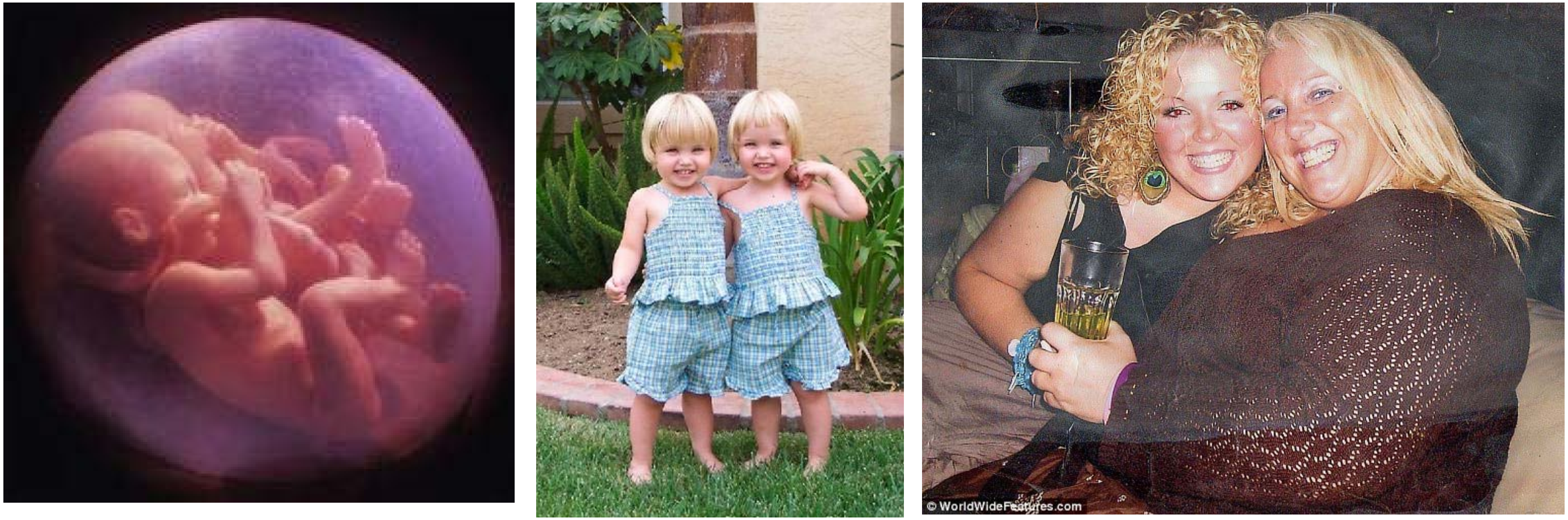
toxins

metabolites

Adult life environment

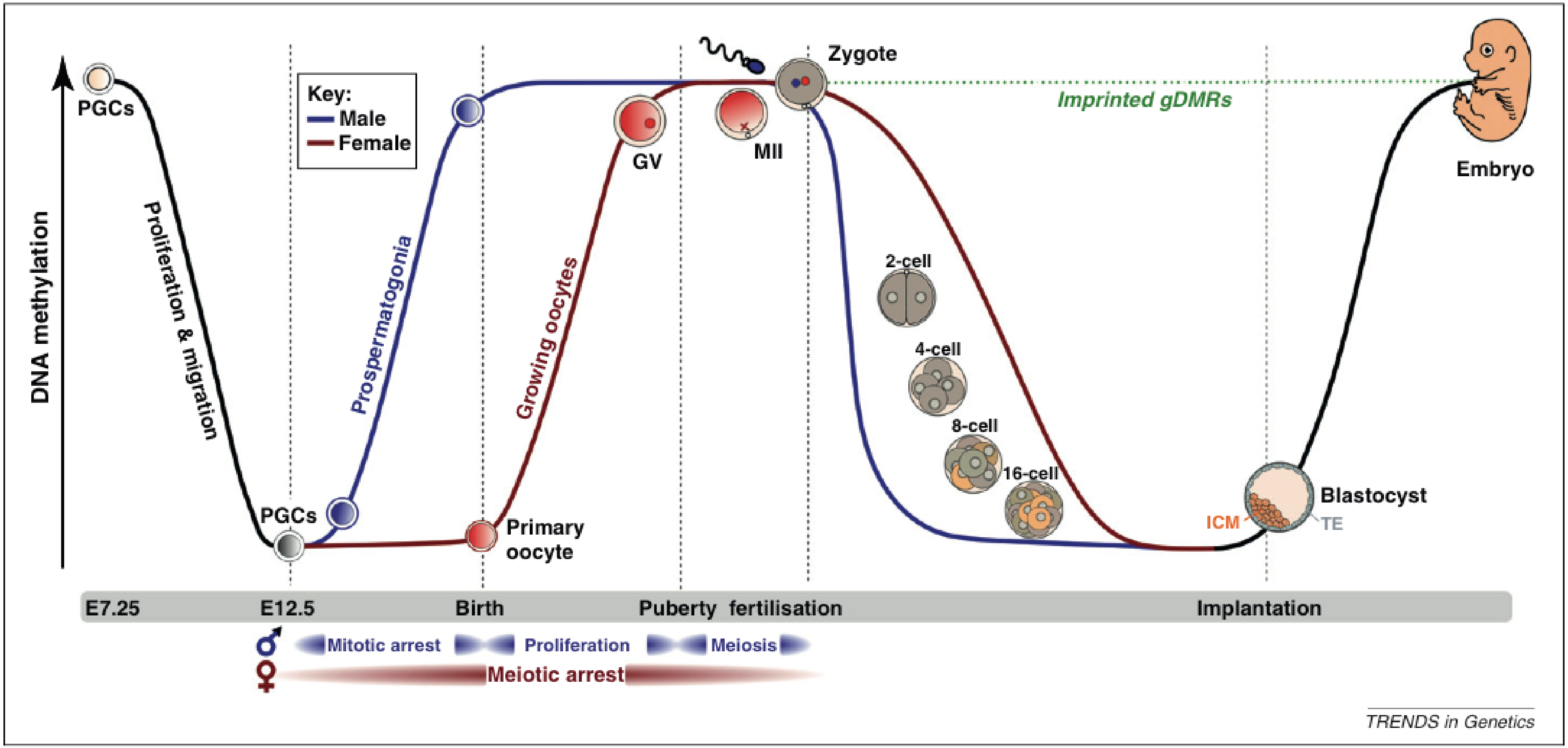
Interindividual epigenetic variability

Fraga et al. (2005) PNAS: Epigenetic differences arise during the lifetime of monozygotic twins.

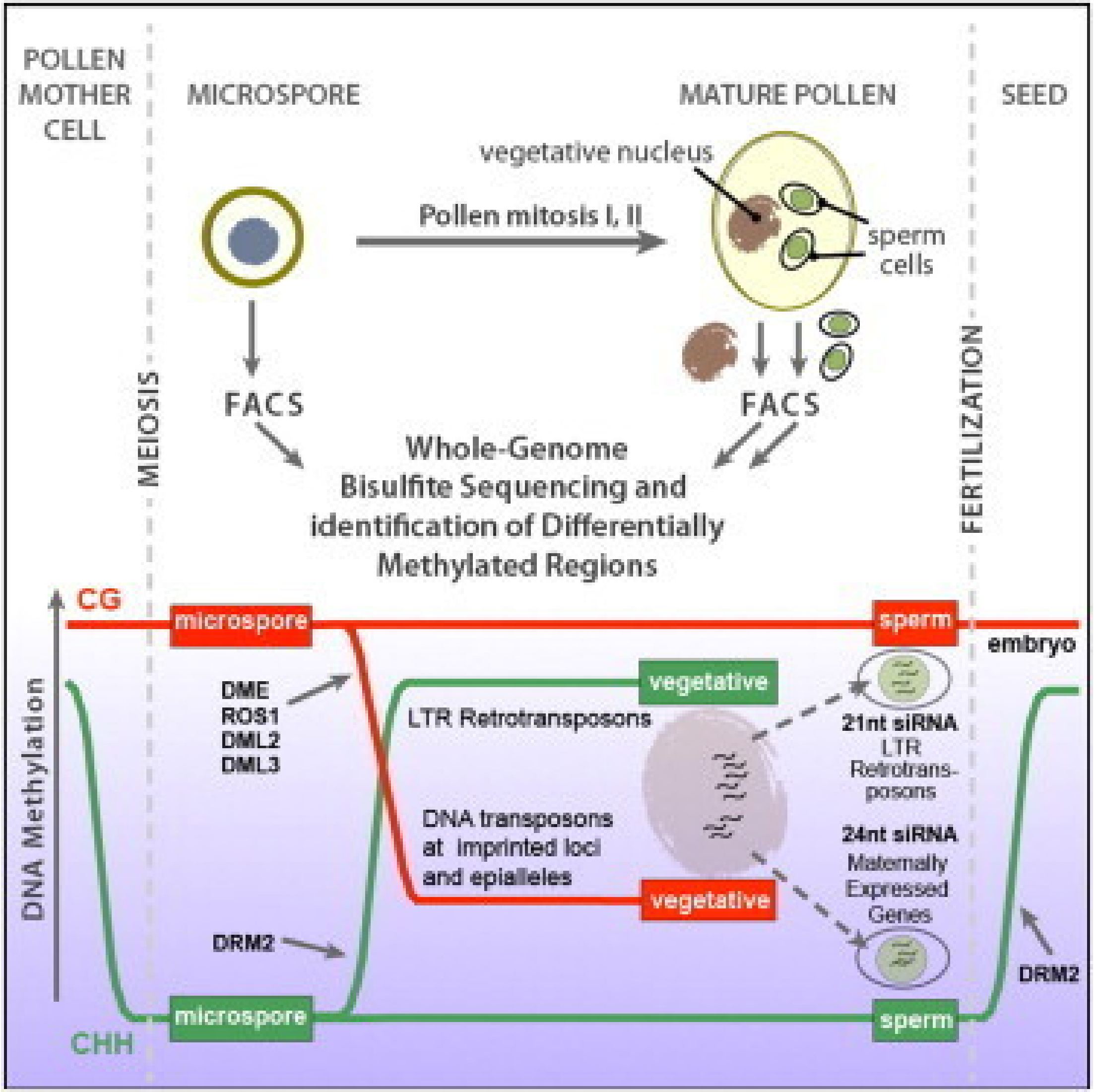


- The amount of differences is proportional to the degree of intra-pair lifestyle dissimilarity: age and environment largely influence epigenetic differences
- **Contribution of the epigenotype to the phenotypic manifestation of the inherited genotype**

Two waves of epigenetic reprogramming in mammals

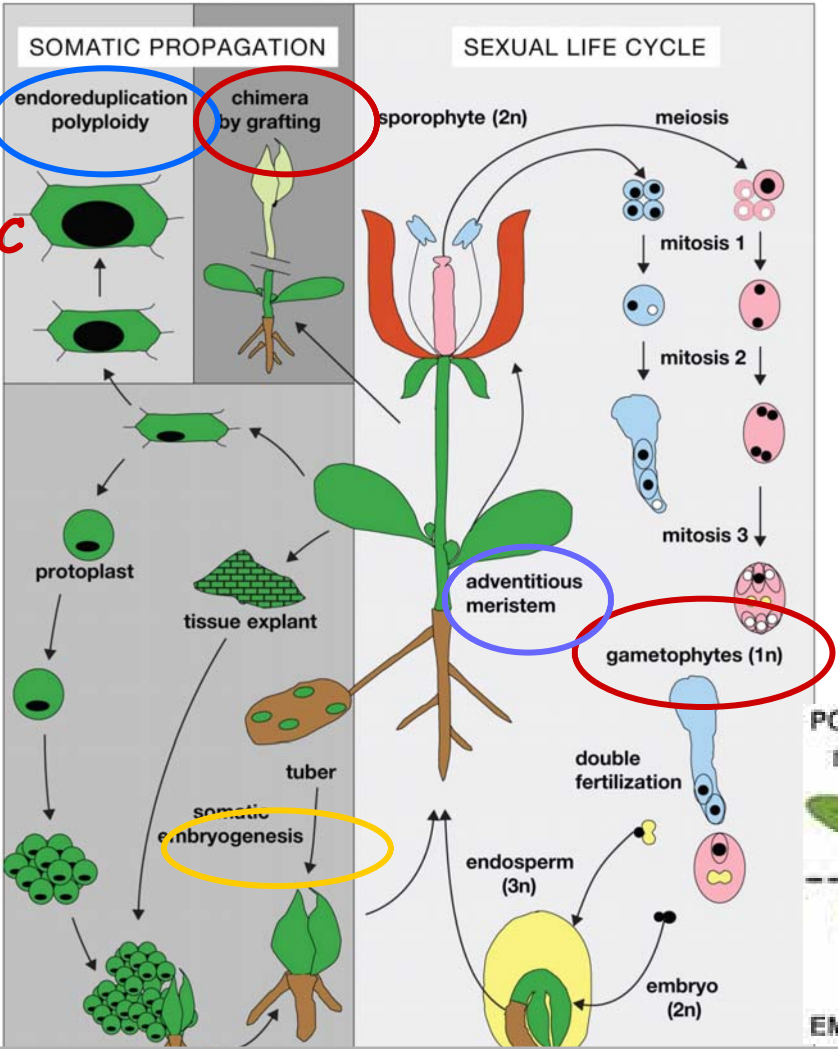


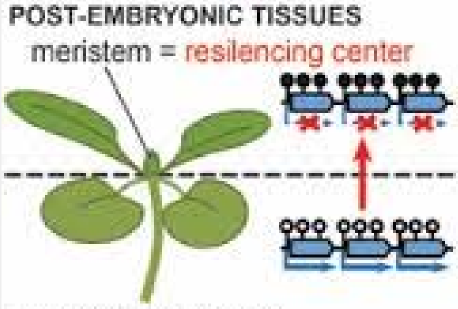
Epigenetic reprogramming in plants



CpG motifs
CpNpG motifs
CpNpN motifs

PLANTS HAVE SPECIFIC LIFE STYLE AND SPECIFIC EPIGENETICS





Epigenetičke oznake se češće nasljeđuju na potomke (EPI-ALELI), ali su fleksibilnije i reverzibilnije nego u kromatinu animalnih genoma1.

