



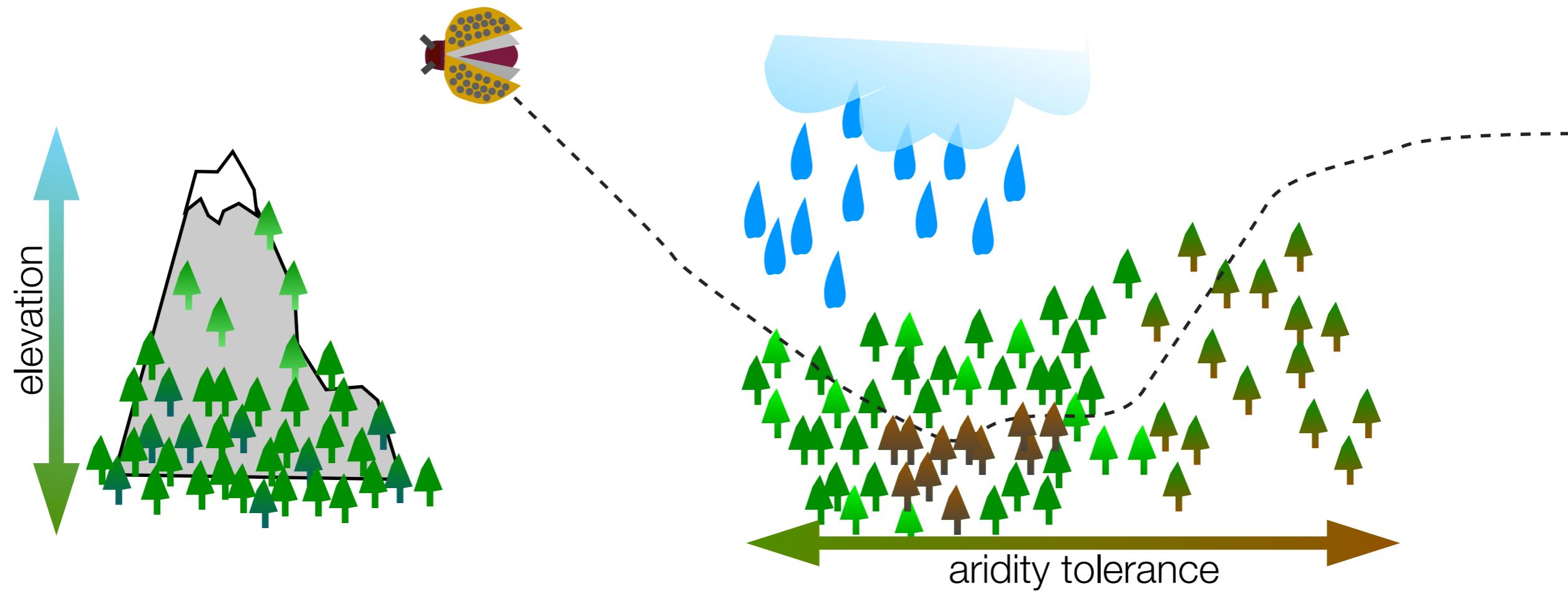
Epigenetics of domestication: A case study of canines

22 September 2016

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Princeton University
Ecology & Evolutionary Biology

Traits in a changing environment

- Abiotic stress (climate, altitude, food/water)
- Biotic stress (pathogens, injury)
- Genomic stress (inbreeding, transposition, selection)



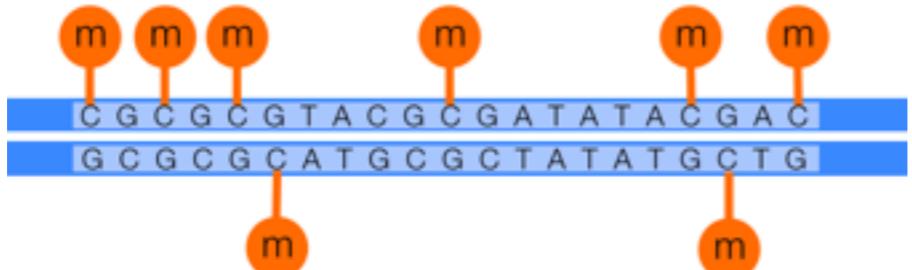


My MZ twin!

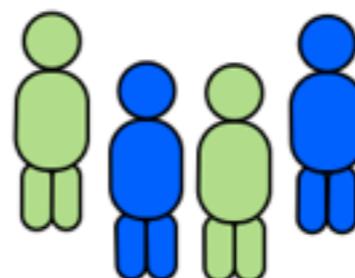
Me!

Ecological epigenetics

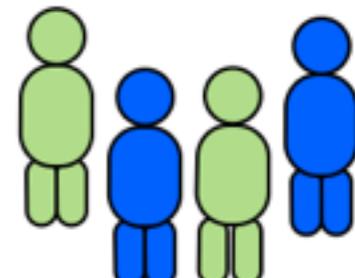
1. DNA methylation



2. Epigenetic methods

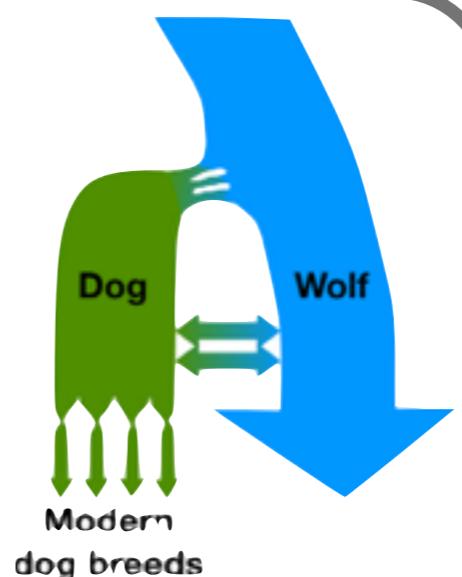


Family 1



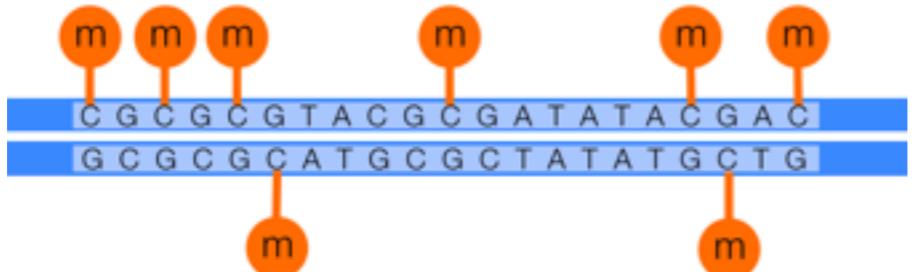
Family 2

3. Comparative methylation of dogs and wolves

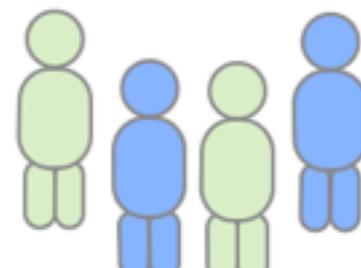


Ecological epigenetics

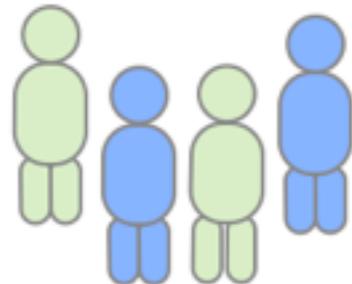
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2. Epigenetic methods

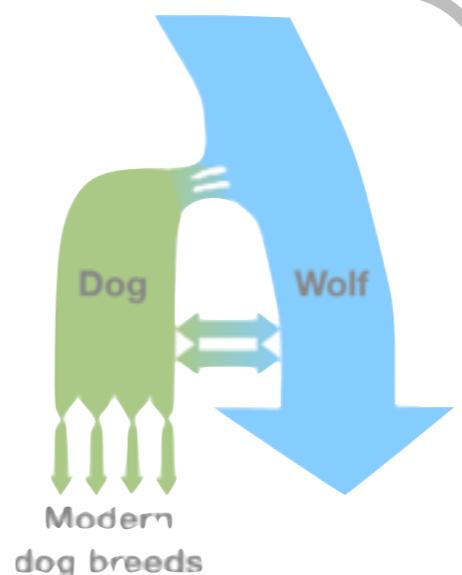


Family 1



Family 2

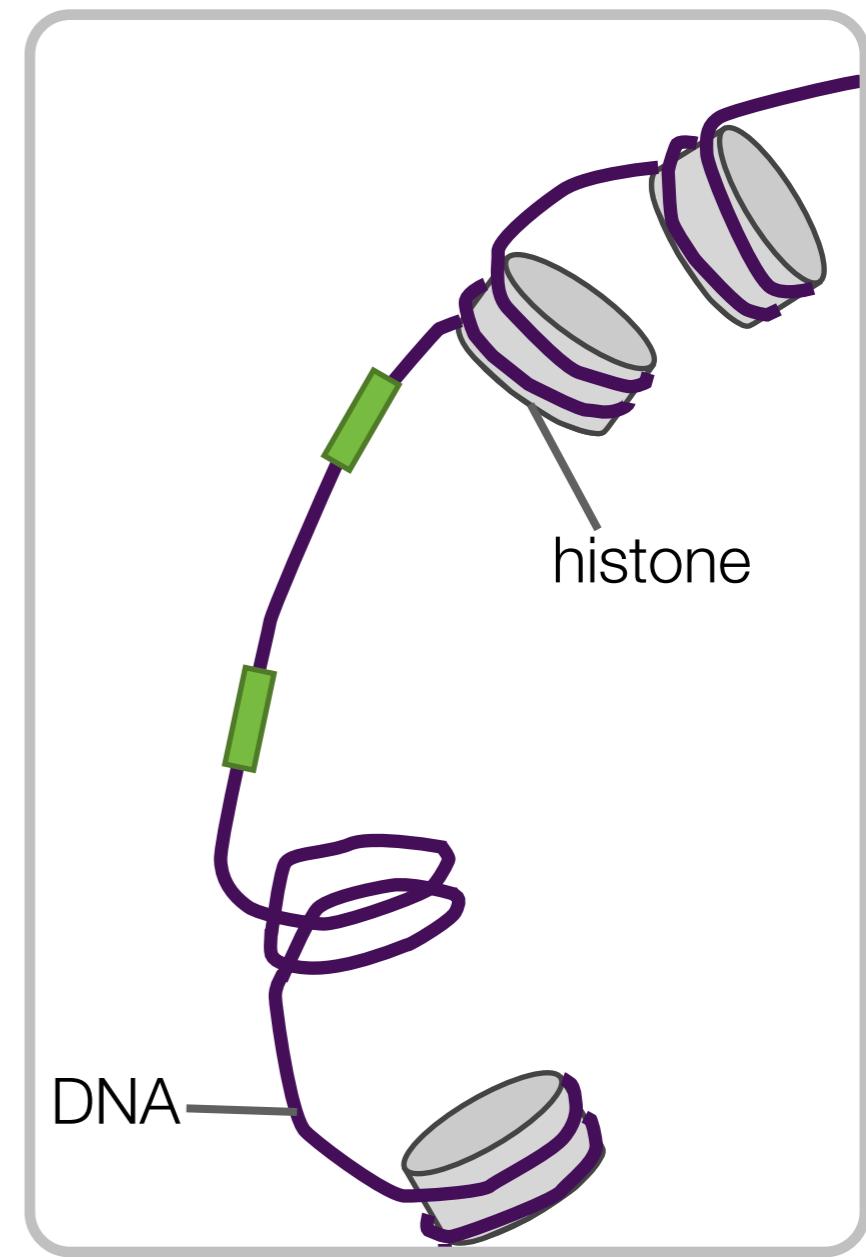
3. Comparative methylation of dogs and wolves



What is epigenetics?

- “*Epi*”-genetics means “above” or “on top of” the DNA sequence
- Epigenetic polymorphisms (or variants) can be heritable, permanent, and reversed

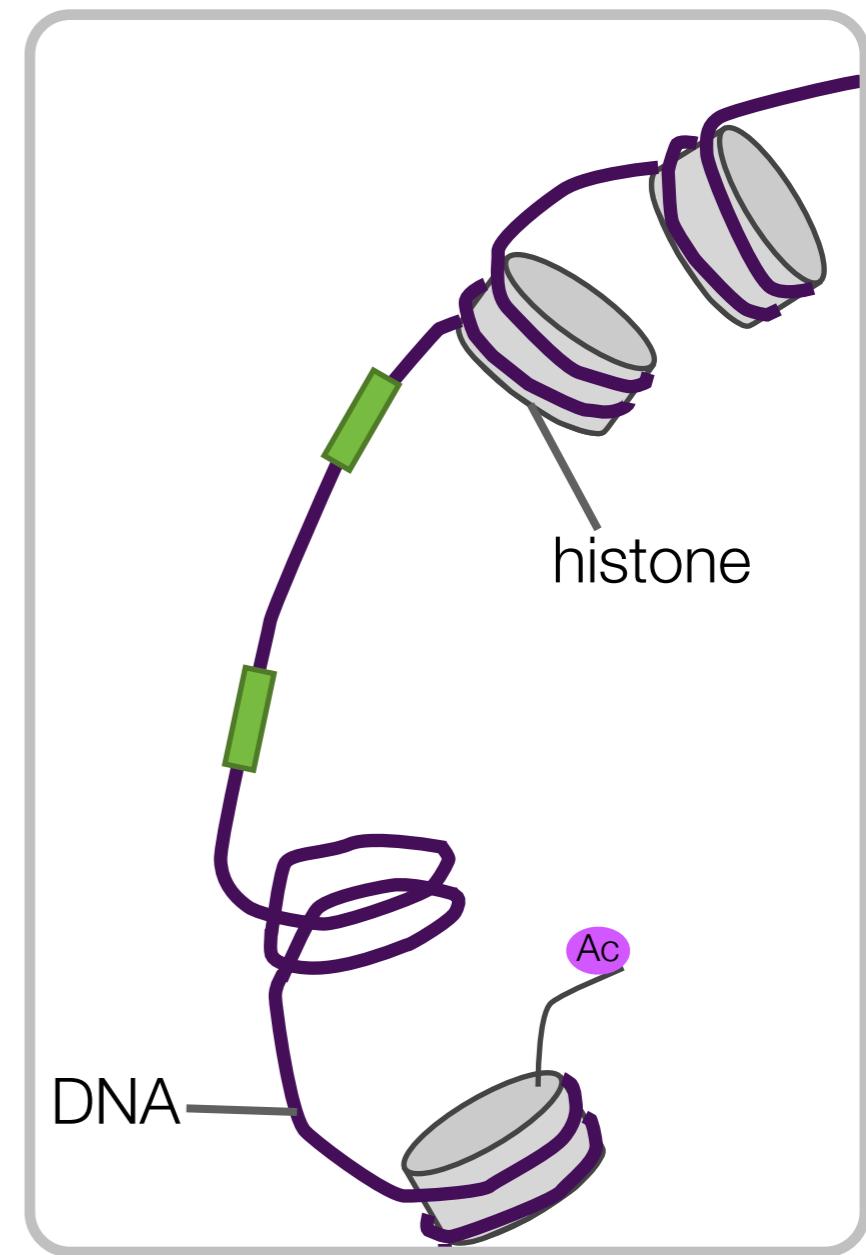
Epigenetic Modification	Activating	Silencing
Nucleosome	Remodeling	



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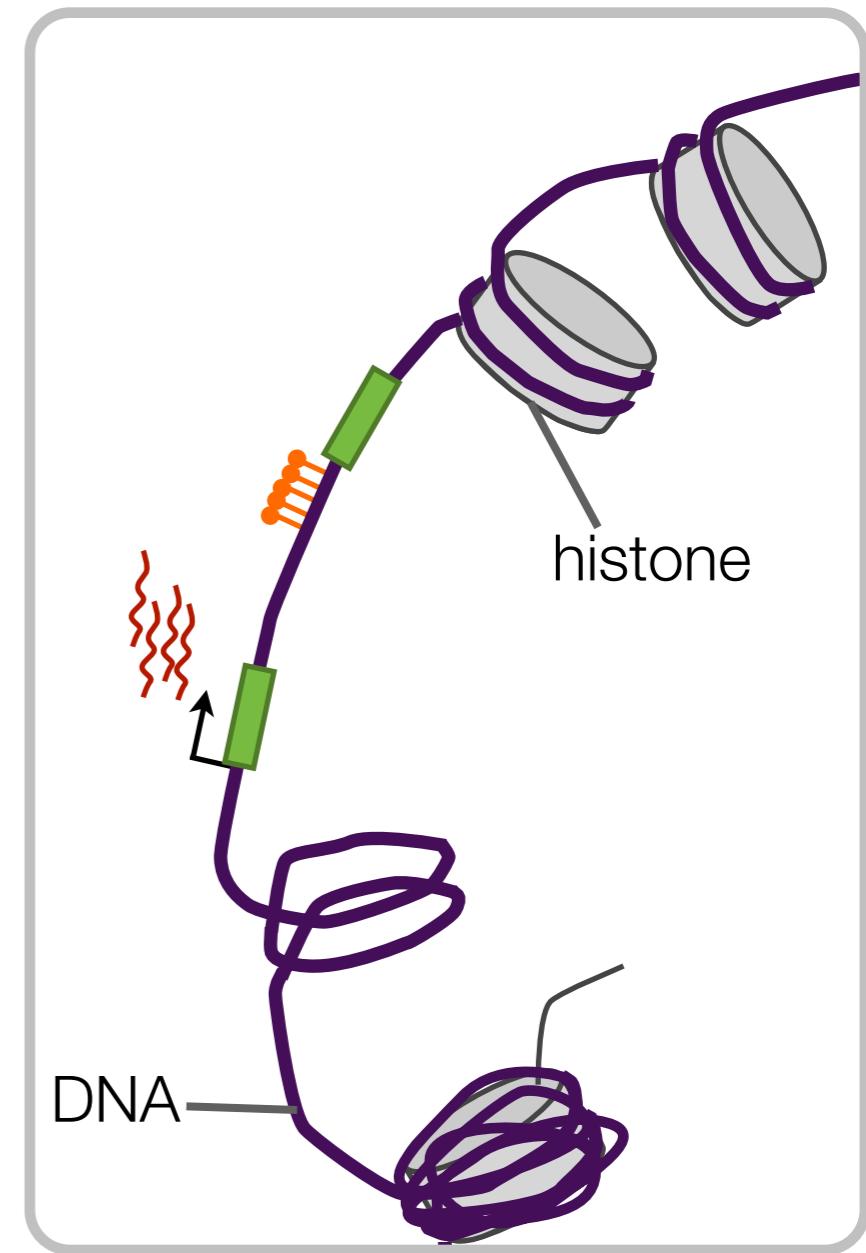
Epigenetic Modification	Activating	Silencing
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Histone	Acetylation	Deacetylation



What is epigenetics?

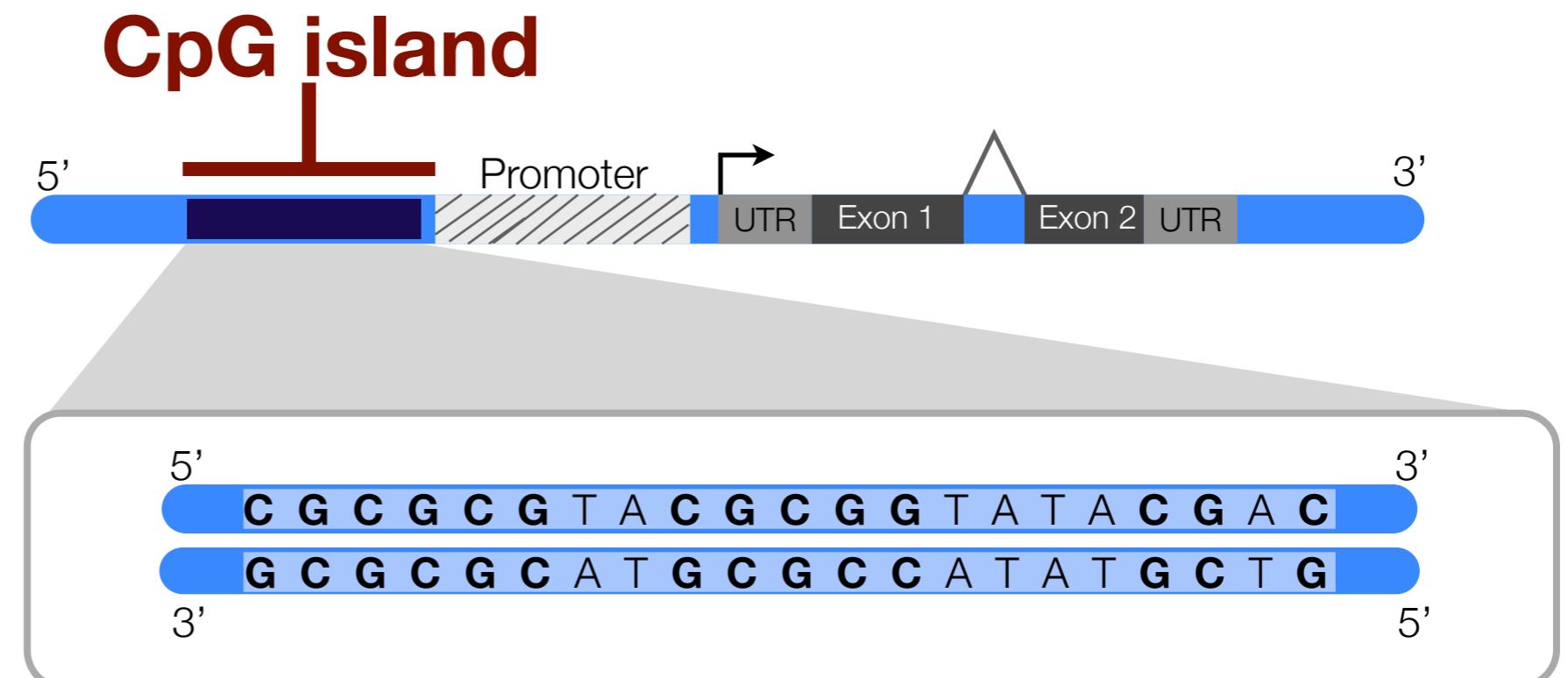
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Epigenetic Modification	Activating	Silencing
Nucleosome	Remodeling	
Histone	Acetylation	Deacetylation
★ DNA	Demethylation	Methylation



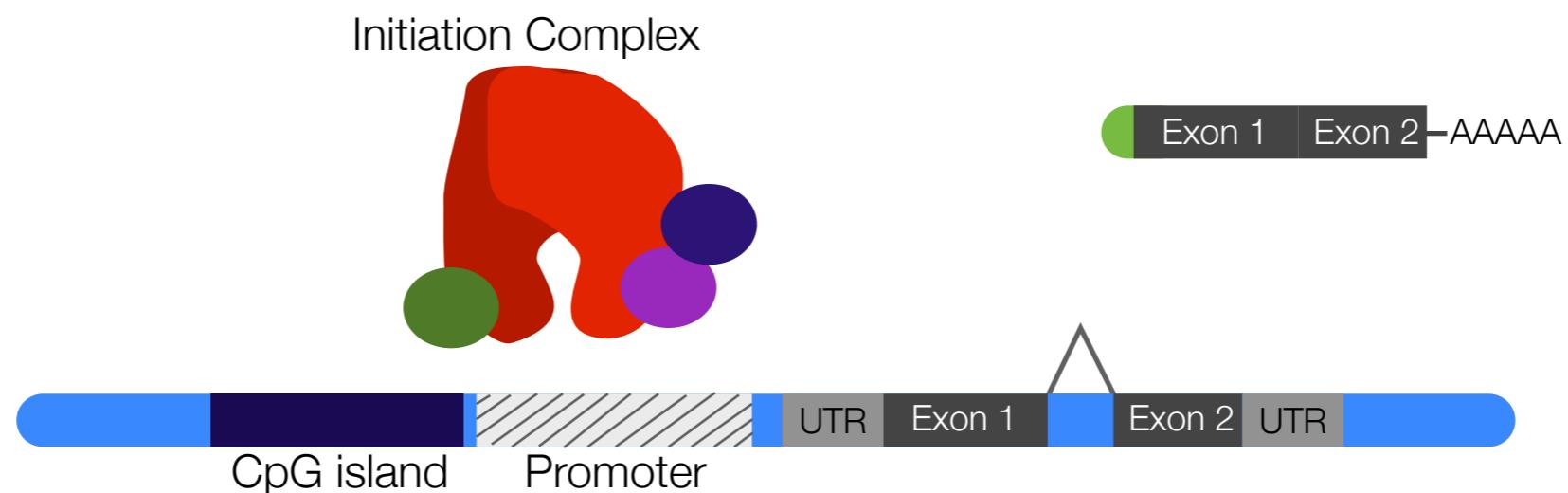
How are genomes structured?