- independent haploid phase (gamethophyte) between meiosis and fertilisation : importance for maintenance of epigenetic information
- no global epigenetic reprogramming - epialleles and transgenerational epigenetic inheritance
- continuous growths through apical and lateral meristems (continuous differentiation)
- vegetative propagation (specialized organs) – somatic embryogenesis and somaclonal variability
- plasmodesmata; vegetative himera (flexible and reversible epigenome)

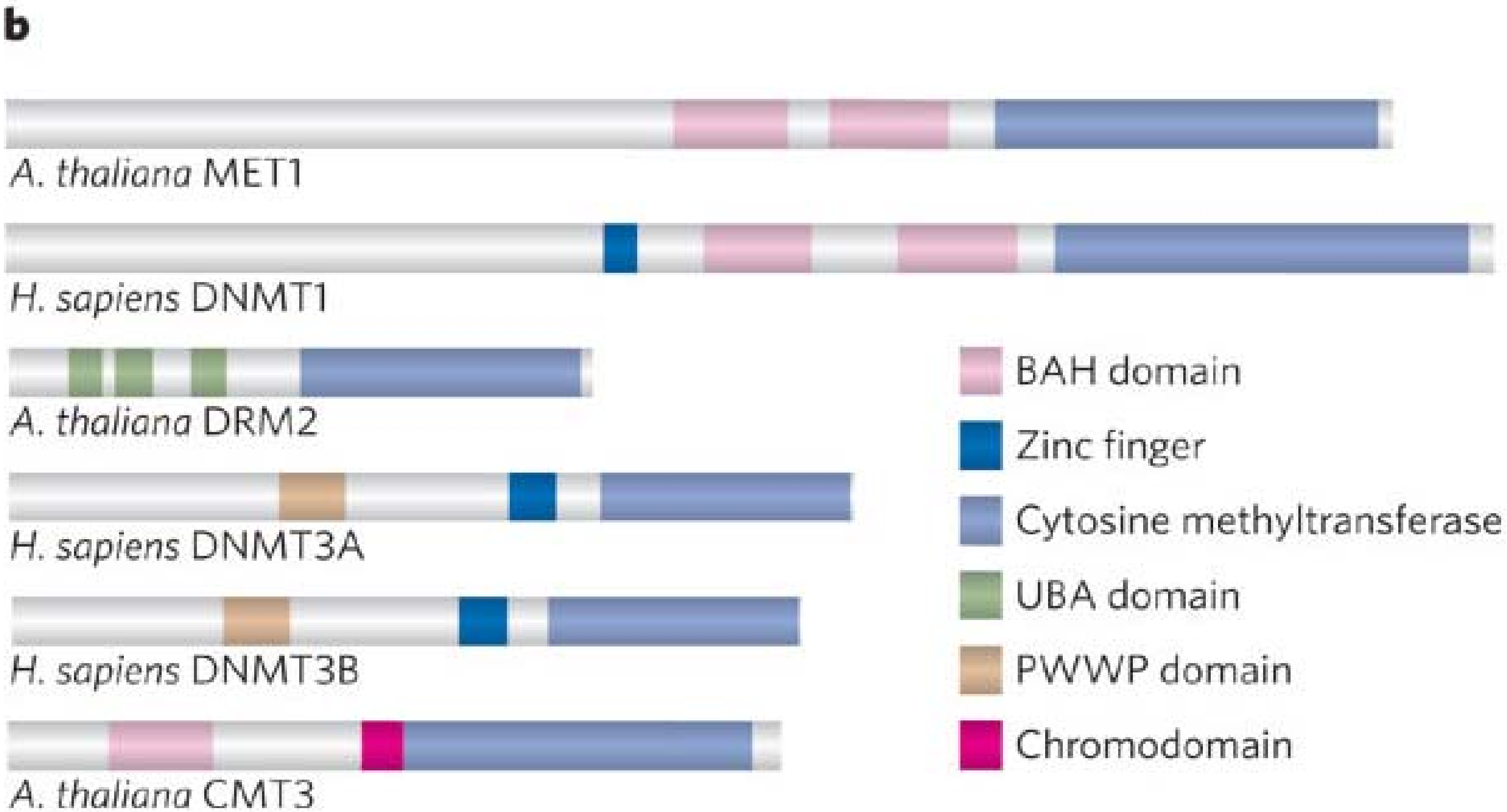
DNA methylatransferases in plants (*Arabidopsis thaliana*) :

MET1 **(**Dnmt1) – maintenance methyltransferase (recognize hemimethylated DNA)

methylation of CpG motifs (can methylate *de novo*), important for plant development

DRM2 **(**Dnmt3a/Dnmt3b) – *de novo* methylation of CpG, CpNpG, CpNpN motifs (methylation of asymetrical motifs), involved in RdDM pathway

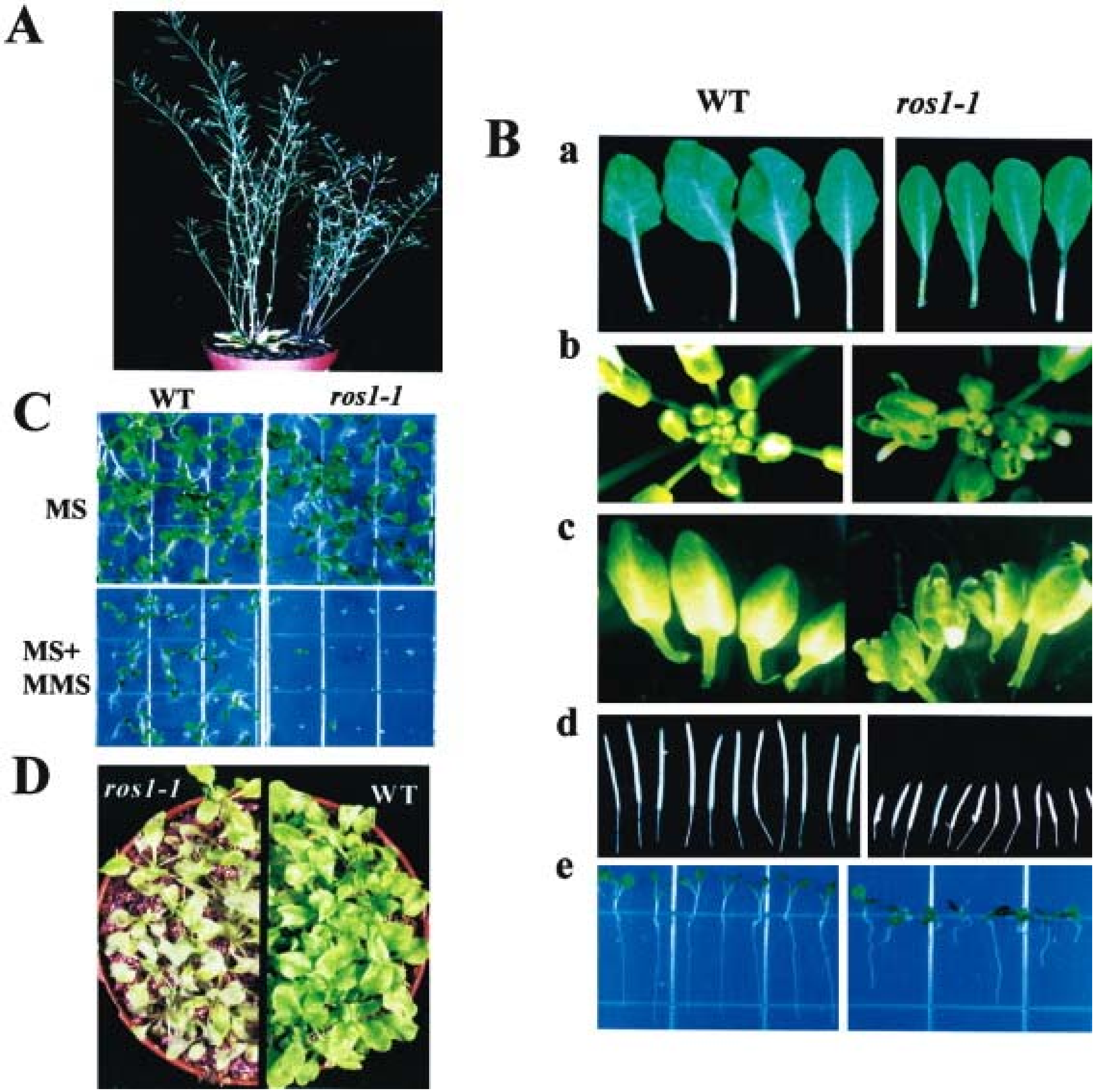
CMT3 – specific for plants; *maintenance and* de novo methyltransferase; methylation of asymetric CpNpG motifs; involved in silencing of retrotransposons