- A *cis*-regulatory element
 - Nearly half of human genes are located close to CpG islands
- CpG islands are short stretches (500bp-2Kb) of DNA
 - Contain up to 50% GC
 - Marks the 5' end of genes



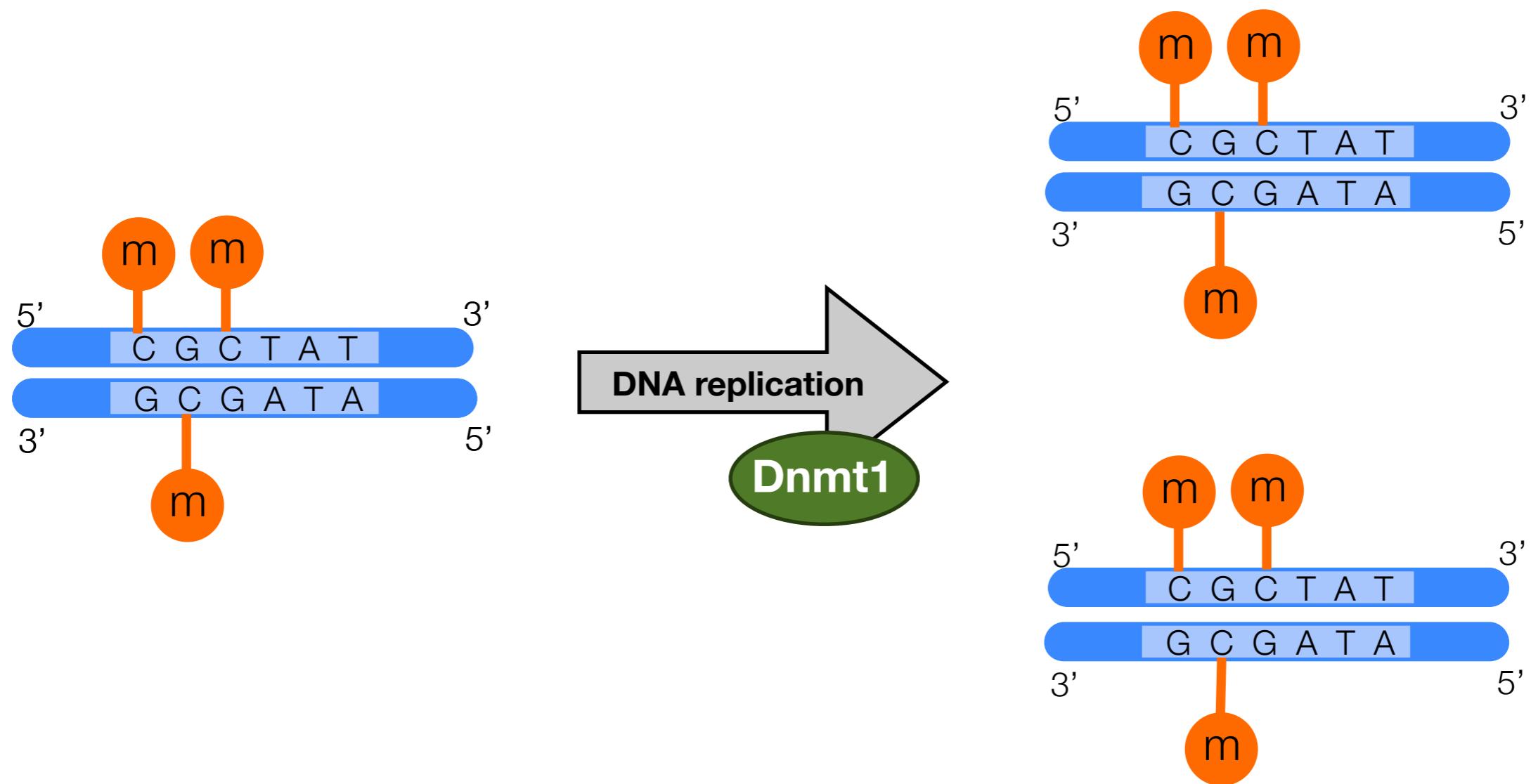
How are genomes structured?

- A *trans*-acting regulatory element
 - The Initiation Complex binds to a DNA motif in the promoter
 - Enzymatic activity to open the double helix and initiation transcription
- Gene transcription produces mRNA to be translated



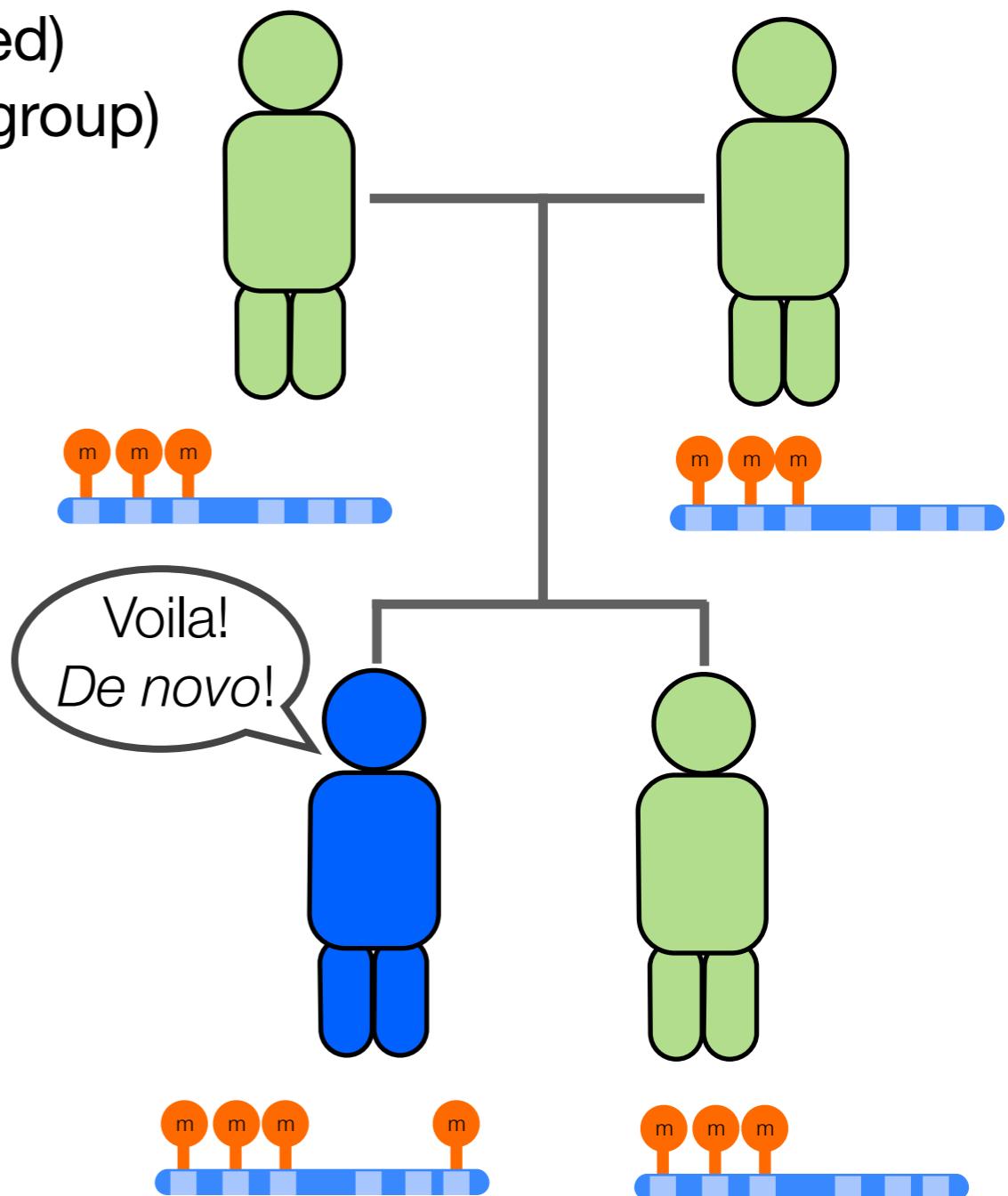
DNA cytosine methylation

- Types of methylation
 - **Maintenance** (DNA replication; conserved)

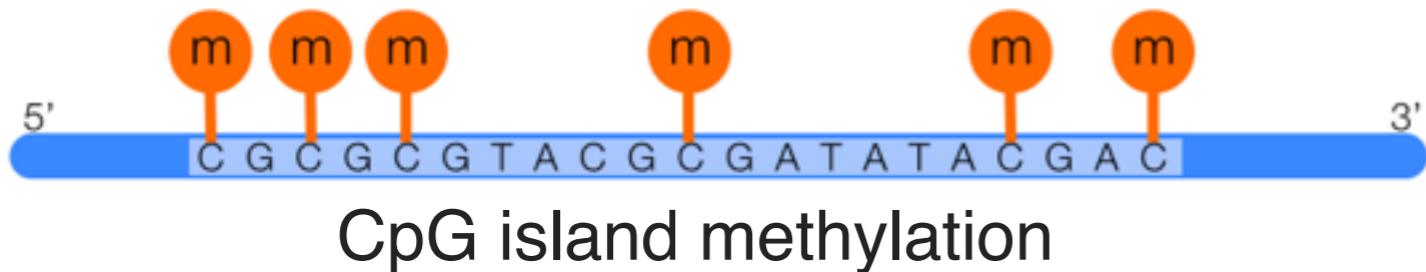


DNA cytosine methylation

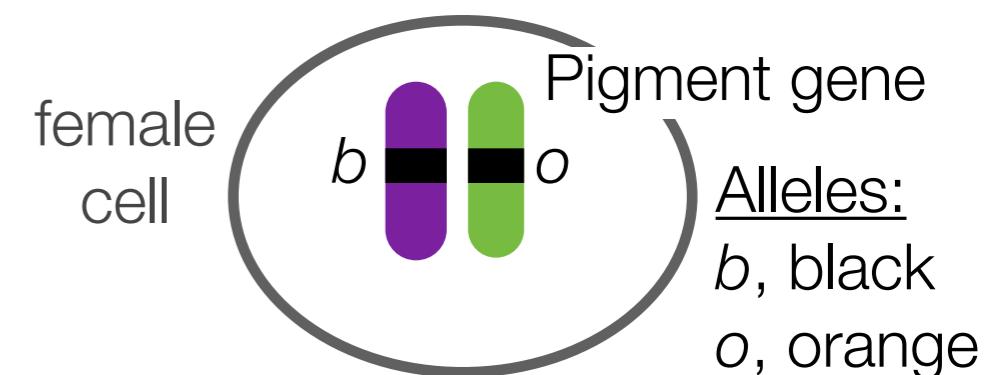
- Types of methylation
 - Maintenance (DNA replication; conserved)
 - **De novo** (new sites acquiring a methyl group)



Dosage compensation

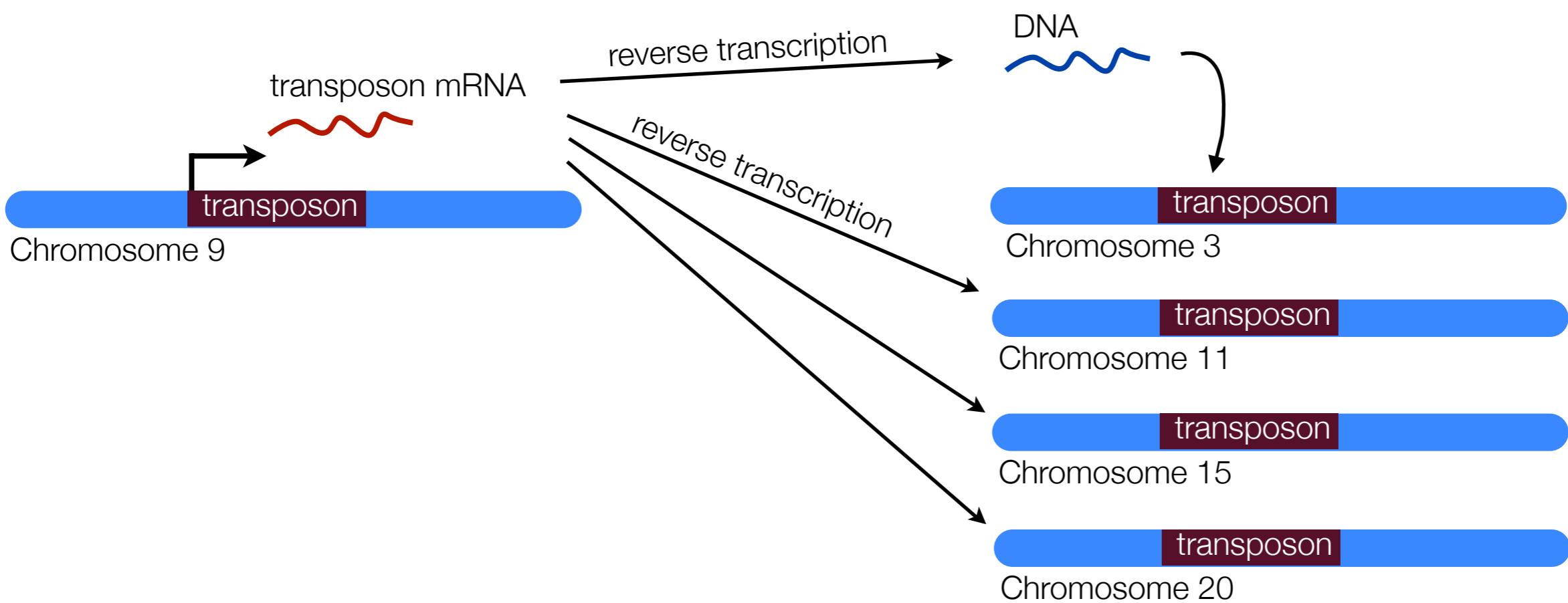


X chromosome origin:
maternal (purple) paternal (green)



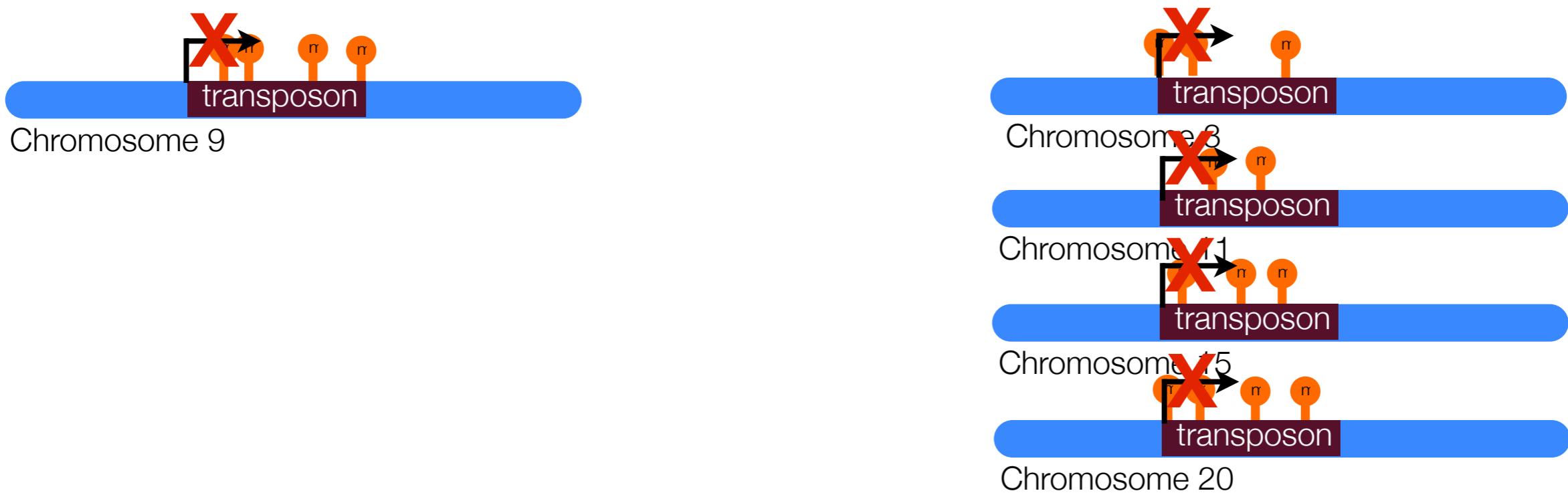
Transposon silencing

- A transposon is a segment of mobile DNA that copies itself and the new copies insert into new locations throughout the genome



Transposon silencing

- Methylation of the transposon prevents their replication and transposition

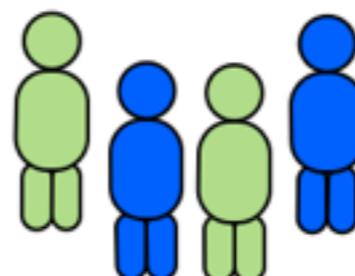


Ecological epigenetics

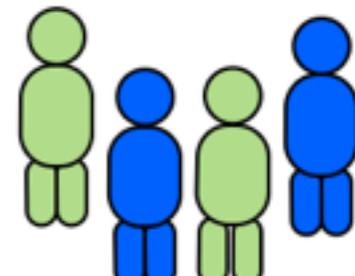
1. DNA methylation



2. Epigenetic methods

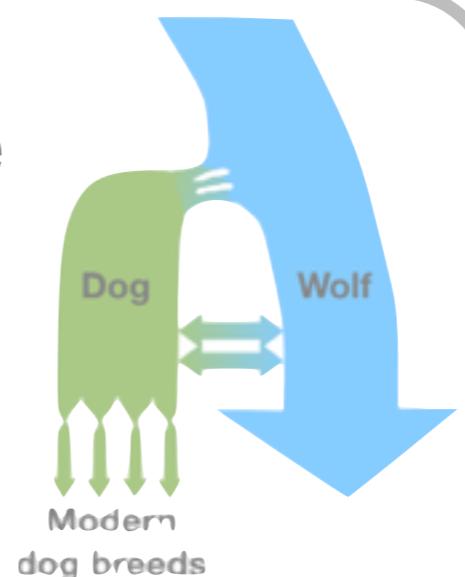


Family 1



Family 2

3. Comparative methylation of dogs and wolves



Studying methylation

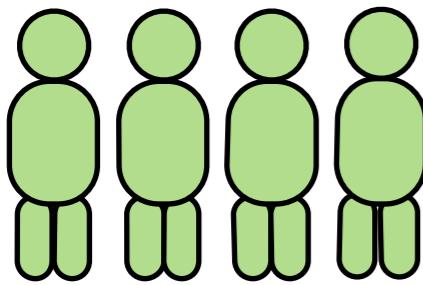
- Disruption of methylation leads to severe disorders
- Lack of imprinting
- Differential methylation may increase disease risk
- CpG islands are “hotspots” for mutation (**CpG** to **TpG**)

Human phenotype	Epigenetic disorder
Angelman Syndrome	Maternal chromosome silenced
Prader-Willi Syndrome	Paternal chromosome silenced
Leukemia	Differential methylation of <i>TAL1</i> and <i>TLX1</i>
Diabetes	Differential methylation of <i>HHEX</i>
Cancers	Differential methylation of <i>IGF2</i>
Esophageal cancer	Differential methylation of <i>FEZ1</i>
Heart malformations	Differential methylation of <i>NKX2</i>

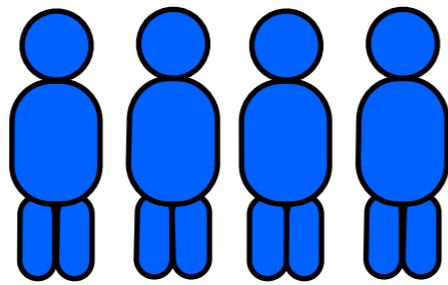
Feinberg and Irizarry (2010) PNAS

Detecting methylated DNA: who?