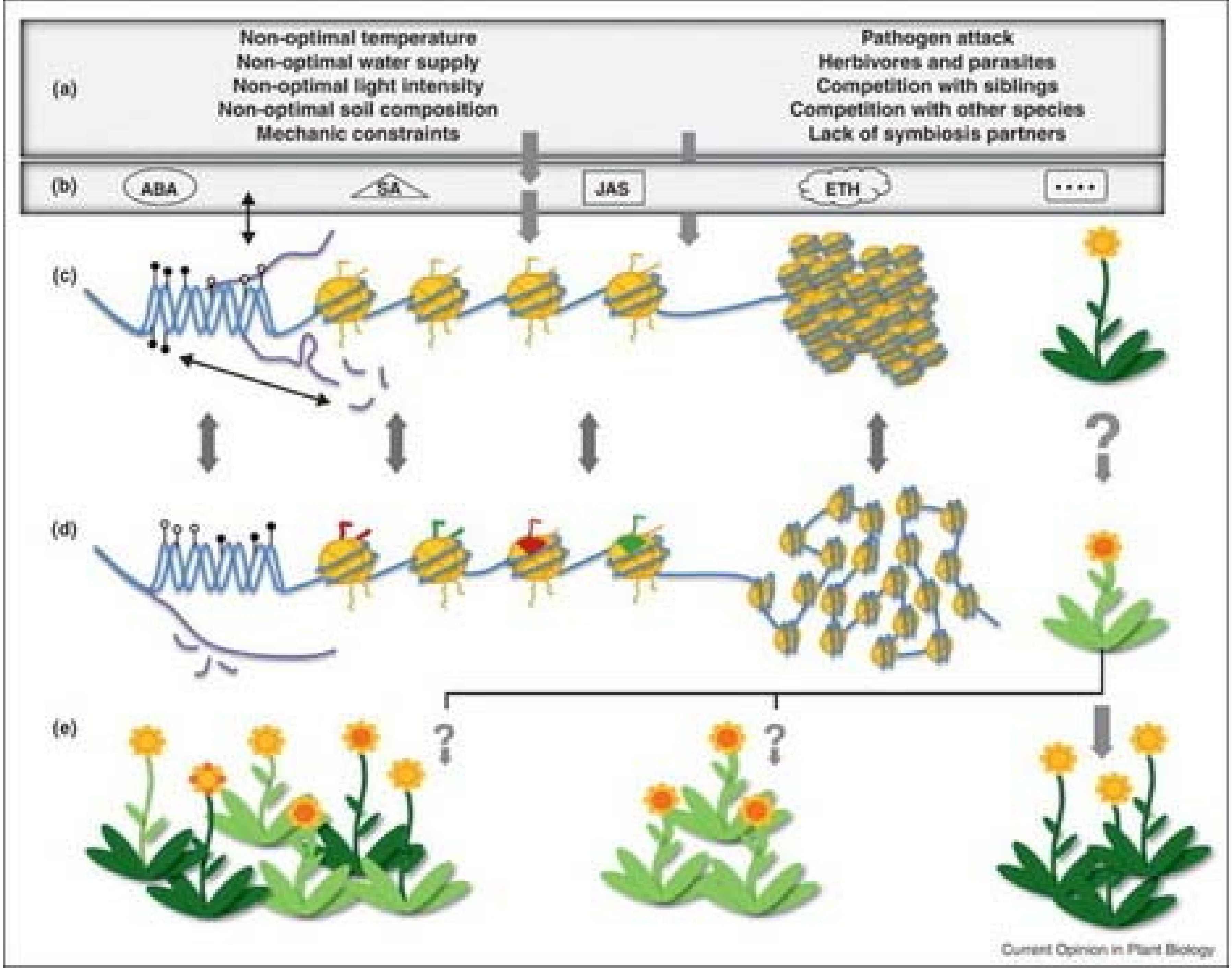


Plant mutants for epigenetic effectors

Columbia *MET1*

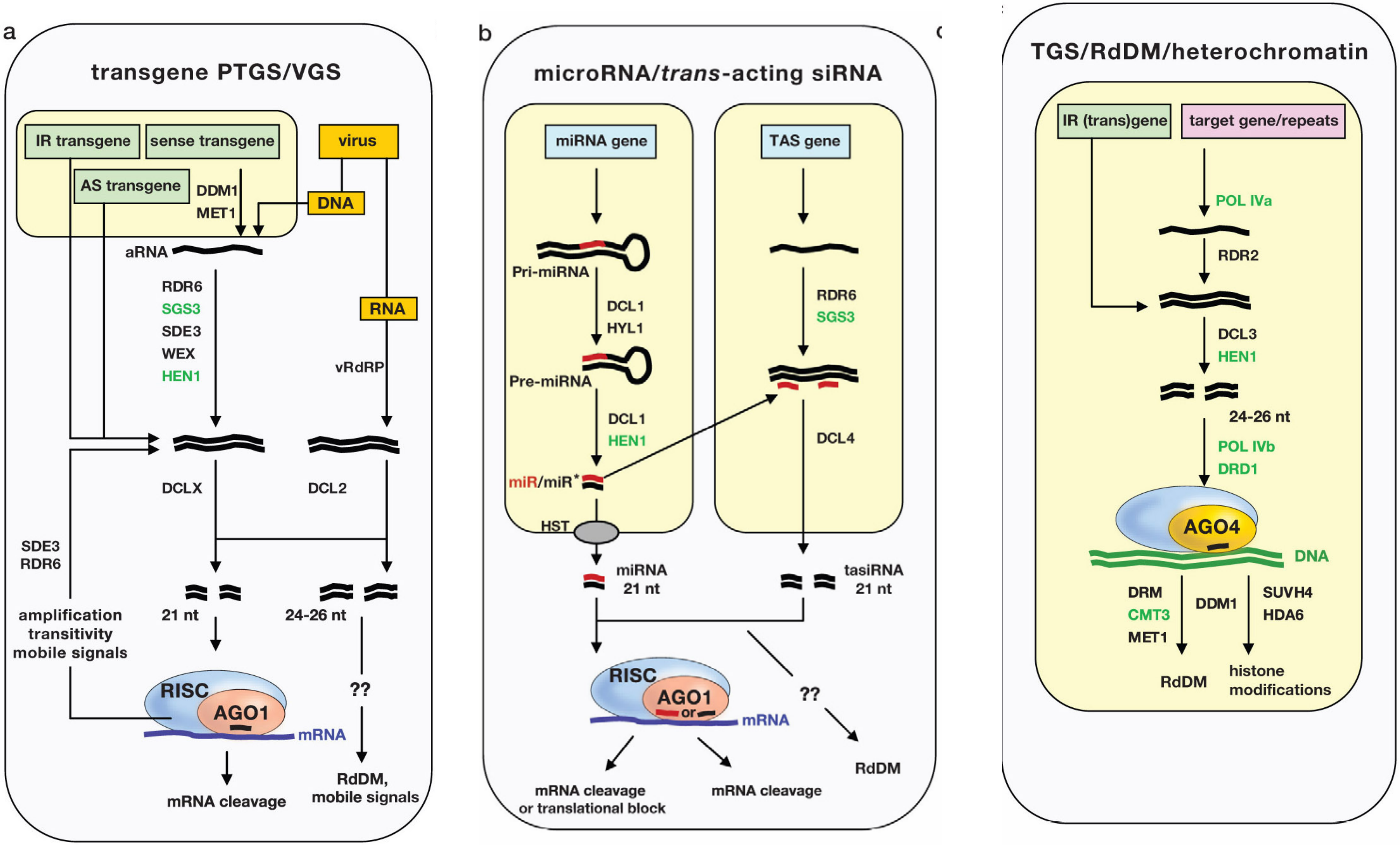
Columbia *met1-1*





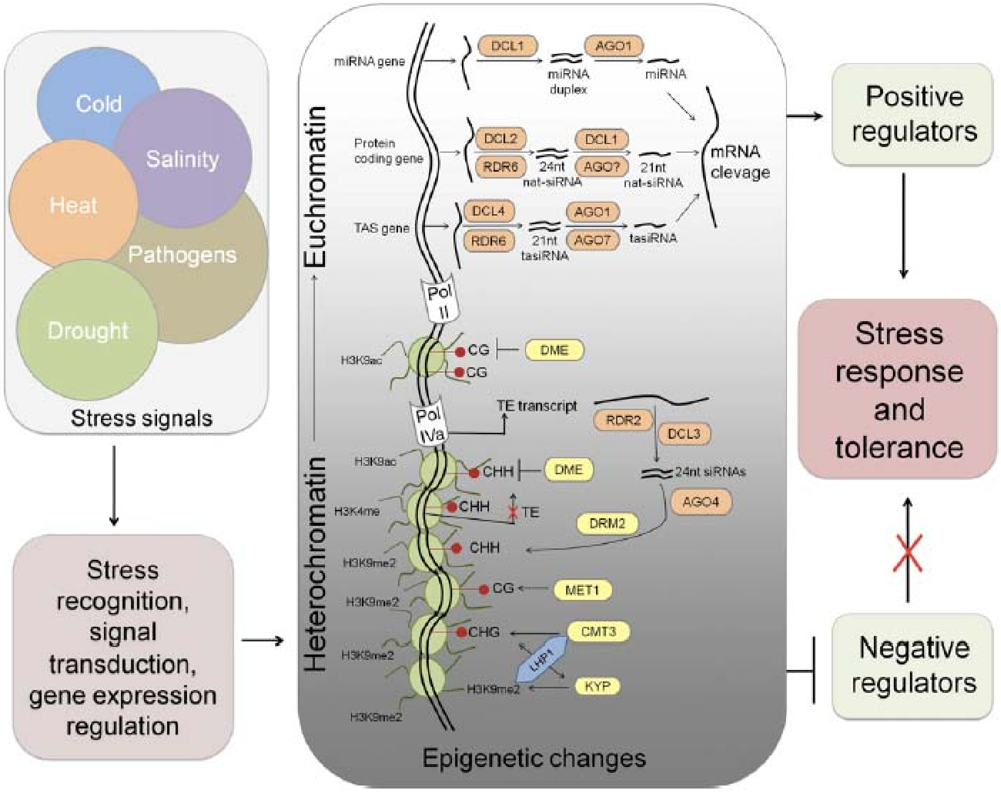
Current Opinion in Plant Biology

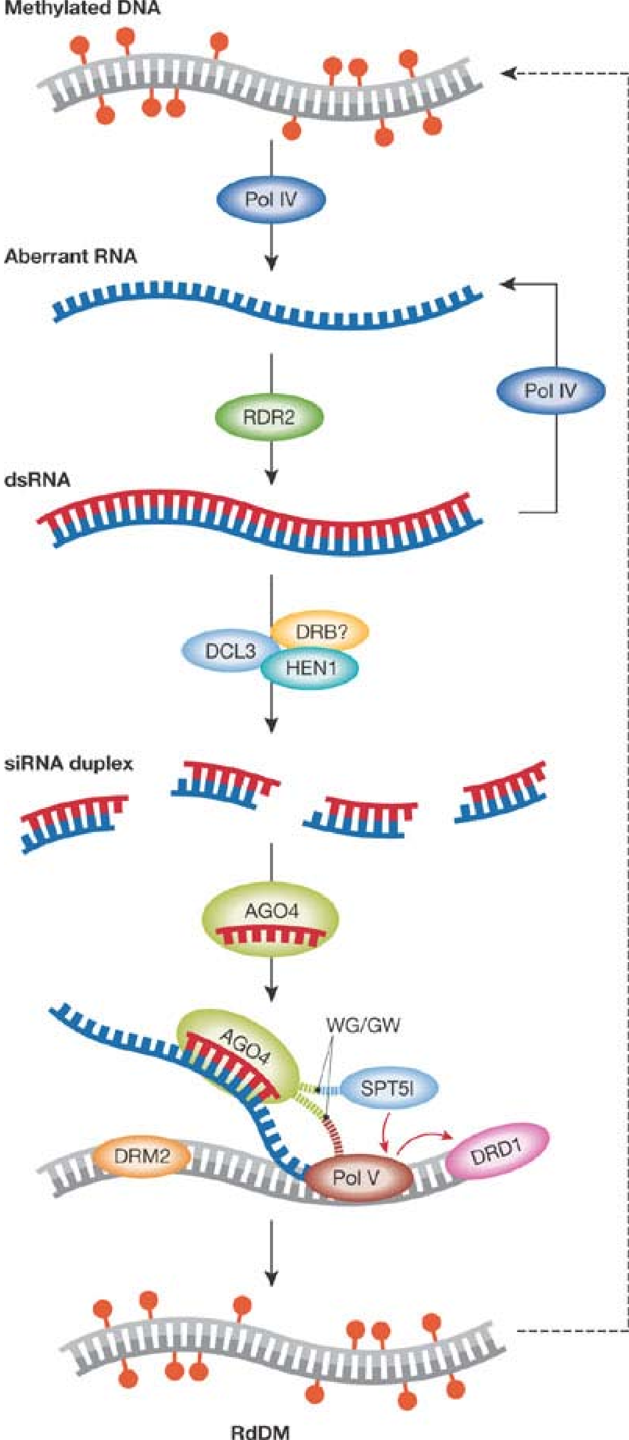
Involvement of TGS and PTGS in stress signaling



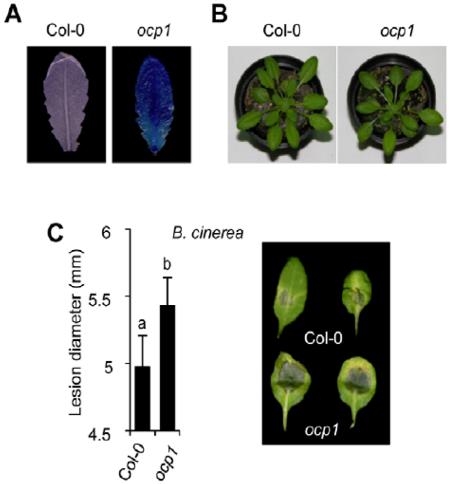
RNA directed DNA methylation (RdDM)

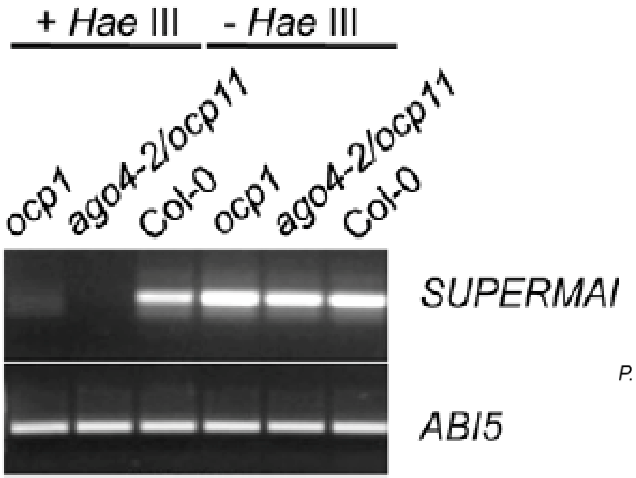
- 30% of cytosines in *Arabidopsis* genome is dependent on the RNA-depended silencing pathway
- PolIV and PolV – specific for plants (involved in DNA methylation)
- Involved in silencing of ME, gene regulation and STRESS SIGNALING

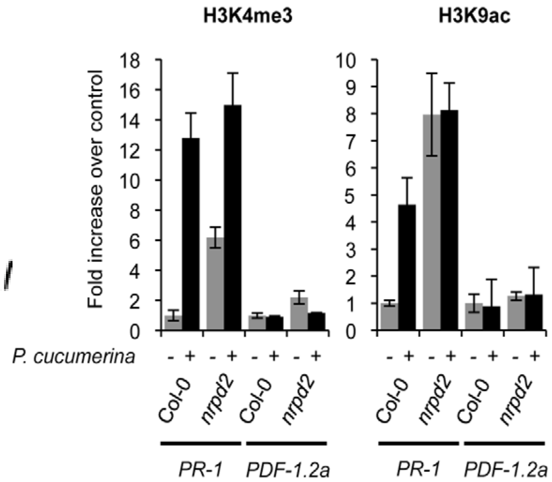




Overlap between plant stress signaling and epigenetic mechanisms (RdMD involving ncRNAs)

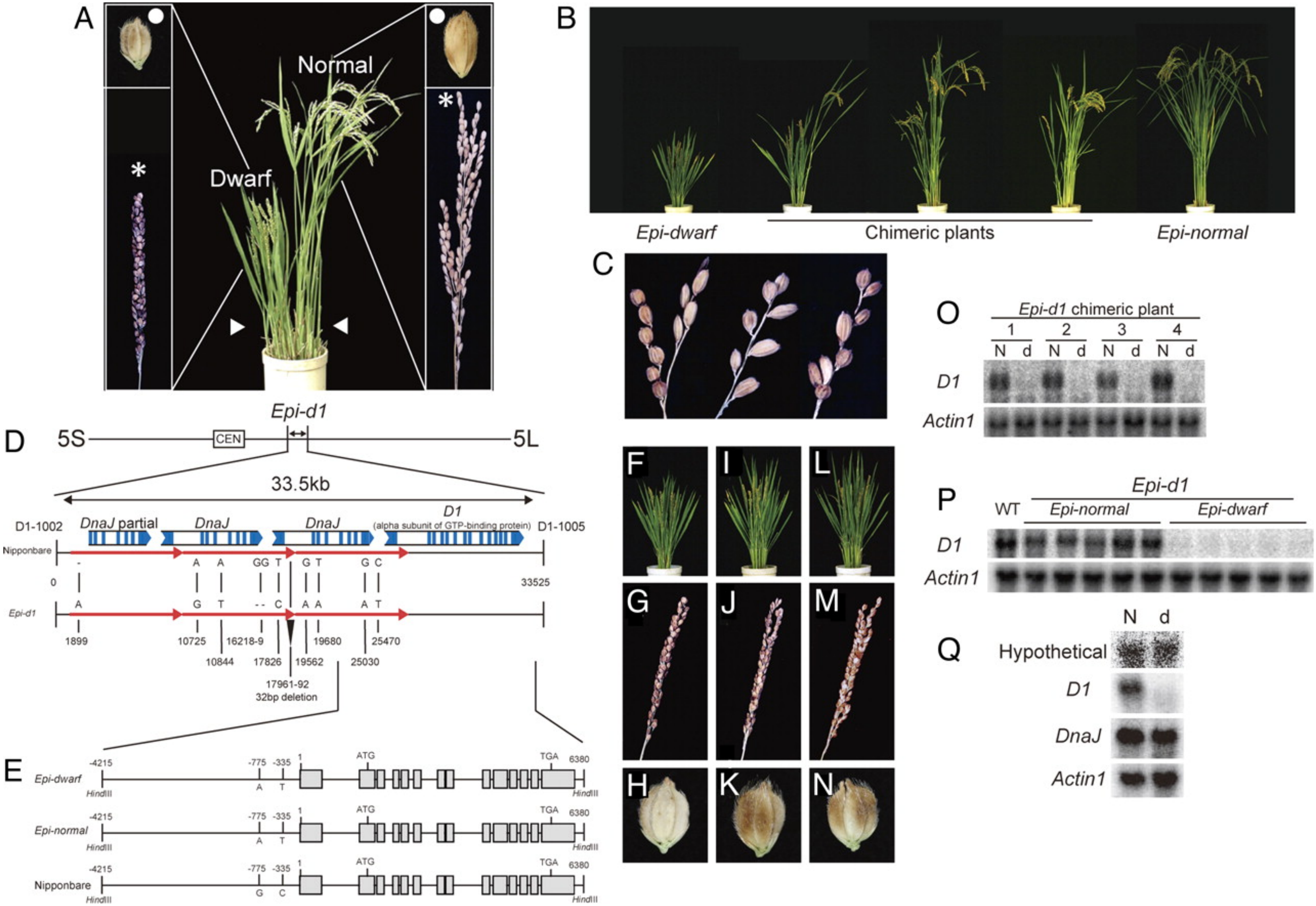






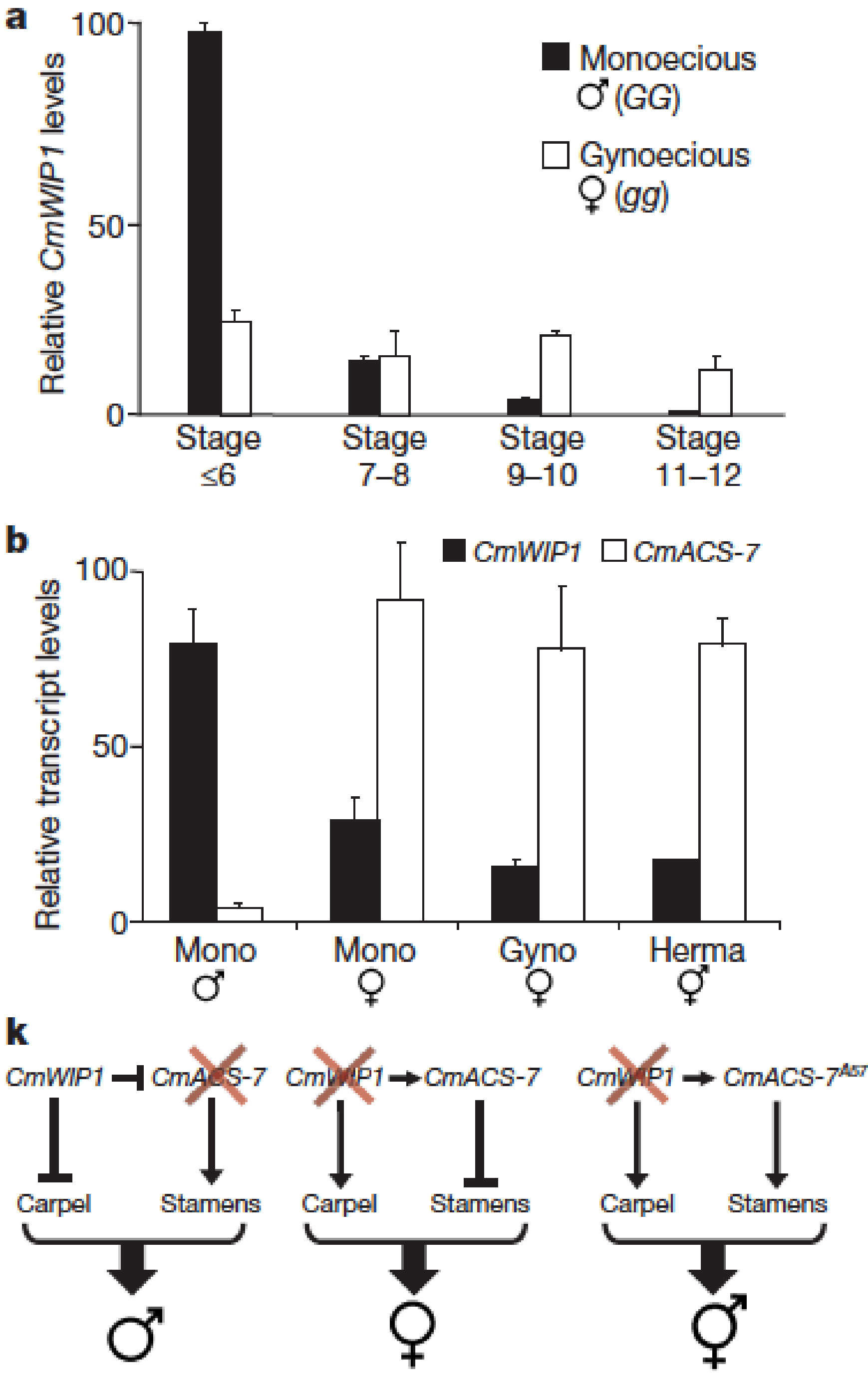
- *Arabidopsis* mutant for NRPD2 gene – constitutive expression for salicil acid
- Mechanistical link between stress signaling and RdMD
- RdMD mutant plants have compromised imune response to pathogene fungi
- Overlap between stress signaling using plant hormons and epigenetic mechanisms (RdDM) involving action of ncRNAs

Plant epi-mutants



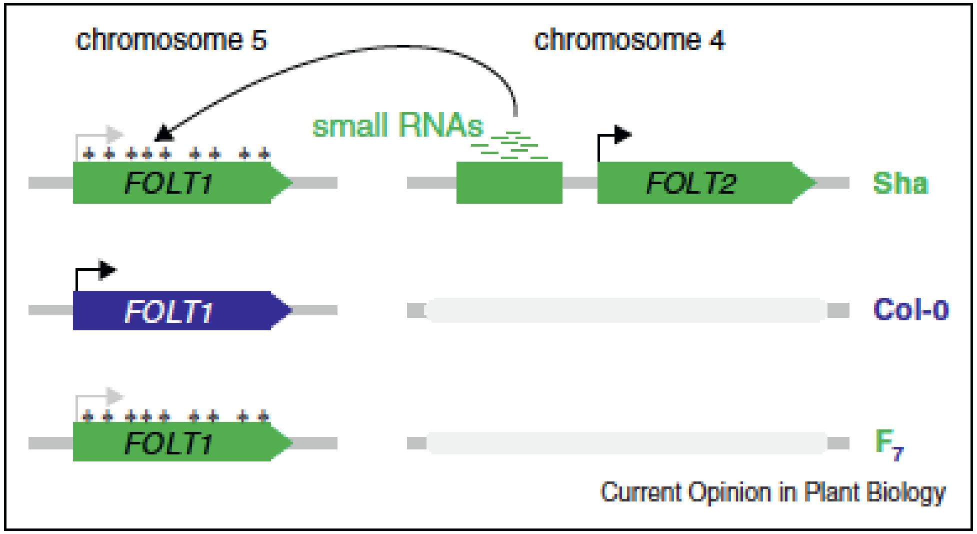
Metastable epialelles

- sex determination mutant in melon *Cucumis melo*
- a transposon-induced epigenetic change in promoter of CmWIP1 gene
- removal of TE from CmWIP1 promoter reactivates the gene activity



Martin et al. 2009 Nature

Epialeles or genetic mutation?