Population-based approach

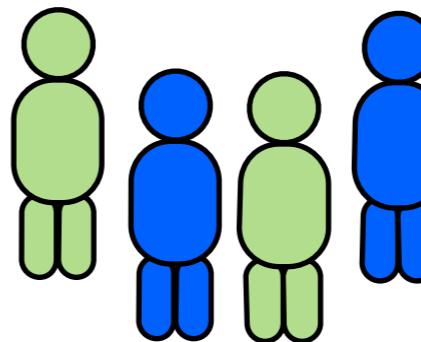


Case cohort

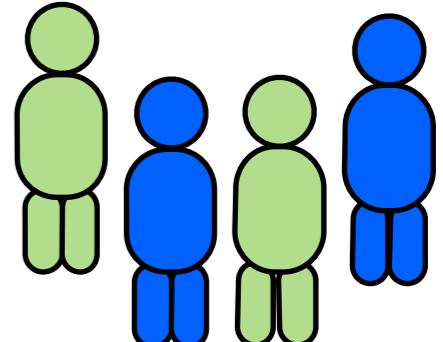


Control cohort

Families: inheritance-based approach

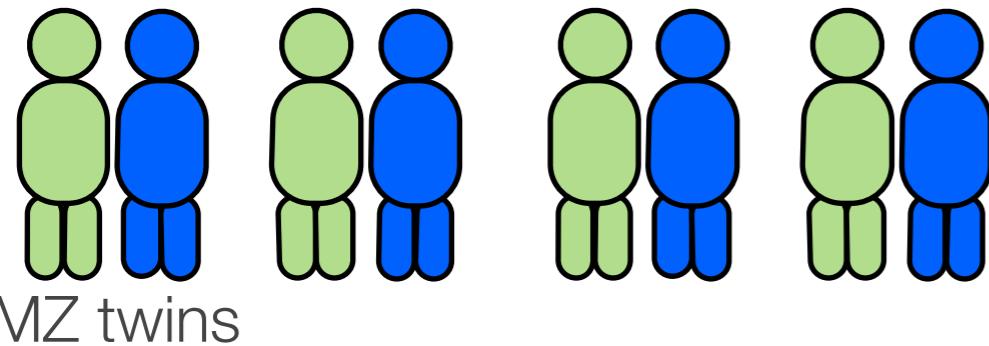


Family 1



Family 2

Twin-based study



MZ twins

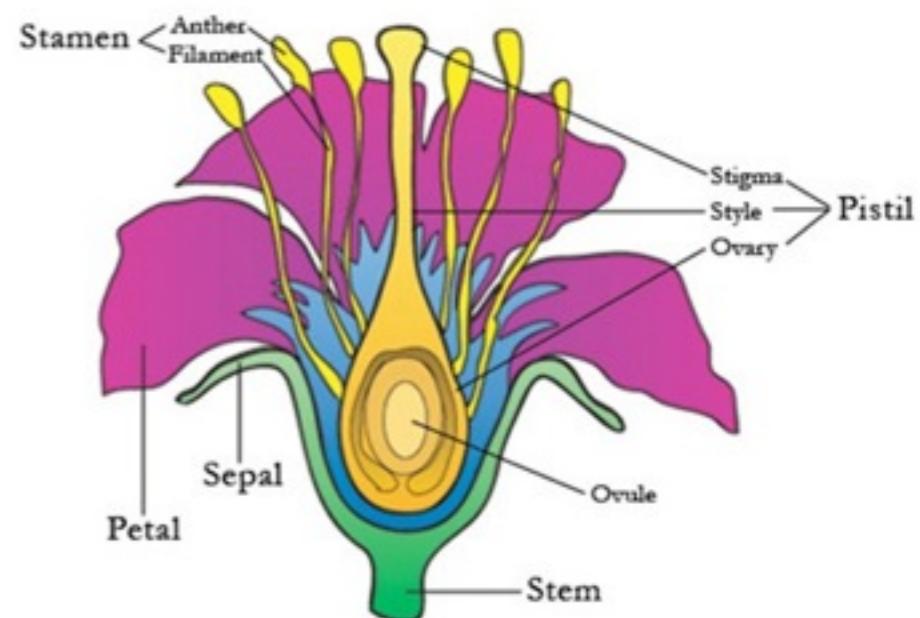
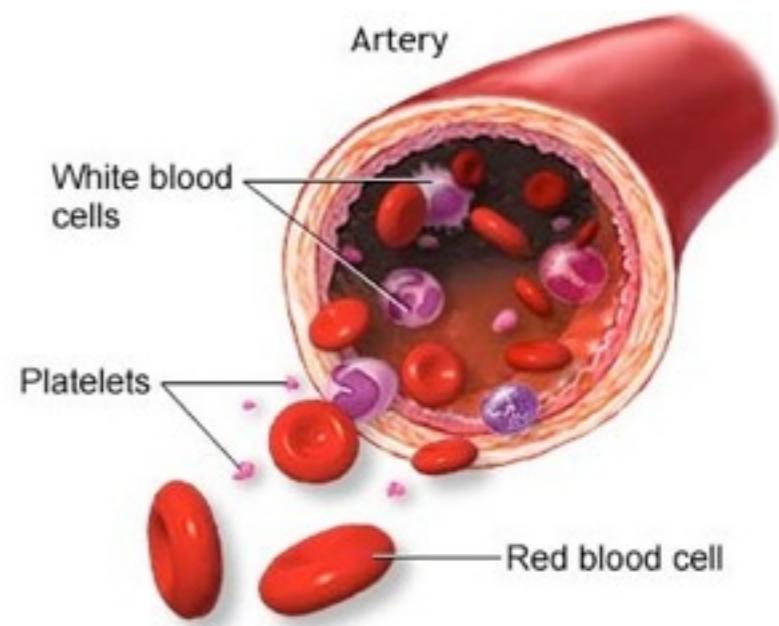
Longitudinal study



Temporal-based analysis

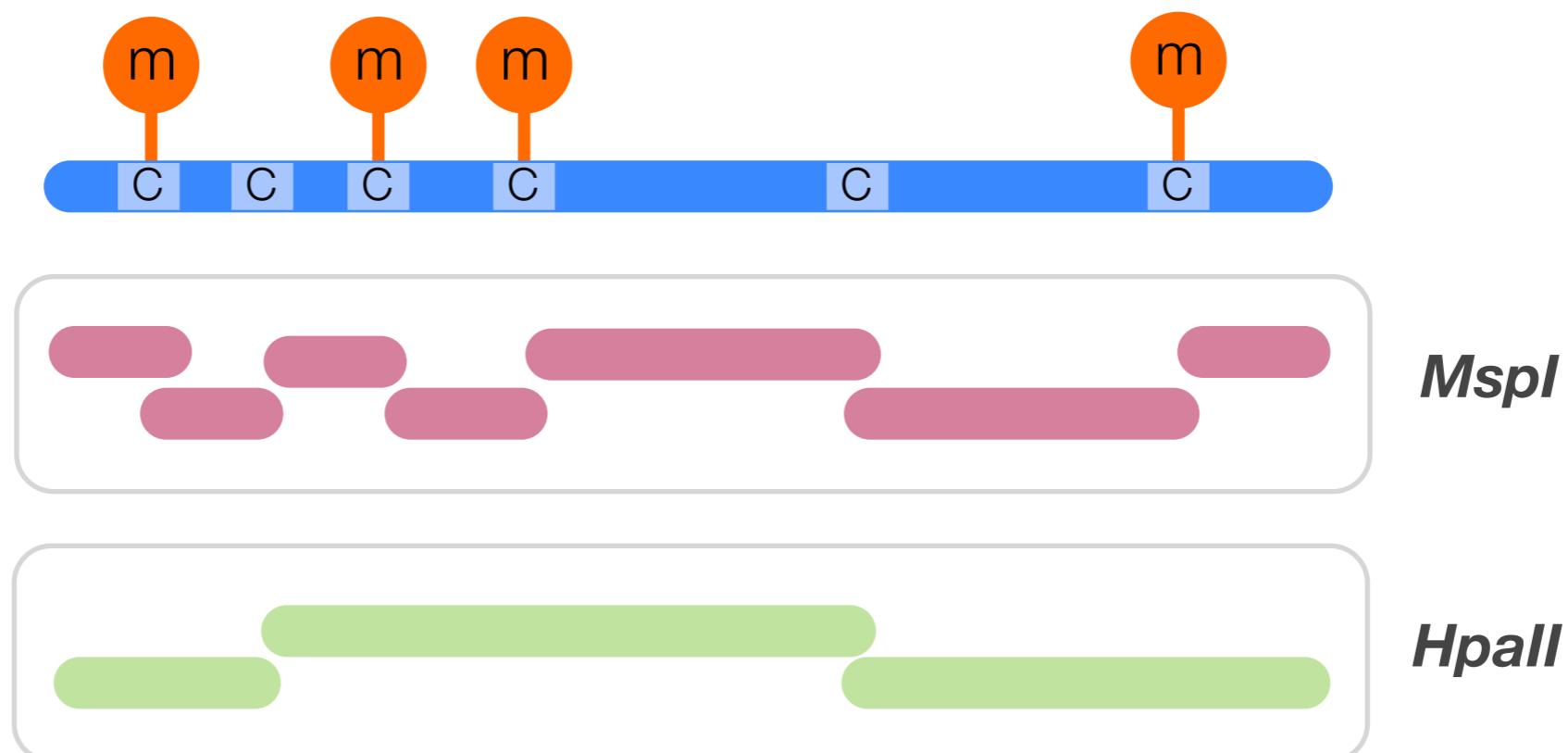
Detecting methylated DNA: what?

- Whole blood
 - Red blood cells (enucleated in mammals) and white blood cells (nucleated immune system cells) in plasma
 - Hormones and other proteins
- Specific tissues and/or cell lines
 - Liver, brain, etc.
 - Cancer vs. normal cells
 - Leaves, shoots, roots, flowers, etc.



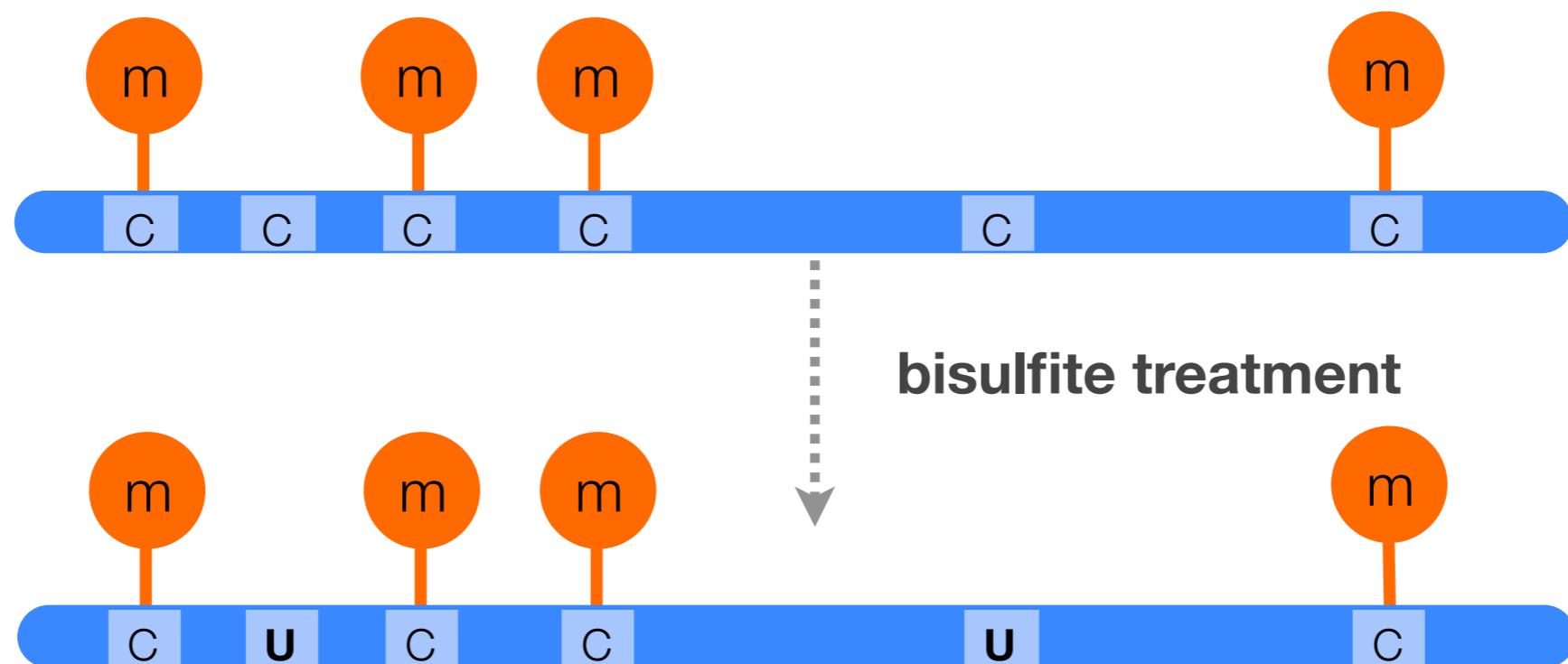
Detecting methylated DNA: how?