Current Opinion in Plant Biology

Durand et al. 2012 Curr Biol

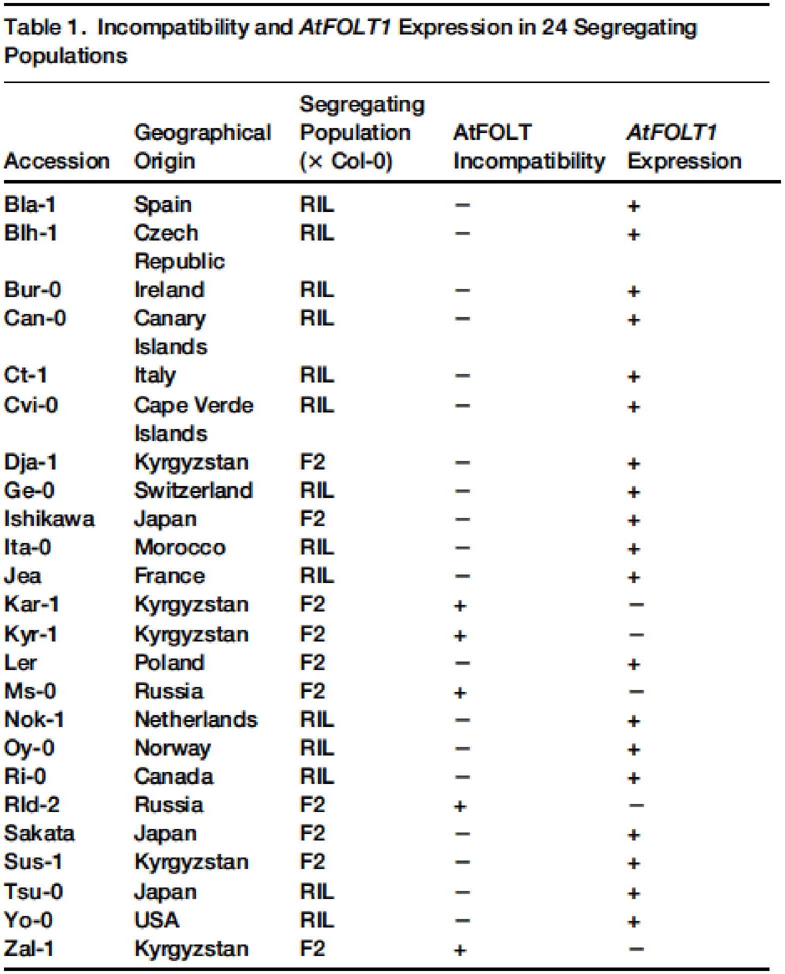


Table 1. Incompatibility and *AtFOLT1* Expression in 24 Segregating Populations

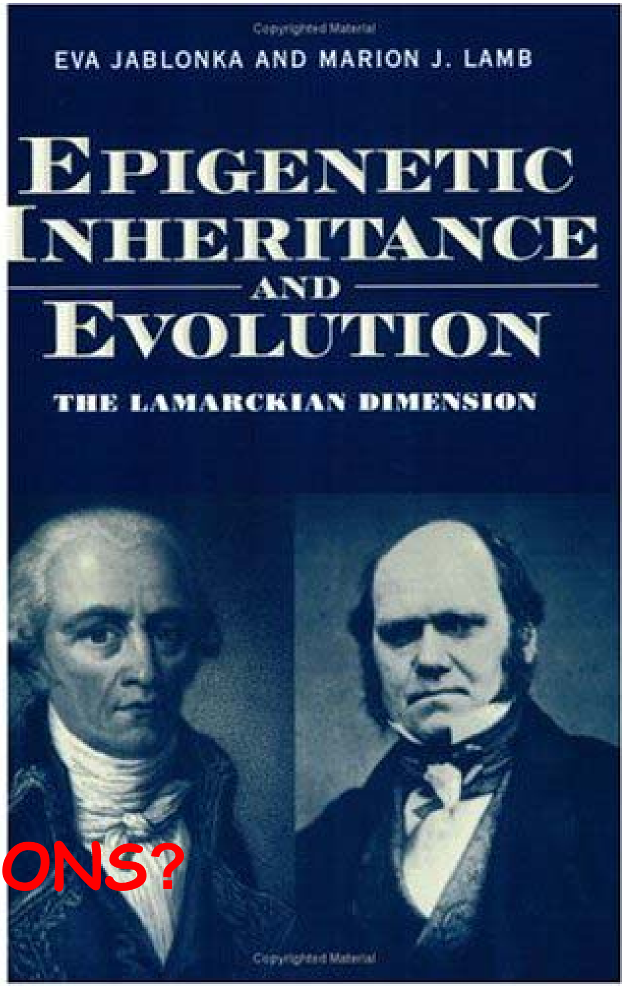
Accession	Geographical Origin	Segregating Population (× Col-0)	AtFOLT Incompatibility	*AtFOLT1* Expression
Bla-1	Spain	RIL	−	+
Blh-1	Czech Republic	RIL	−	+
Bur-0	Ireland	RIL	−	+
Can-0	Canary Islands	RIL	−	+
Ct-1	Italy	RIL	−	+
Cvi-0	Cape Verde Islands	RIL	−	+
Dja-1	Kyrgyzstan	F2	−	+
Ge-0	Switzerland	RIL	−	+
Ishikawa	Japan	F2	−	+
Ita-0	Morocco	RIL	−	+
Jea	France	RIL	−	+
Kar-1	Kyrgyzstan	F2	+	−
Kyr-1	Kyrgyzstan	F2	+	−
Ler	Poland	F2	−	+
Ms-0	Russia	F2	+	−
Nok-1	Netherlands	RIL	−	+
Oy-0	Norway	RIL	−	+
Ri-0	Canada	RIL	−	+
Rld-2	Russia	F2	+	−
Sakata	Japan	F2	−	+
Sus-1	Kyrgyzstan	F2	−	+
Tsu-0	Japan	RIL	−	+
Yo-0	USA	RIL	−	+
Zal-1	Kyrgyzstan	F2	+	−

Rapid establishment of genetic incompatibility through natural epigenetic variation

Epialleles in adaptation and evolution

NEO-LAMARCKISM and EPIGENETICS: “SOFT-INHERITANCE”

- Lamarck
- Trofim Lyscenko
- **Conrad Waddington** – *intrinsic dynamics of development - the “epigenetic landscape” is the real source of novel non-random variations for evolution*



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EPIGENETIC INHERITANCE AND EVOLUTION

THE LAMARCKIAN DIMENSION

Copyrighted Material

- Environment can induce stable change of epigenetic state in a genome – the molecular mechanism that enables environment to have permanent biological efect

WHAT ABOUT NATURAL POPULATIONS?

Analysis of global genome methylation

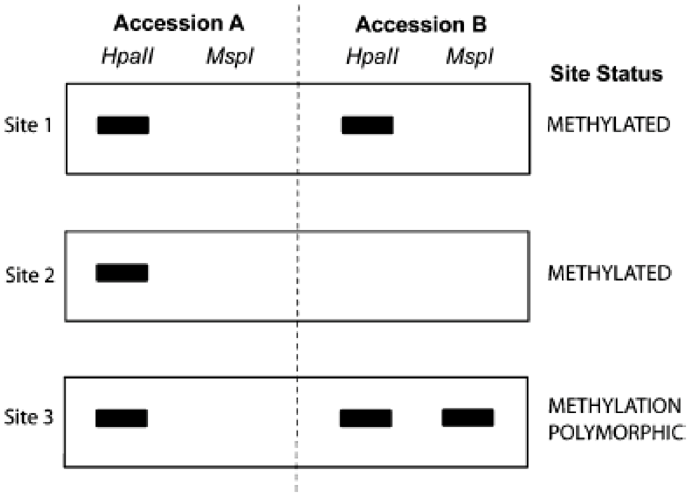
- Methods based on differential sensitivity of restriction enzymes (isoshizomeres) such as *Msp*I/*Hpa*II to methylation : MSAP (methylation sensitive amplified polymorphism)

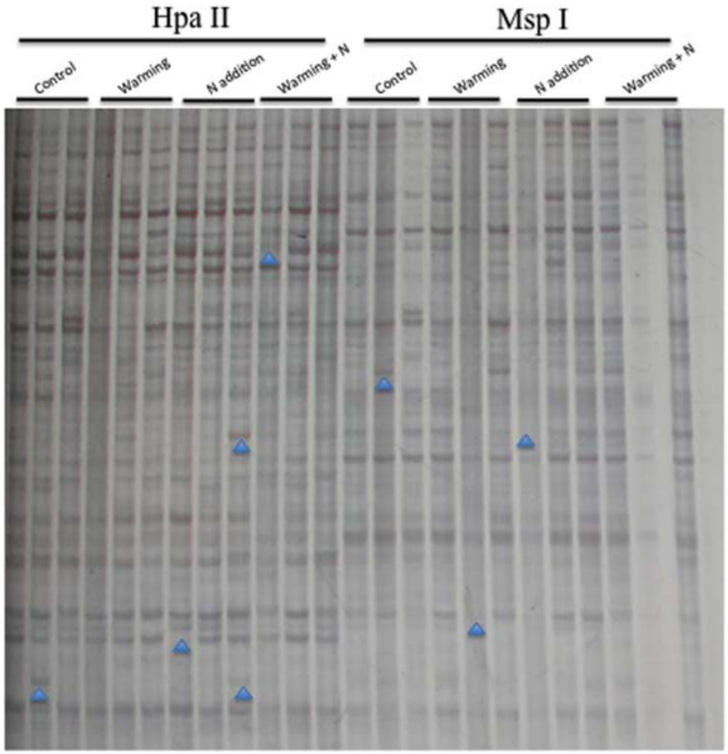
MSAP = Methylation Sensitive Amplified Polymorphism

Methylation event	Sequence	HpaII	MspI
I	CCGG GGCC	+	+
II	^{5m}CCGG GGCC	+	-
III	C^{5m}CGG G GCC	-	+
IV	C^{5m}C GG G G^{5m}CC	-	+
V	^{5m}C^{5m}C G G G G^{5m}C^{5m}C	-	-

EcoRI = GAATTC
HpaII/MspI = CCGG

***EcoRI/HpaII* = genetički podatci**
***EcoRI/MspI* = epigenetički podatci**

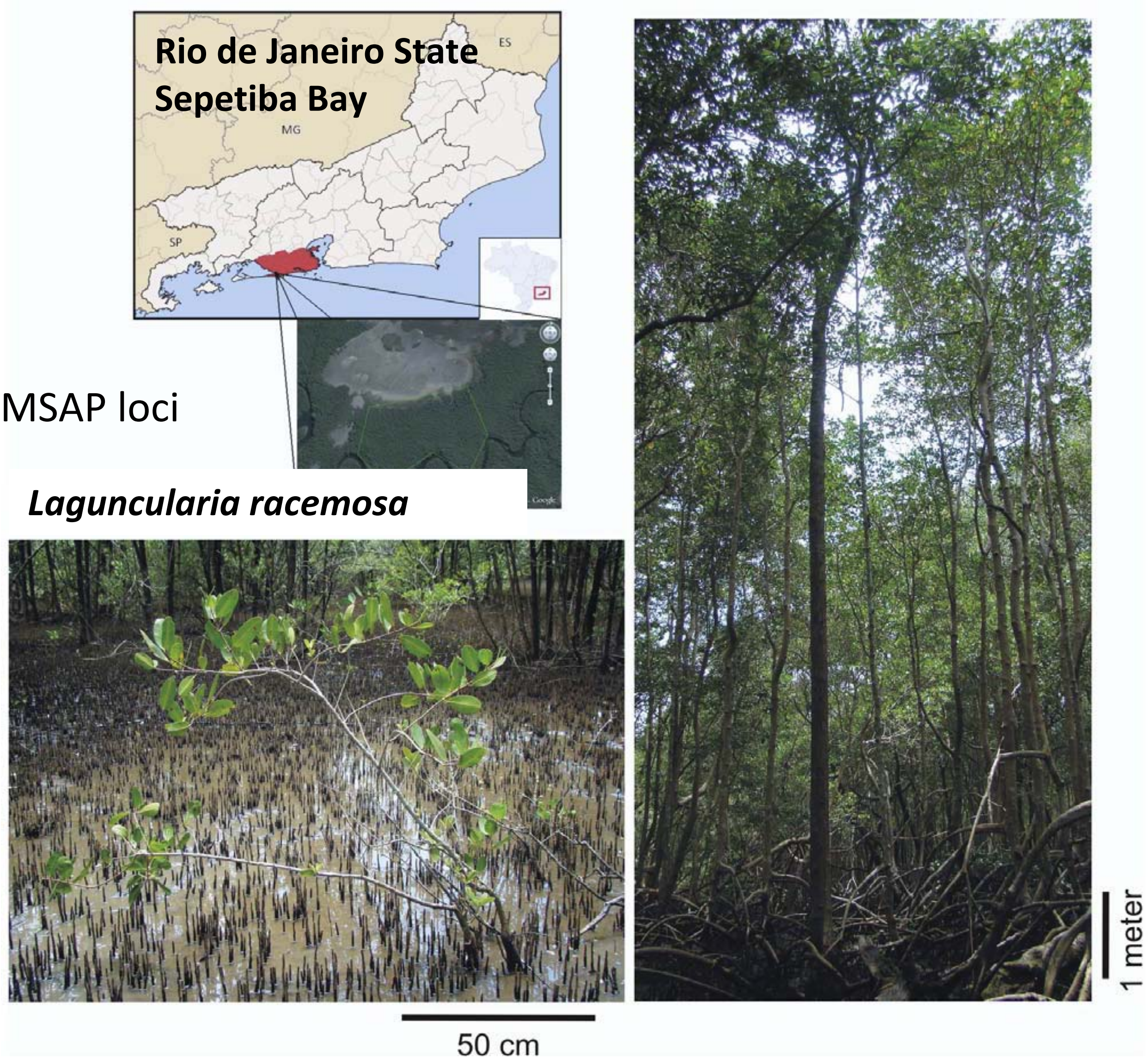




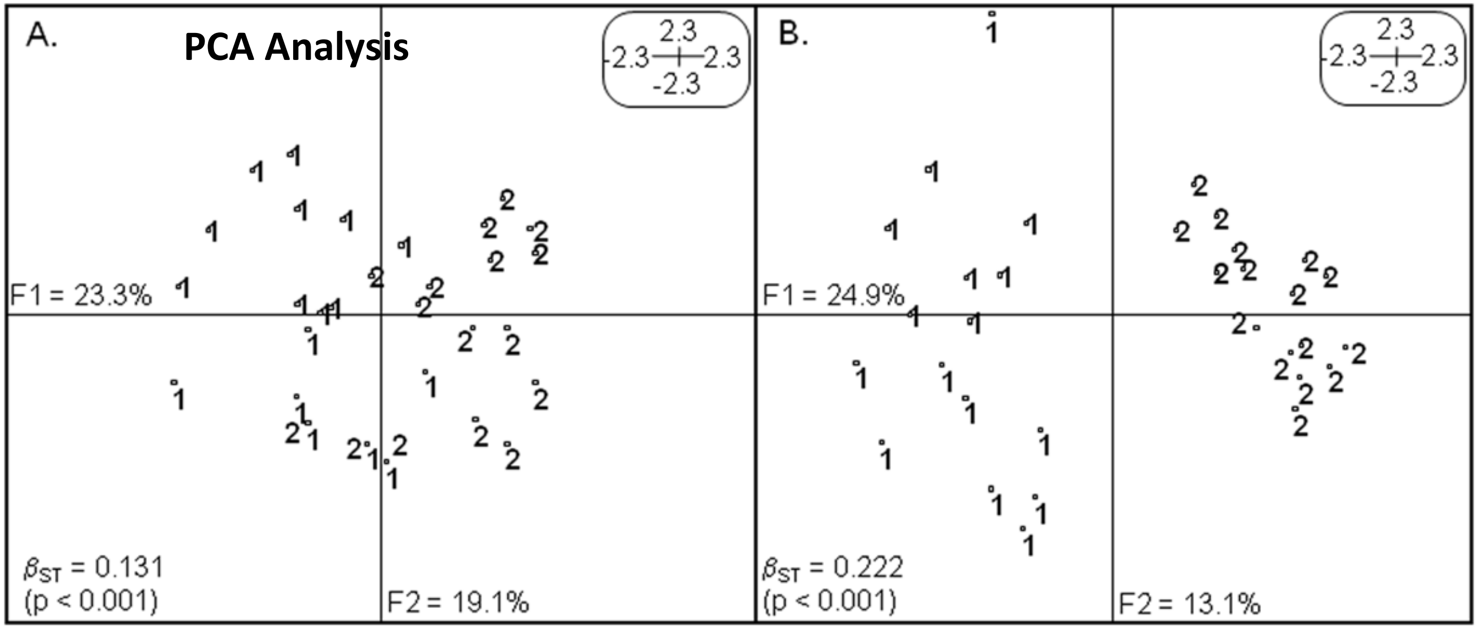
	TX number/accession number																					
ID no.	34	168	98	116	493	6	119	303	2089	230	141	461	1009	44	656	706	724	210	Delcott 344	Tamcott CAMD-E	B444	B559
G1.384	1	1	1	1	1	1	?	1	1	1	1	0	1	1	1	0	0	?	1	1	1	1
G1.367	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	?	?
G1.307	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	0	0	0	0	0	?	?
G1.192[b]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
G2.428	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G2.151[b]	0	0	0	1	0	?	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
G3.491	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1
G3.482	0	1	1	0	1	?	1	0	0	1	1	1	0	0	0	1	0	0	0	0	?	?
G3.430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
G3.392[c]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
G3.292	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
G3.274[b]	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G3.160[b]	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
G3.154	1	1	1	1	1	?	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G4.491[b]	1	0	1	1	1	?	1	?	1	1	1	1	1	1	0	1	1	1	1	1	1	1
G4.368	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
G4.288	?	1	1	1	1	1	1	?	0	1	?	0	?	1	?	0	1	1	1	1	?	?
G4.194	1	?	0	0	?	1	?	1	1	0	1	1	0	?	1	1	?	?	?	?	?	0
G4.172	0	0	0	0	1	0	1	0	?	0	0	0	0	0	1	0	0	0	0	0	?	?
G4.165	0	1	1	1	1	1	1	1	1	1	0	0	0	0	?	?	?	1	0	0	0	0
B1.424	1	?	1	1	?	?	?	0	?	1	0	0	0	1	1	1	1	1	1	1	0	?
B1.218	0	0	0	1	0	?	?	0	0	0	0	0	0	0	0	0	0	?	0	1	?	?
B2.493	0	0	0	0	0	1	1	0	0	0	0	0	0	?	0	0	0	?	0	0	0	?
B2.465	?	?	?	?	?	1	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?
B2.232[c]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
B2.158	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
B3.416	0	1	1	0	?	?	0	?	1	0	0	1	?	1	0	?	0	?	0	?	0	?
B3.359[b]	0	1	0	0	1	0	1	1	1	1	0	1	0	1	1	0	0	0	1	1	1	1
B3.208[b]	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0
B4.499	0	0	0	0	0	1	1	?	0	0	0	0	0	0	1	0	0	?	0	0	0	1
B4.327	?	0	0	?	?	?	?	?	?	?	0	?	1	0	?	?	?	?	?	?	0	0
B4.325	0	?	?	0	0	1	1	1	0	0	0	0	?	?	0	0	0	0	0	0	?	?
B4.175	1	?	1	1	0	?	0	0	0	0	?	0	0	?	0	1	0	?	1	1	1	0

Epigenetic change as a long term strategy in plant adaptation

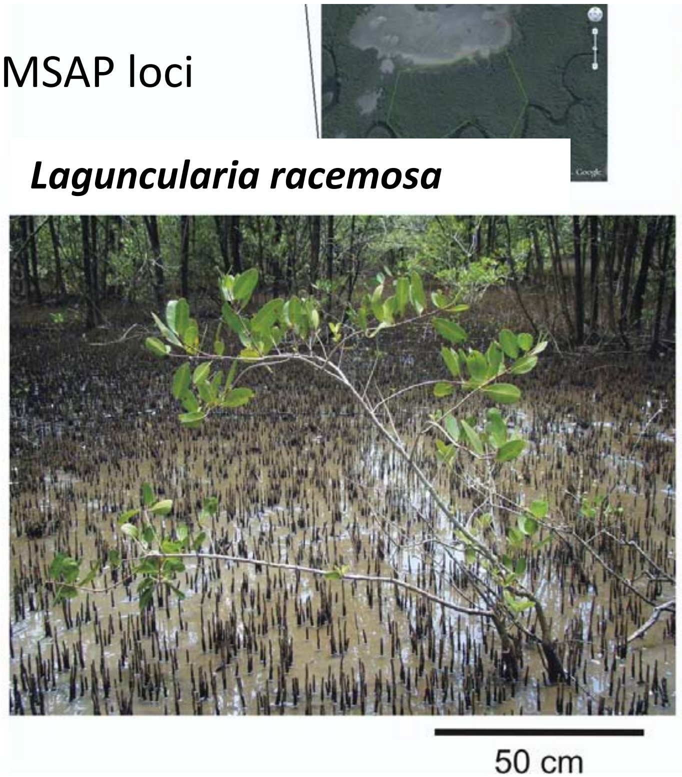
- Analysis of 183 informative MSAP loci



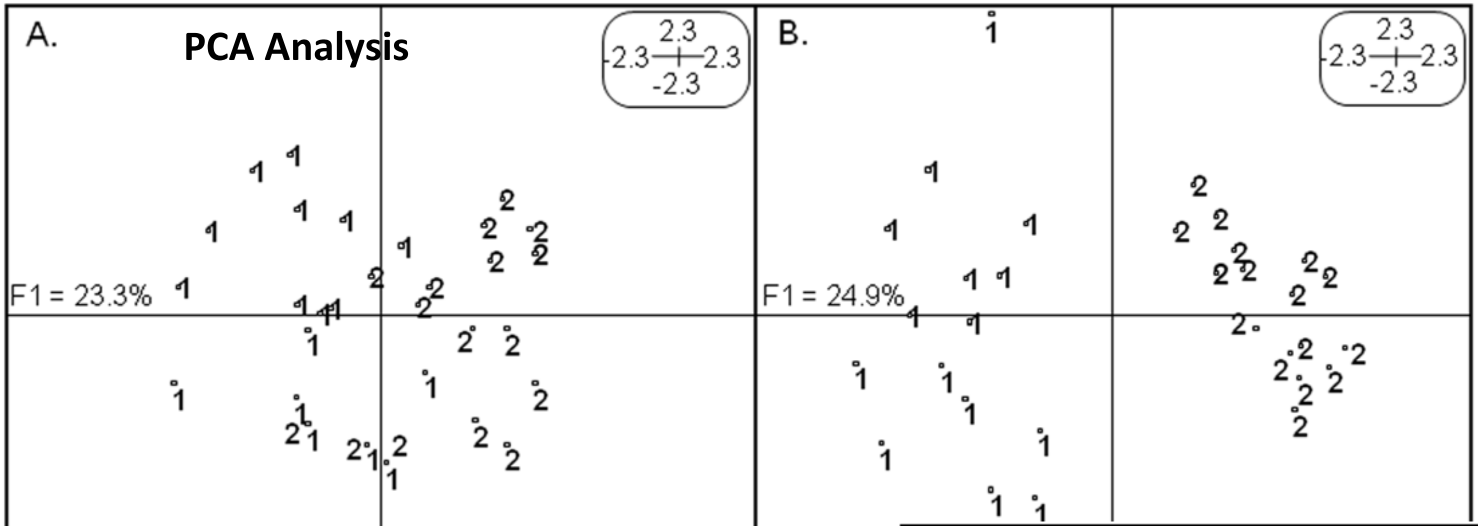
rategy in



- Analysis of 183 informative MSAP loci


Laguncularia racemosa

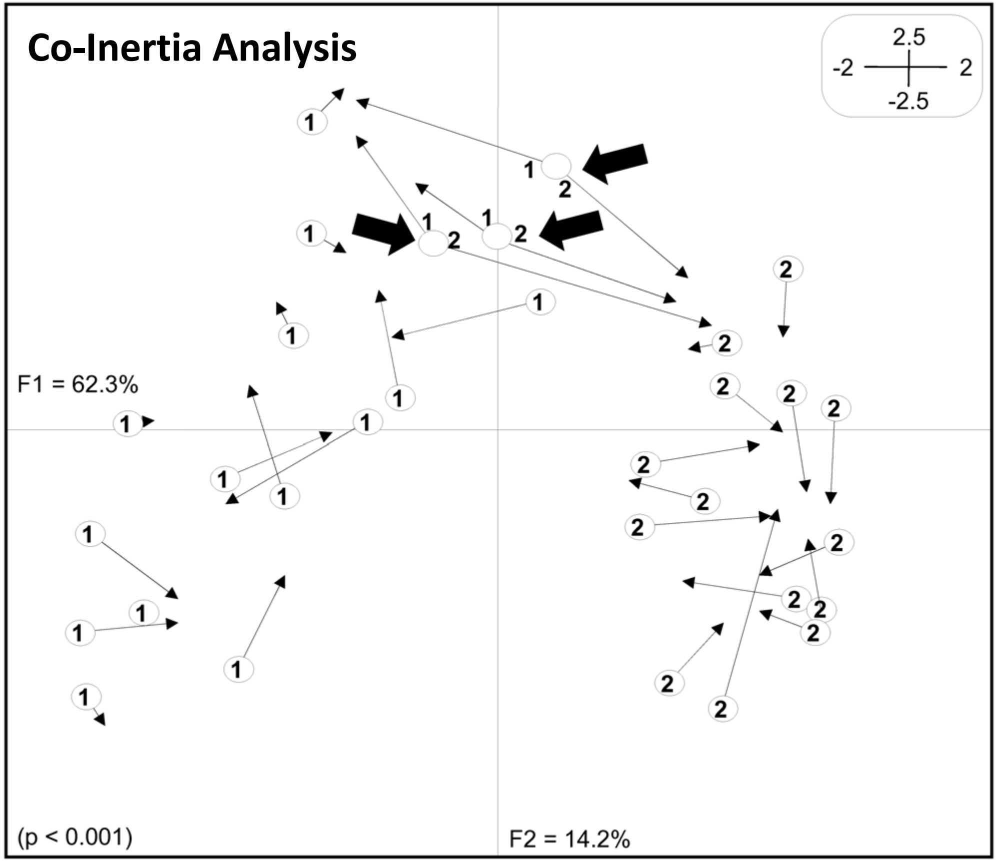
rategy in



PCA Analysis

- Analysis of 183 informative MSAP loci

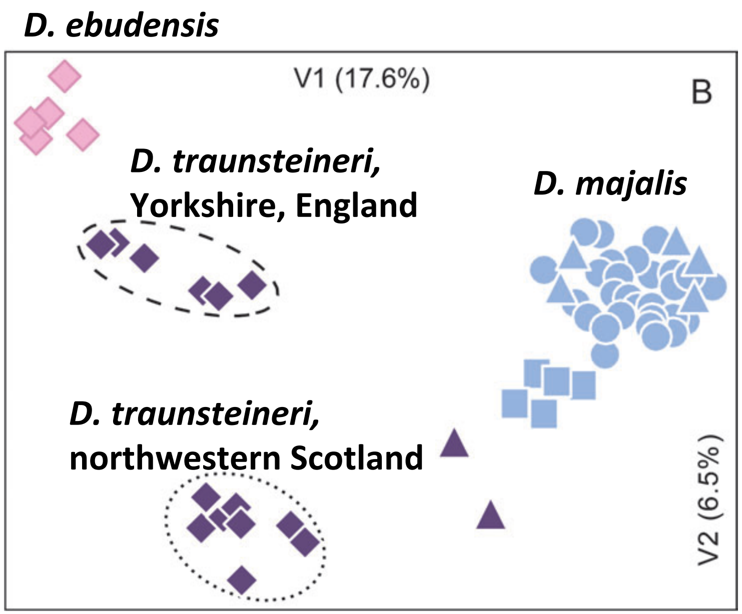
Laguncularia



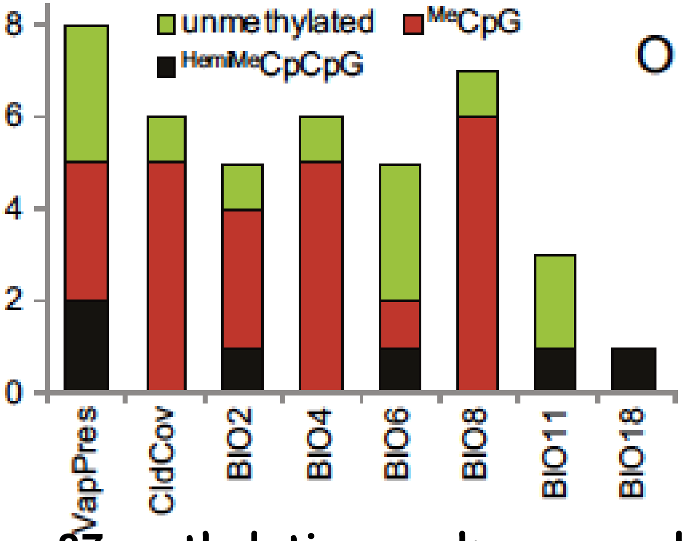
Co-Inertia Analysis

50 cm

Environmental allopatry through epigenetic changes

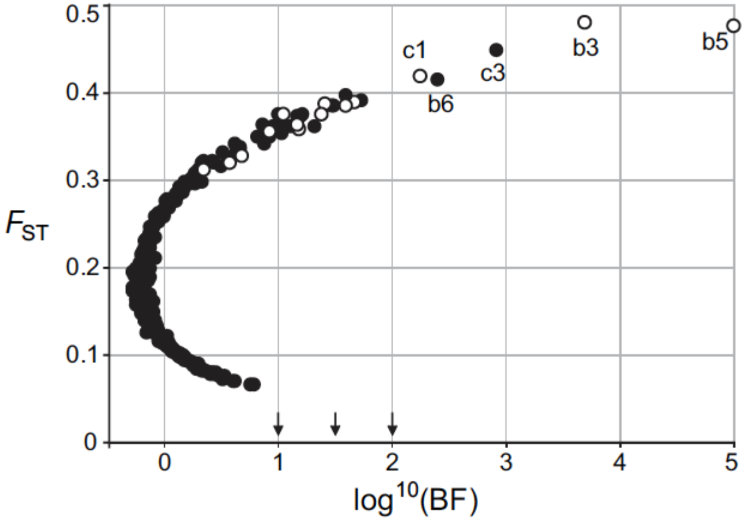


- Alloplyploid *Dactylorhiza* sibling species - hybridisation and polyploydisation in evolutionary history
- Morphological similarities and highly similar genetic constitution (AFLP)
- Epigenetic differences (MSAP) provoked by genome duplication