- **Methylation-sensitive restriction enzymes** (REs) with subsequent sequencing
 - *MspI* cuts the DNA sequence {CCGG} regardless of it's methylation status
 - *HpaII* cannot cut methylated {CCGG}



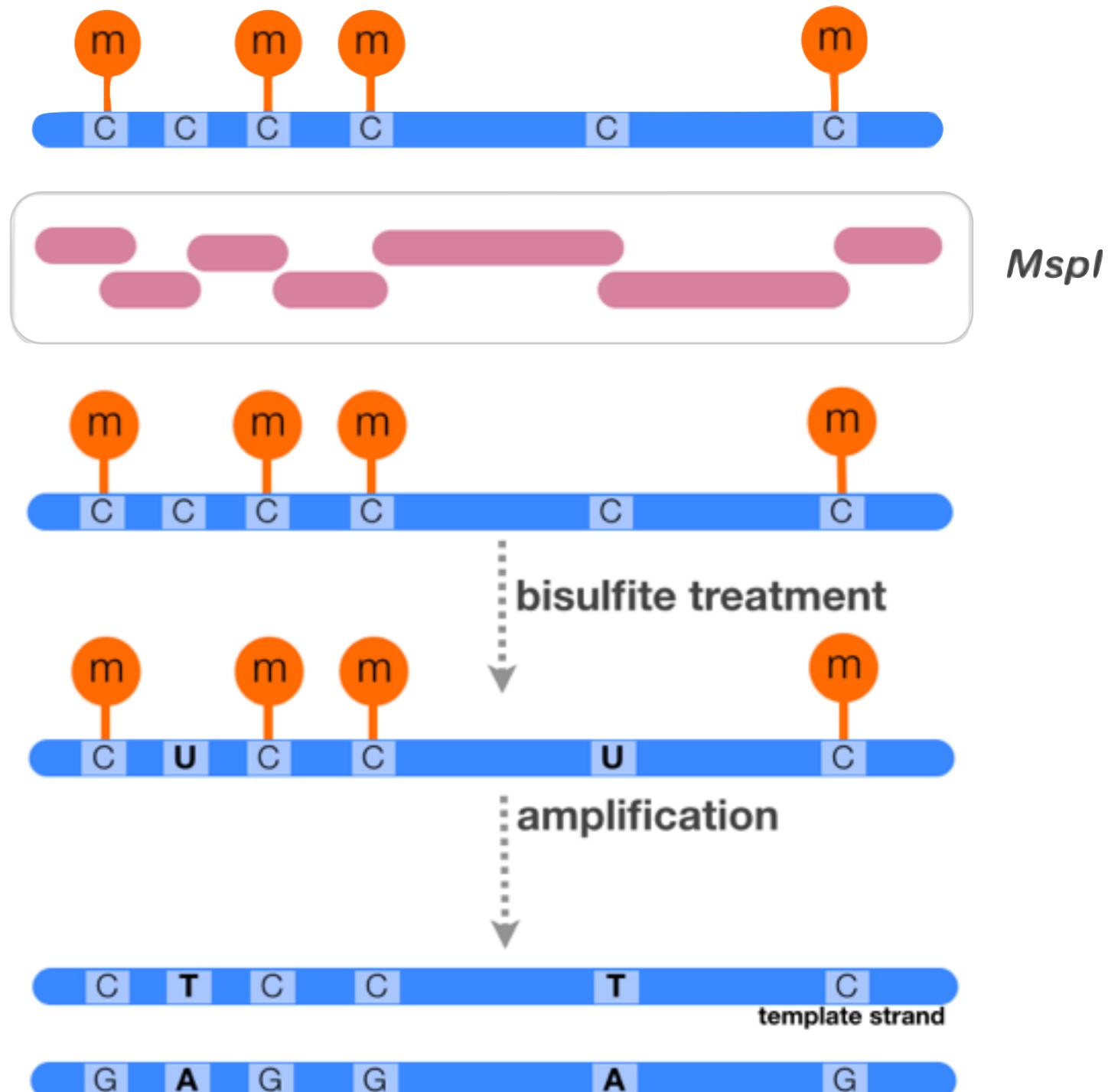
Detecting methylated DNA: how?

- **BS-seq:** Sequencing-based method after DNA conversion through bisulfite (BS) treatment
 - BS deaminates unmethylated cytosines
 - Sequence of converted DNA to infer methylation status of cytosines
 - Whole genome approach



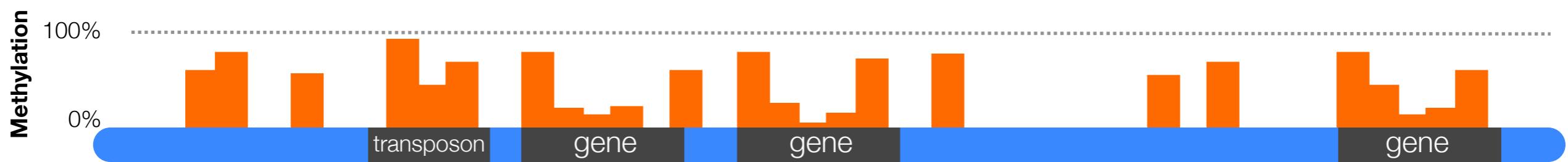
Detecting methylated DNA: how?

- **Reduced representation bisulfite sequencing (RRBS)**
targeted enrichment of CpG islands followed by conversion and high throughput sequencing
- Targets CpG islands by *MspI*
- <5% of genome
- Putatively functionally-relevant loci
- Pooling available to maximize economics of sequencing

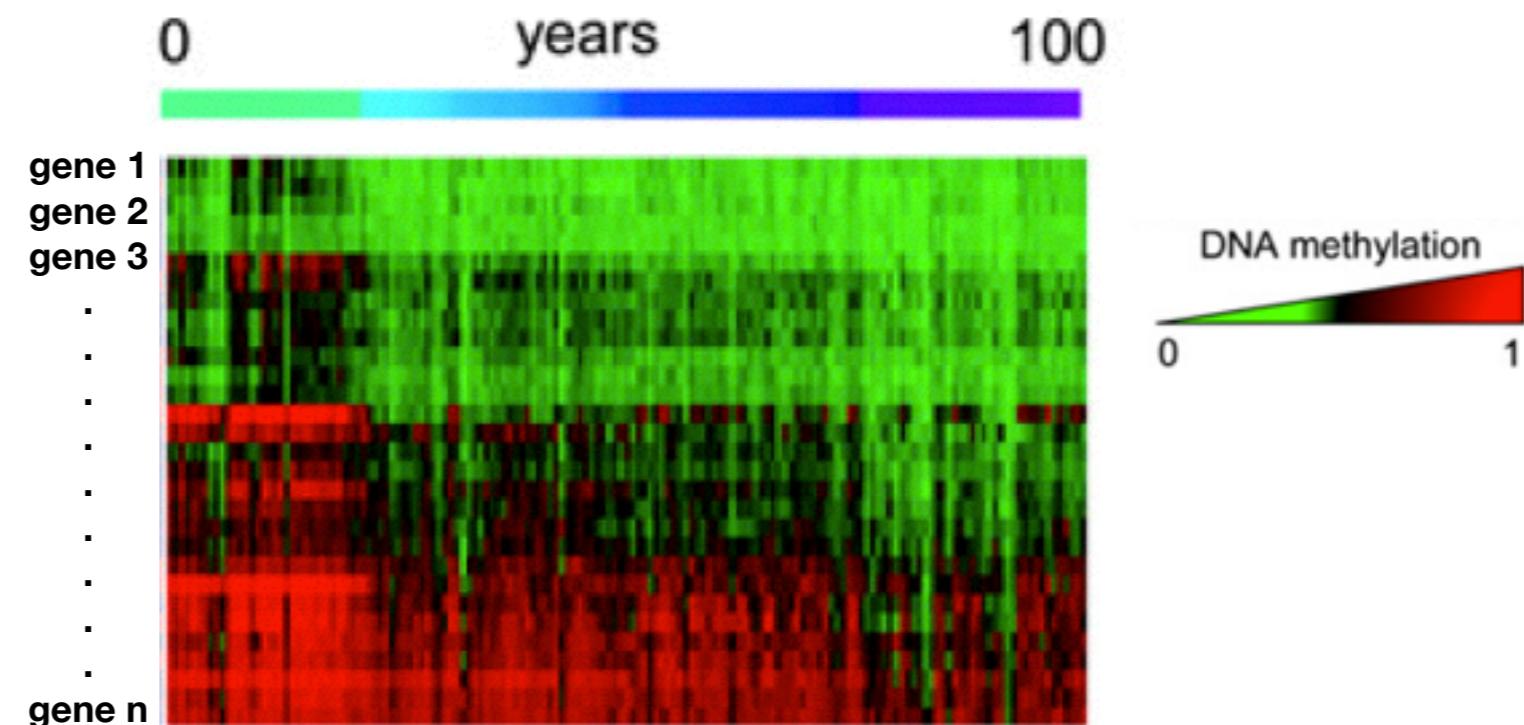


Detecting methylated DNA

- Bisulfite treated DNA followed by sequencing



- Assessing methylation of many genes from the same individual as they aged

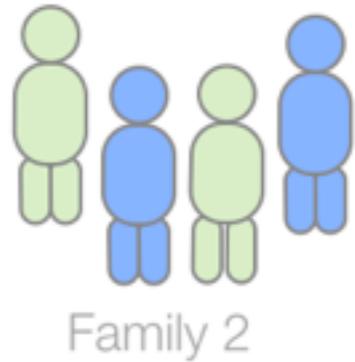
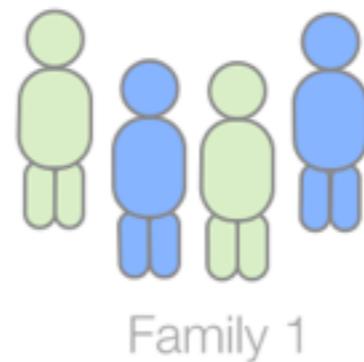


Ecological epigenetics

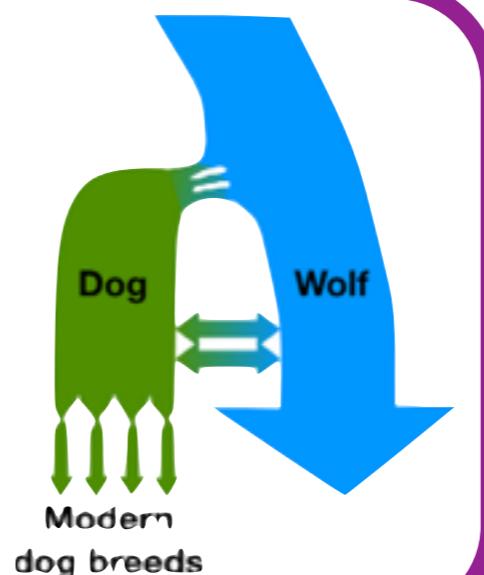
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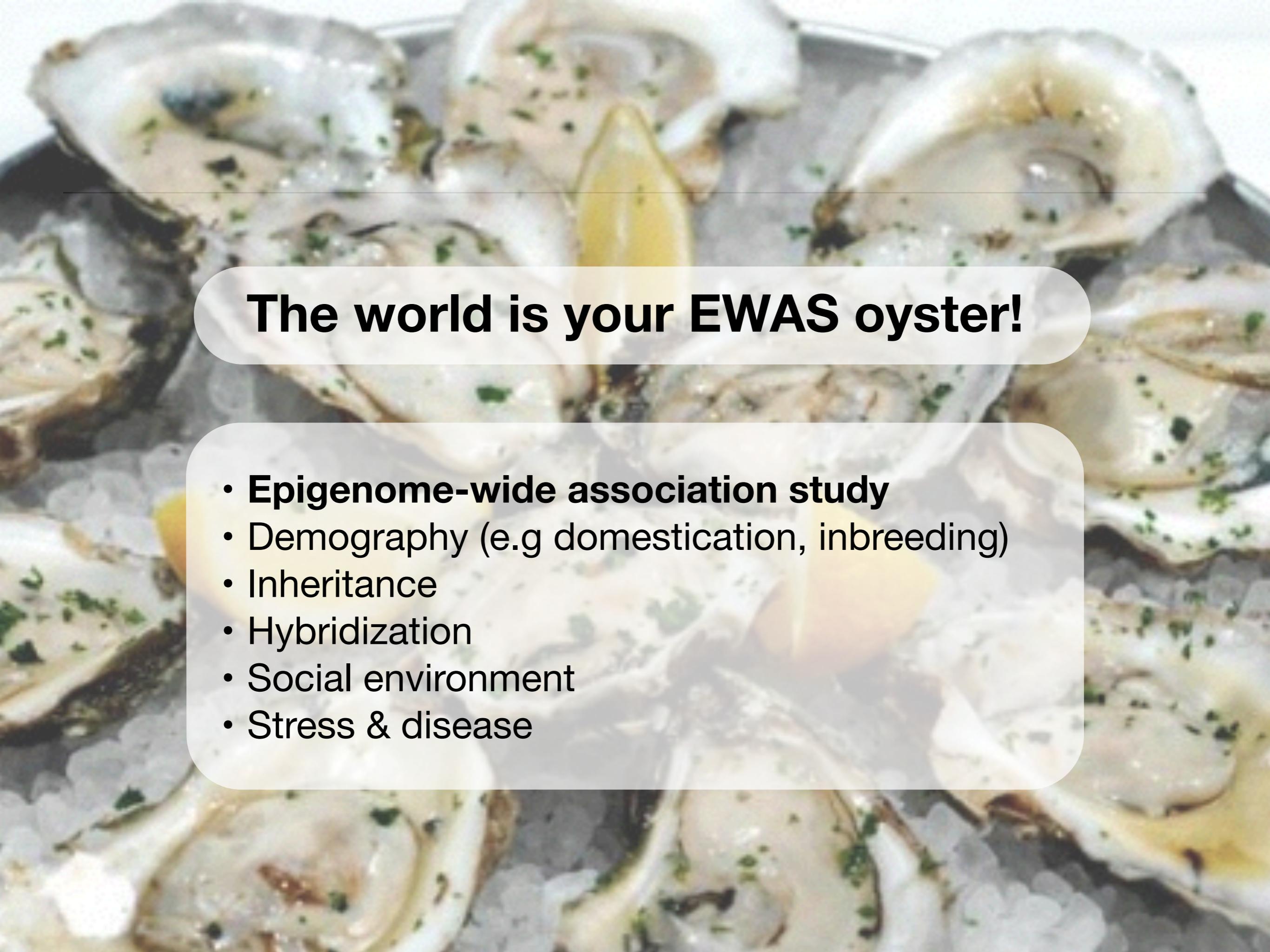


2. Epigenetic methods



3. Comparative methylation of dogs and wolves





The world is your EWAS oyster!

- **Epigenome-wide association study**
- Demography (e.g domestication, inbreeding)
- Inheritance
- Hybridization
- Social environment
- Stress & disease

Analytical pipeline

- 1x101nt HiSeq sequence data
- Demultiplexing (or deplexing) pooled indexed reads (custom perl scripts)
- Trimming low-quality nucleotides and clip adapters specific to RRBS using
cut adapt
(<https://cutadapt.readthedocs.org/en/stable/>)

Bisulfite sequencing (RRBS)

When trimming reads that come from a library prepared with the RRBS (reduced representation bisulfite sequencing) protocol, the last two 3' bases must be removed in addition to the adapter itself. This can be achieved by using not the adapter sequence itself, but by adding two wildcard characters to its beginning. If the adapter sequence is **ADAPTER**, the command for trimming should be:

```
cutadapt -a NNADAPTER -o output.fastq input.fastq
```

Details can be found in [Babraham bioinformatics' "Brief guide to RRBS"](#). A summary follows.

Analytical pipeline

- 1x101nt HiSeq sequence data
- Demultiplexing (of deplexing) pooled indexed reads (custom perl scripts)
- Trimming low-quality nucleotides and clip adapters specific to RRBS using
cut adapt
(<https://cutadapt.readthedocs.org/en/stable/>)

You have
access to
data from 4
wolves

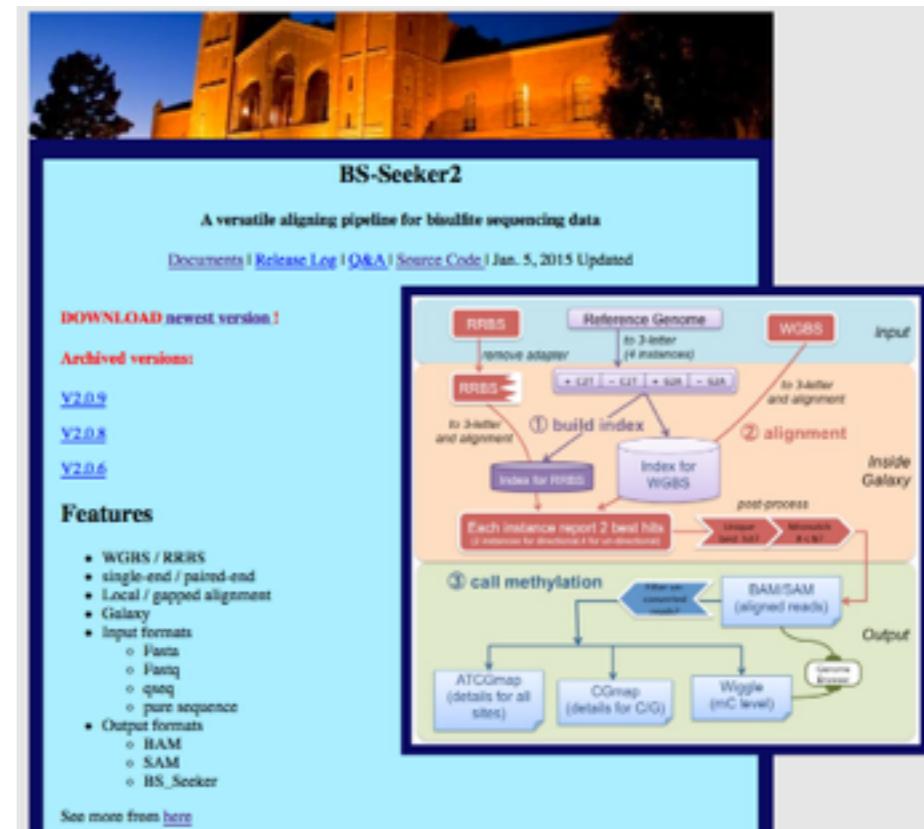
```
/path/to/cutadapt-1.8.1/bin/cutadapt
-a NNAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC
--minimum-length 20
-q 20
-o 005F_trimmed.fastq
005F.fastq
```

Analytical pipeline

- Map/align and call methylation with BS-seeker2 (http://pellegrini.mcdb.ucla.edu/BS_Seeker2/)
 - Single or paired end data
 - Whole genome BS or RRBS
 - Uses Bowtie2 <---- <http://bowtie-bio.sourceforge.net/index.shtml>
 - Imports qseq/FASTQ/FASTA formats
 - Outputs BAM/SAM, **CGmap**, ATCGmap
 - Python command line

• Input files

- Genome reference file formatted as FASTA
- Executables (BS-Seeker2 and bowtie2)
- Your data formatted as FASTQ



Analytical pipeline: BS-Seeker2

1. Pre-process (build) the indexed reference genome

- For RRBS, you need to specify "-r" in the parameters
- Specify LOW_BOUND and UP_BOUND for the range of fragment lengths according your protocol (e.g. 280bp to 400bp)

```
python bs_seeker2-build.py  
-f /work/reference/canfam31/canfam31.fa      # path to ref genome  
--aligner=bowtie2  
-p /Applications/bowtie2-2.0.2                # path to bowtie2  
-r  
-l 50  
-u 300
```

- This builds a new reference genome in **~/BS-Seeker/reference_genome**

Analytical pipeline: BS-Seeker2

2. Align data using module to call bowtie2

```
python bs_seeker2-align.py
-i 002M.fastq
-r                                         # for RRBS
--low=50
--up=300
-g canfam31.fa                            # specify ref genome
--aligner=bowtie2
-p /Applications/bowtie2-2.0.2
-o 002M_aligned.bam                         # name outfile
```

Analytical pipeline: BS-Seeker2

2. Align data using module to call bowtie2

```
-0.12.7/bowtie -e 200 --quiet --nrc --sam-nohead -k 2 --best -p 2 --nomaqrou  
nd --sam /u/home/mcdb/ldorozco/BS-Seeker/reference_genome/chr1.fa_rrbs_40_25  
0_bowtie/C_C2T -f /tmp/bs_seeker2_file1.bam_-bowtie-TMP-5mXR1e/Trimed_C2T.fa  
.tmp-2321955 /tmp/bs_seeker2_file1.bam_-bowtie-TMP-5mXR1e/C_C2T_m5.mapping.tm  
p-2321955  
[2013-01-30 14:36:08] Aligning reads is done  
[2013-01-30 14:36:08] Extracting alignments is done  
[2013-01-30 14:36:08] Done: /tmp/bs_seeker2_file1.bam_-bowtie-TMP-5mXR1e/file  
1-s-1 (1)  
  
[2013-01-30 14:36:08] 0 Number of raw reads: 10000  
[2013-01-30 14:36:08] 0 Number of CGG/TGG tagged reads: 9946 (0.995)  
[2013-01-30 14:36:08] 0 Number of raw reads with CGG tag: 5965 (0.597)  
[2013-01-30 14:36:08] 0 Number of raw reads with TGG tag: 3981 (0.398)  
[2013-01-30 14:36:08] 0 Number of CGG/TGG reads having adapter removed: 0  
[2013-01-30 14:36:08] 0 Number of unique-hits reads for post-filtering: 201  
[2013-01-30 14:36:08] 0 ----- 182 uniquely aligned reads, passed fragment ch  
eck, with mismatches <= 5  
[2013-01-30 14:36:08] 0 Mapability= 1.8200%  
[2013-01-30 14:36:08] -----  
[2013-01-30 14:36:08] M Methylated C in mapped reads  
[2013-01-30 14:36:08] M mCG 61.466%  
[2013-01-30 14:36:08] M mCHG 0.965%  
[2013-01-30 14:36:08] M mCHH 1.154%  
[2013-01-30 14:36:08] -----  
[2013-01-30 14:36:08] ----- END -----  
(END) □
```

Analytical pipeline: BS-Seeker2

3. Call methylation per site from aligned reads

```
python bs_seeker2-call_methylation.py  
-i 002M_aligned.bam  
-o YNP_002M          # prefix for multiple outfiles that will be produced  
--db /path/to/BSseeker2/bs_utils/reference_genomes/  
canfam31.fa_rrbs_50_300_bowtie2/      # path to build ref
```

Analytical pipeline: BS-Seeker2

3. Call methylation per site from aligned reads

```
python bs_seeker2-call_methylation.py  
-i 002M_aligned.bam  
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--db /path/to/BSseeker2/bs_utils/reference_genomes/  
canfam31.fa_rrbs_50_300_bowtie2/      # path to build ref
```

- Outfiles produced

```
zcat YNP_002M.ATCGmap.gz | head -10  
zcat YNP_002M.CGmap.gz | head -10
```

Analytical pipeline: BS-Seeker2

CGmap file: Methylation calls

chr01	C	4438	CHG	CC	0.25	1	4
chr01	C	4439	CG	CG	0.888361045131	7106	7999
chr01	C	4442	CHH	CA	0.0138779785284	106	7638
chr01	C	4444	CHH	CA	0.00390084308544	31	7947
chr01	C	4446	CHH	CA	0.00330999363463	26	7855
chr01	G	4449	CHH	CT	0.0	0	1
chr01	C	4451	CG	CG	0.82903951976	6629	7996
chr01	G	4452	CG	CG	1.0	1	1
chr01	G	4454	CHH	CT	0.0	0	2
chr01	G	4456	CHH	CA	0.0	0	2

- (1) chromosome
- (2) nucleotide on Watson (+) strand
- (3) position
- (4) context (CG/CHG/CHH)
- (5) dinucleotide-context (CA/CC/CG/CT)
- (6) methylation-level = #_of_C / (#_of_C + #_of_T).
- (7) #_of_C (methylated C, the count of reads showing C here)
- (8) = #_of_C + #_of_T (all Cytosines, the count of reads showing C or T here)