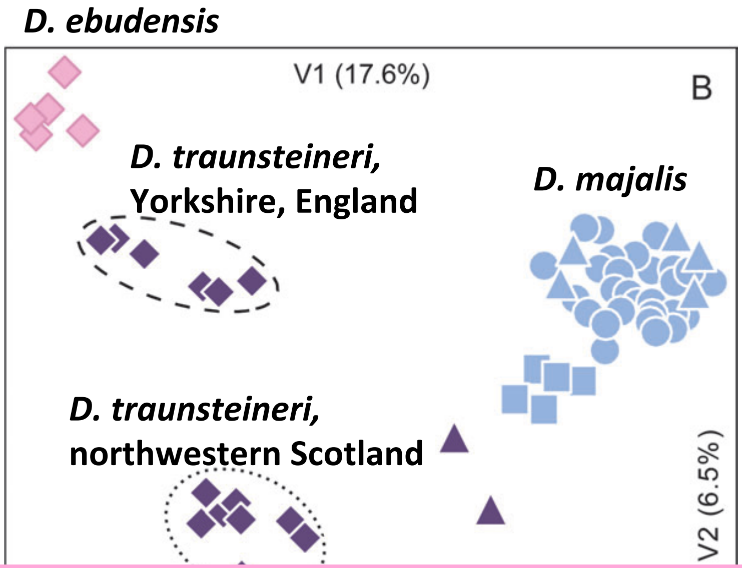


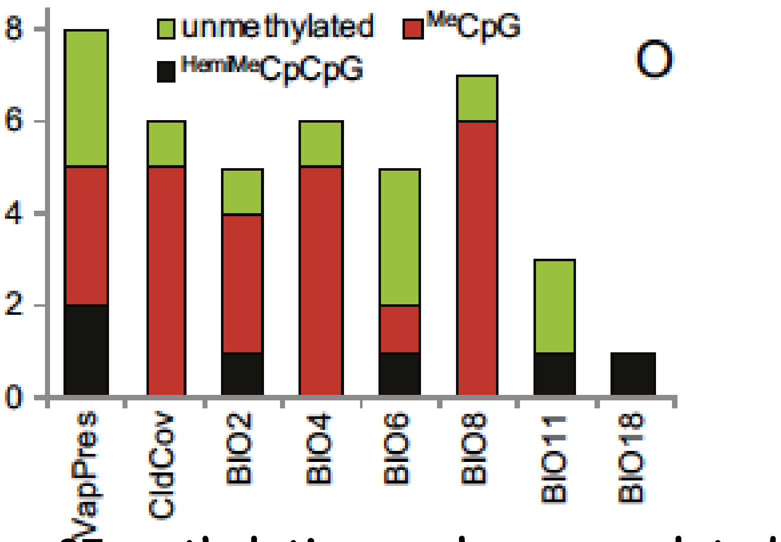
87 methylation markers correlated with environmental variables (temp/water availability)

BayeScan analysis



Environmental allopatry through epigenetic changes

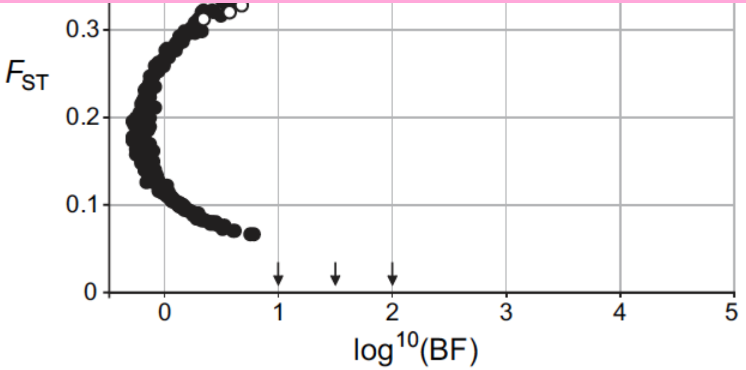




87 methylation markers correlated

- Ecological divergence largely the result of adaptation achieved by epigenetic effects that modulate gene expression (cDNA- AFLP) under environmental influence.

- h
h
-
genetic constitution (AFLP)
- Epigenetic differences (MSAP) provoked by genome duplication

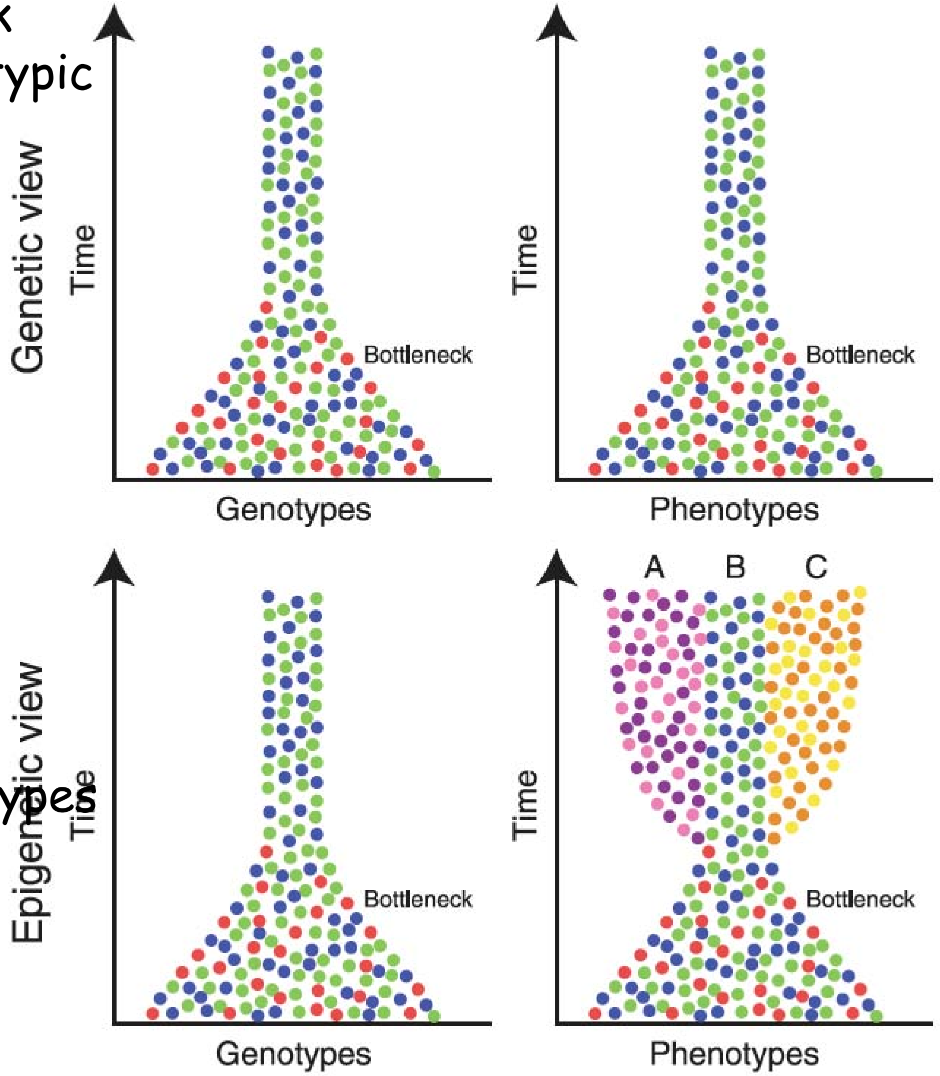


Epigenetic view of the genetic bottleneck

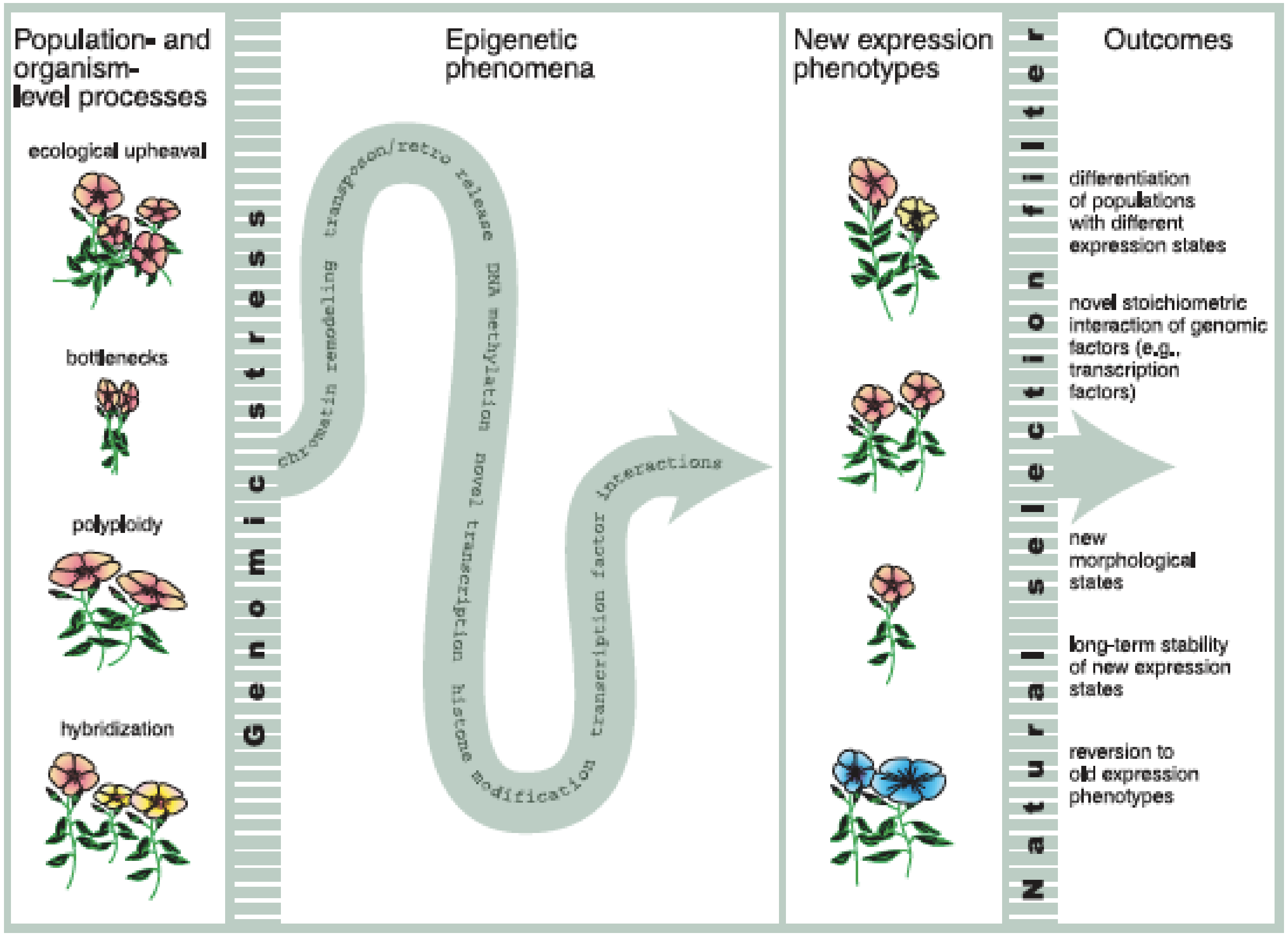
- Interspecies hybridisation and polyploidisation provoke genomic distruption and genomic shock which have effect of genetic bottleneck
- Promotion and fixation of new phenotypic variants in small populations

Winnowing of genotypes and phenotypes in requirements of genetic drift and natural selection

Winnowing process is ameliorated by novel epigenetically mediated phenotypes

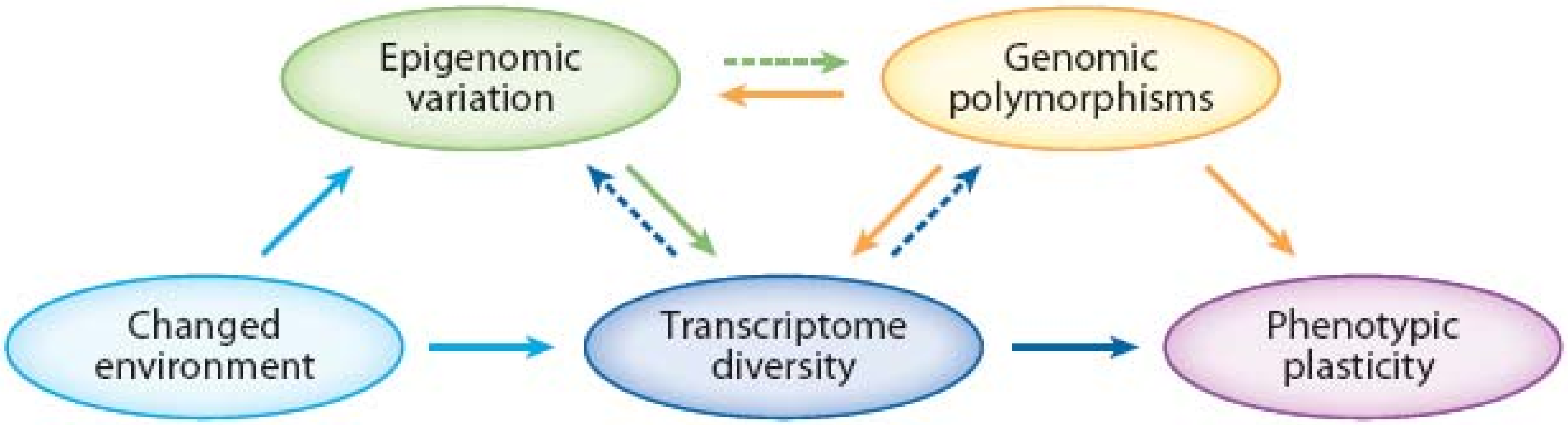


Epigenetic variation contributes to evolution



In this view, a bottleneck could provide the stimulus for evolutionary novelty, mediated by epigenetic response to genomic stress/ecological upheaval, where new phenotypic variability can rapidly be achieved and fixed within a population.

Interaction of genome, epigenome and transcriptome diversity in response to changed environment in creation of phenotype



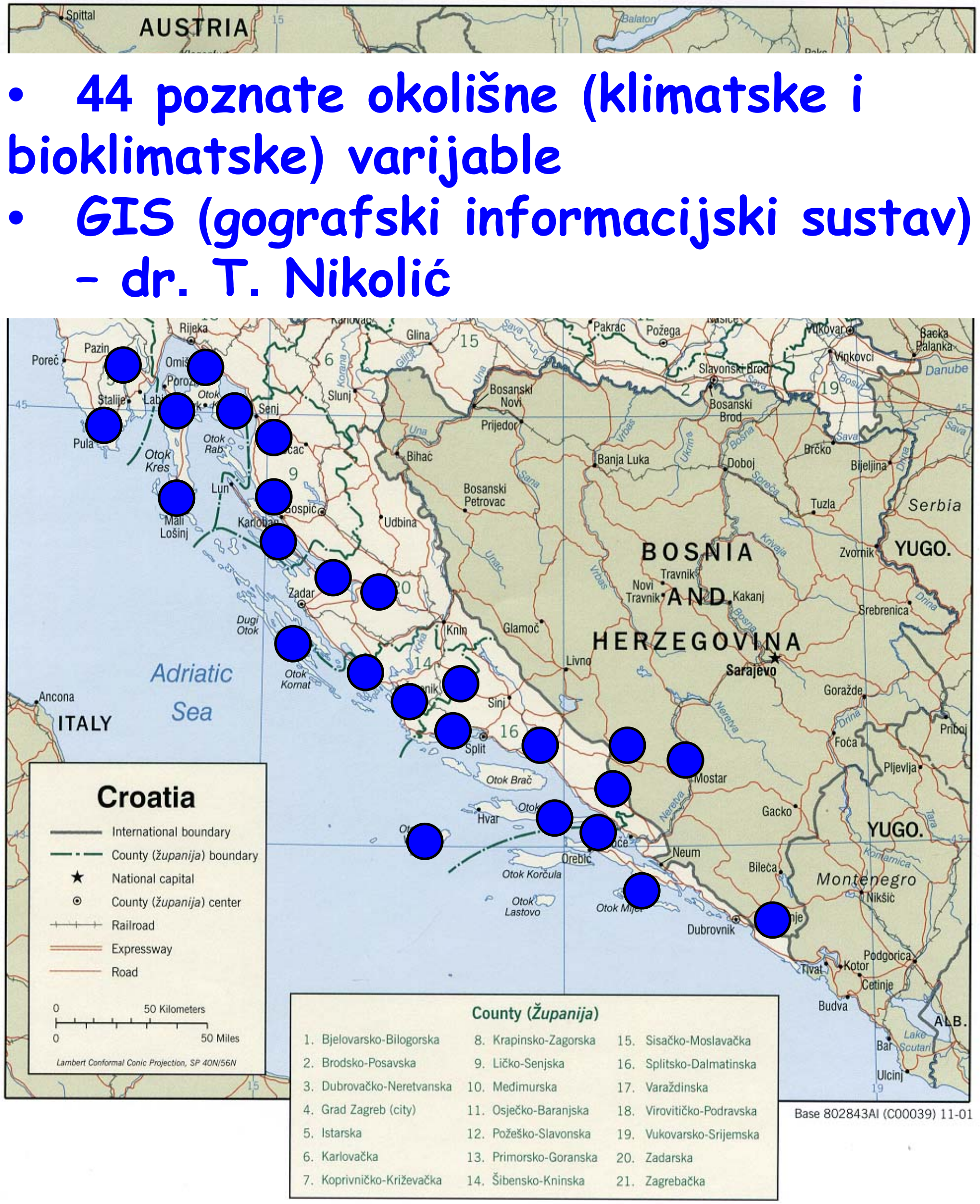
Plasticity and dynamics of plant (epi)genomes

Epigenetic phenomena in natural variation and microevolution in *Salvia* species

- 3 croatian endemic salvia species: *S. officinalis* L., *S. brachyodon* Vandas, *S.* x *auriculata* Mill.
- *S.* x *auriculata* Mill. (hybrid between *S. officinalis* L. and *S. fruticosa*) – Vis
- Each collection site will be geocoded with high precision by GPS and abiotic factors will be generated by GIS
- Variables - altitude, slope, exposition (min, max, mean for each variable) will be determined

LJEKOVITA KADULJA (*Salvia officinalis* L.)

- 44 poznate okolišne (klimatske i bioklimatske) varijable
- GIS (gografski informacijski sustav) – dr. T. Nikolić

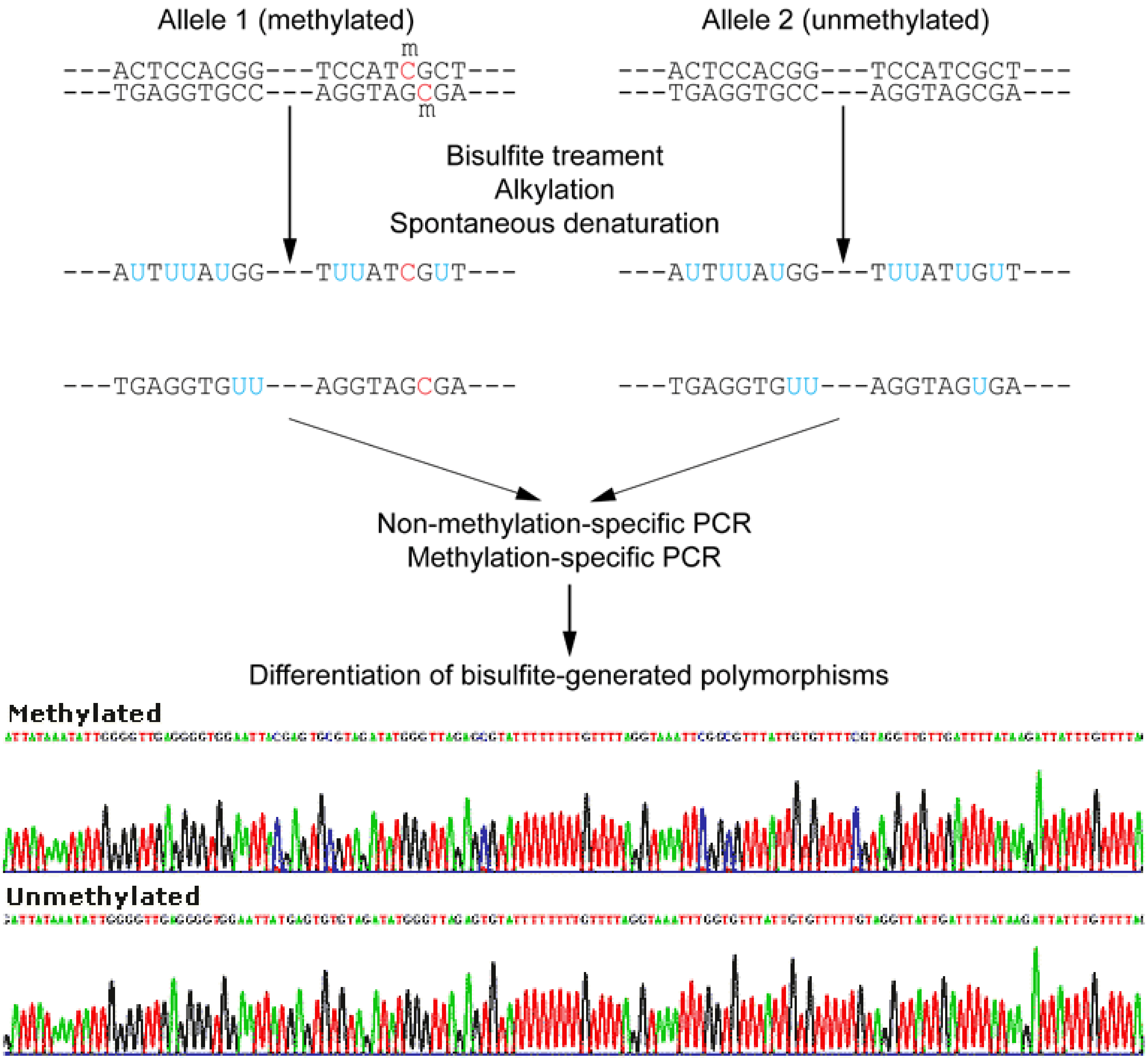


25 populacija po 25 biljaka

1 Šušnjevica
2 Kamenjak
3 Krk
4 Stara Baška
5 Cres
6 Lošinj
7 Vratnik
8 Karlobag
9 Pag
10 Dugi Otok
11 Otišina
12 Pirovac
13 Zrmanja
14 Šparadići
15 Vinišće
16 Unešić
17 Biokovo
18 Runovići
19 Mostar
20 Međugorje
21 Hvar
22 Vis
23 Pelješac
24 Mljet
25 Konavle

Ongoing studies

- Genetic (AFLP) versus epienetic diversity (MSAP)
- *S. officinalis* L. – widely distributed along eastern Adriatic cost
- *S. brachyodon* Vandas – genetic bottleneck, steoendemic plant with very narrow range size (Mount Ilija, Pelješac, and Mount Orijen, Montenegro)
- *S.* x *auriculata* Mill – hybridisation events, Vis is the only site of appearance
- Informative epialleles – estimation of methylation level by pyrosequencing


Allele 1 (methylated)
Allele 2 (unmethylated)
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
Bisulfite treament
Alkylation
Spontaneous denaturation
---AUTUUAUGG---TUUATCGUT---
---AUTUUAUGG---TUUATUGUT---
---TGAGGTGUU---AGGTAGCGA---
---TGAGGTGUU---AGGTAGUGA---
Non-methylation-specific PCR
Methylation-specific PCR
Differentiation of bisulfite-generated polymorphisms
Methylated
Unmethylated

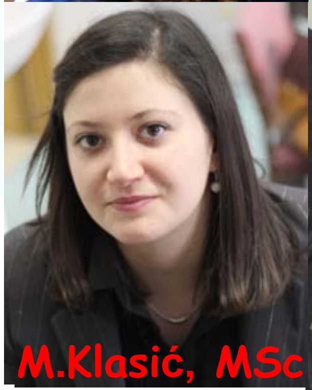
SVEUČILIŠTE U
ZAGREBU

Epi-group


A. Vojta, PhD


P. Dobrinić,MSc


D. Markulin,
MSc


M.Klasić, MSc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Vedrana Vičić, MSc


I. Samaržija,
PhD

Hrvatska zaklada
za znanost

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HRZZ-EpiSalvia, HRZZ-IgGGlyco