Analytical pipeline: BS-Seeker2

ATCGmap file:

chr01	C	4438	CHG	CC	0	3	1	0	0	0	0	0	0	0	0.25
chr01	C	4439	CG	CG	0	893	7106	0	0	0	0	0	0	0	0.888361045131
chr01	G	4440	CG	CG	0	0	0	8000	0	0	0	0	0	0	na
chr01	G	4441	CHG	CC	0	0	0	7999	2	0	0	0	0	0	na
chr01	C	4442	CHH	CA	0	7532	106	0	364	0	0	0	0	0	0.0138779785284
chr01	A	4443	--	--	7970	1	0	31	1	0	0	0	0	0	na
chr01	C	4444	CHH	CA	48	7916	31	9	0	0	0	0	0	0	0.00390084308544
chr01	A	4445	--	--	7938	27	1	38	0	0	0	0	0	0	na
chr01	C	4446	CHH	CA	32	7829	26	118	0	0	0	0	0	0	0.00330999363463
chr01	A	4447	--	--	7974	10	1	22	0	0	0	0	0	0	na

- (1) chromosome
- (2) nucleotide on Watson (+) strand
- (3) position
- (4) context (CG/CHG/CHH)
- (5) dinucleotide-context (CA/CC/CG/CT)

- (6) - (10) plus strand
- (6) # of reads from Watson strand mapped here, support A on Watson strand
- (7) # of reads from Watson strand mapped here, support T on Watson strand
- (8) # of reads from Watson strand mapped here, support C on Watson strand
- (9) # of reads from Watson strand mapped here, support G on Watson strand
- (10) # of reads from Watson strand mapped here, support N

- (11) - (15) minus strand
- (11) # of reads from Crick strand mapped here, support A on Watson strand and T on Crick strand
- (12) # of reads from Crick strand mapped here, support T on Watson strand and A on Crick strand
- (13) # of reads from Crick strand mapped here, support C on Watson strand and G on Crick strand
- (14) # of reads from Crick strand mapped here, support G on Watson strand and C on Crick strand
- (15) # of reads from Crick strand mapped here, support N

- (16) methylation_level = #C/(#C+#T) = C8/(C7+C8) for Watson strand, =C14/(C11+C14) for Crick strand;
"nan" means none reads support C/T at this position.

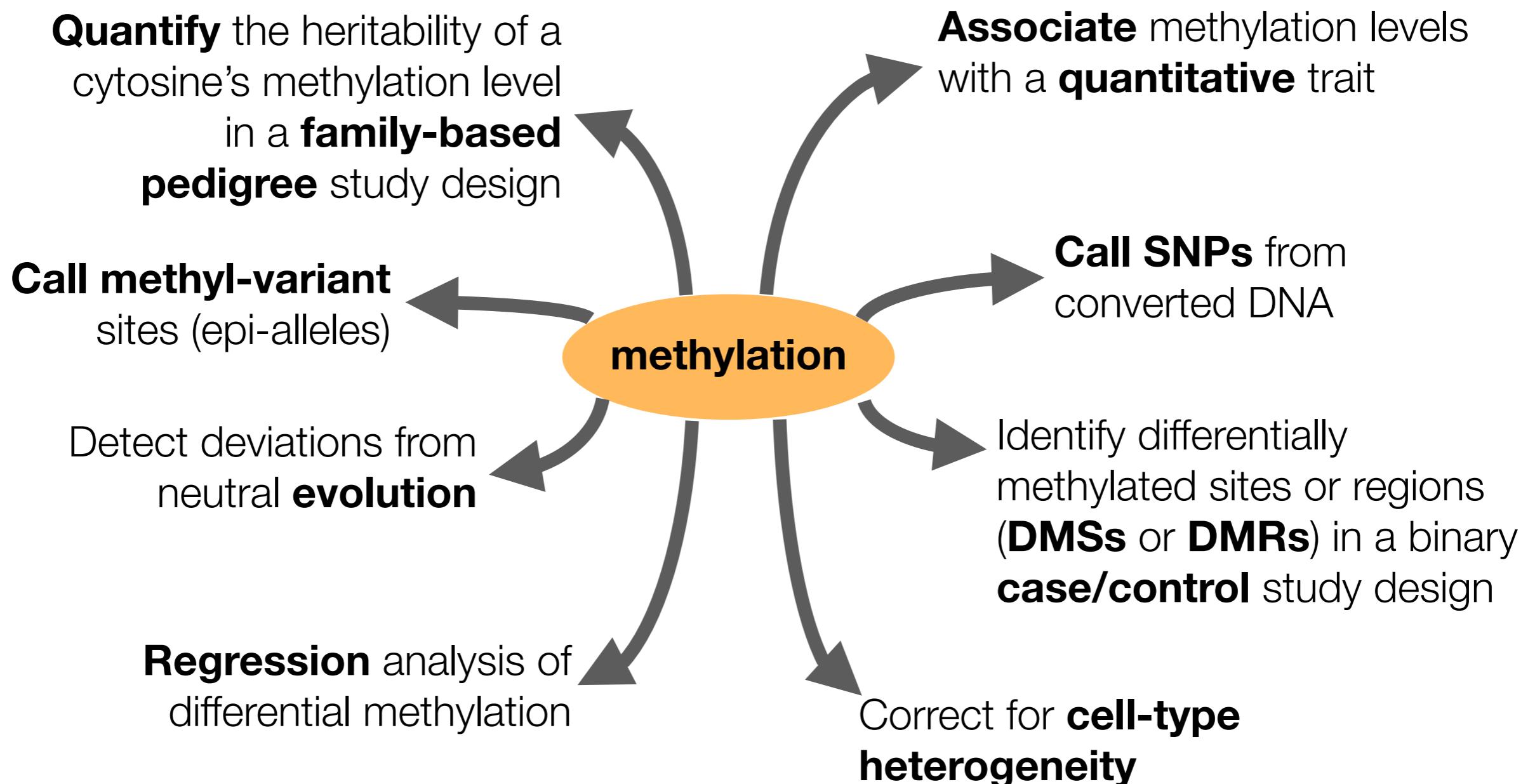


The world is your EWAS oyster!

- Different methylation sequence aligners
- Pedigree-based family/inheritance analysis
- Regression analysis and model selection
- Calling SNPs from methylation data
- More....!

and data type!

Software choice is dependent upon your question



Software (<http://omictools.com/differentially-methylated-regions-category>)

- BiSeq
- Bisulfighter
- bsseq
- City of Hope Cpg Island Analysis Pipeline
- CpG_MPs
- Dispersion Shrinkage for Sequencing data
- DMAP
- dmrFinder
- DSS-general
- DSS-single
- eDMR
- M3D
- MethPipe
- MethylKit
- methylMnM
- MethylPurify
- methylSig
- metilene
- QDMR
- Qvalue
- reFactor
- Regression Analysis of Differential Methylation
- RRBS-Analyser
- Streamlined Methylation Analysis Pipeline
- swDMR
- WBSA

Additional software

- Differential methylation analysis
 - **MACAU (Tung and Lea are here!)**
 - BiQ Analyzer (<http://biq-analyzer.bioinf.mpi-inf.mpg.de>)
 - MethGo (<https://methgo.readthedocs.org/en/latest/>)
 - SVA (batch effect) (<https://www.bioconductor.org/packages/release/bioc/html/sva.html>)
- Calling SNP genotypes from BS converted DNA
 - BS-SNPer (<https://github.com/hellbelly/BS-Snper>)
 - Bis-SNP (<http://people.csail.mit.edu/dnaase/bissnp2011/>)
- Neutrality testing
 - Wang & Fan (2015) A neutrality test for detecting selection on DNA methylation using single methylation polymorphism frequency spectrum. GBE 7(1),154

Example data for you!

- **Raw fastq** file for 1x101nt HiSeq
 - Yellowstone National park (YNP) wolf RRBS data
 - Pooled data and their barcodes
 - Index 22: CGTACG (wolf 005F)
 - Index 23: GACTGG (wolf 006M)
 - Index 25: ACTCAT (wolf 017F)
 - Index 27: ATTCCG (wolf 021M)
- **Sorted BAM** files
 - Deplexed with a maximum of 2 mismatches
 - Mapped to recent dog genome assembly CanFam3.1
- BS-Seeker2 outfiles
 - **CGmap** files
- **Shared sites** file for the 4 YNP wolves (filtered for 10x and low variation)
 - 1,379,751 cytosines across 38 autosomes + X chromosome



Evolutionary history of phenotypes

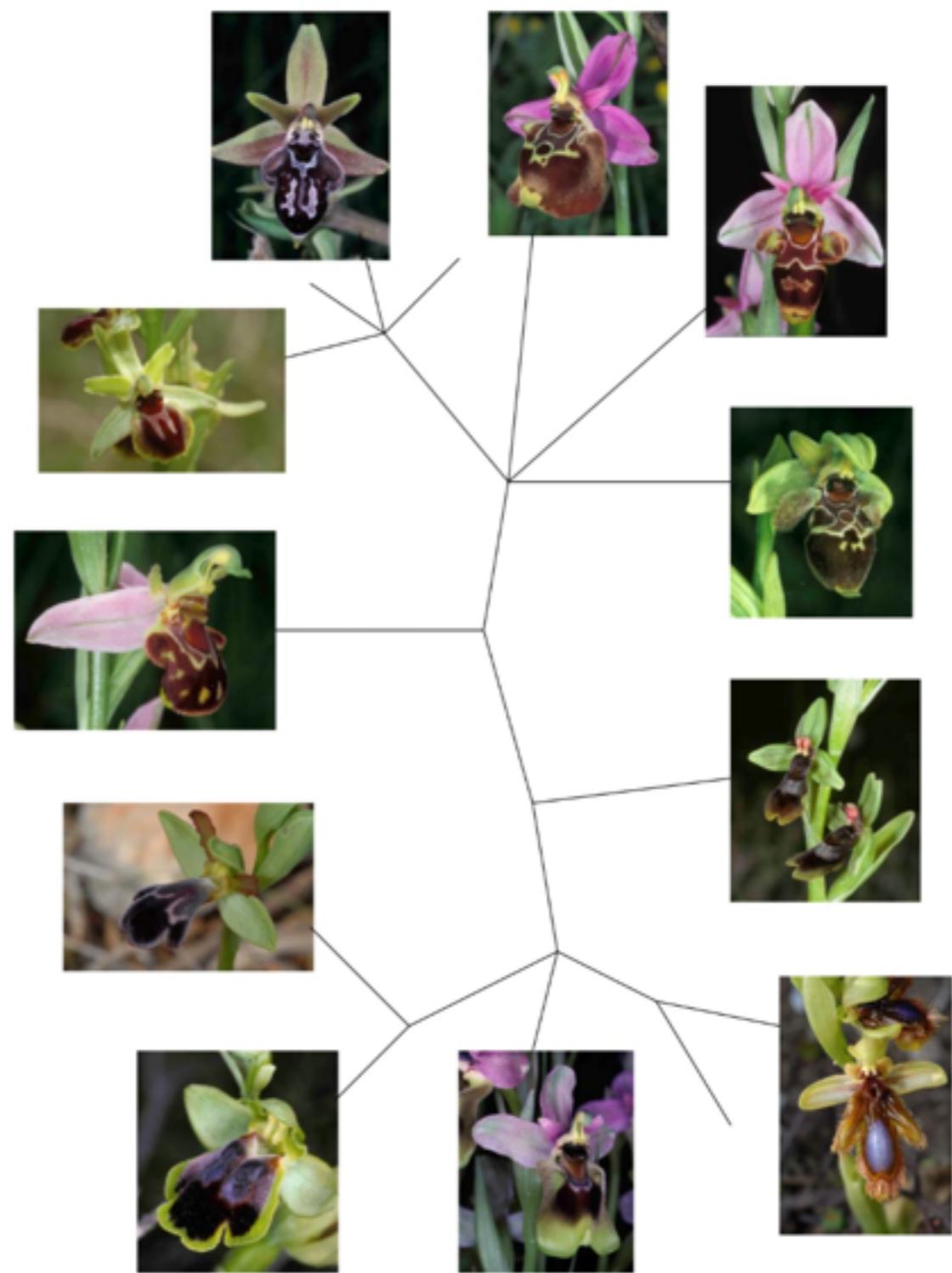
Darwin's finches



African cichlids



Bee orchids



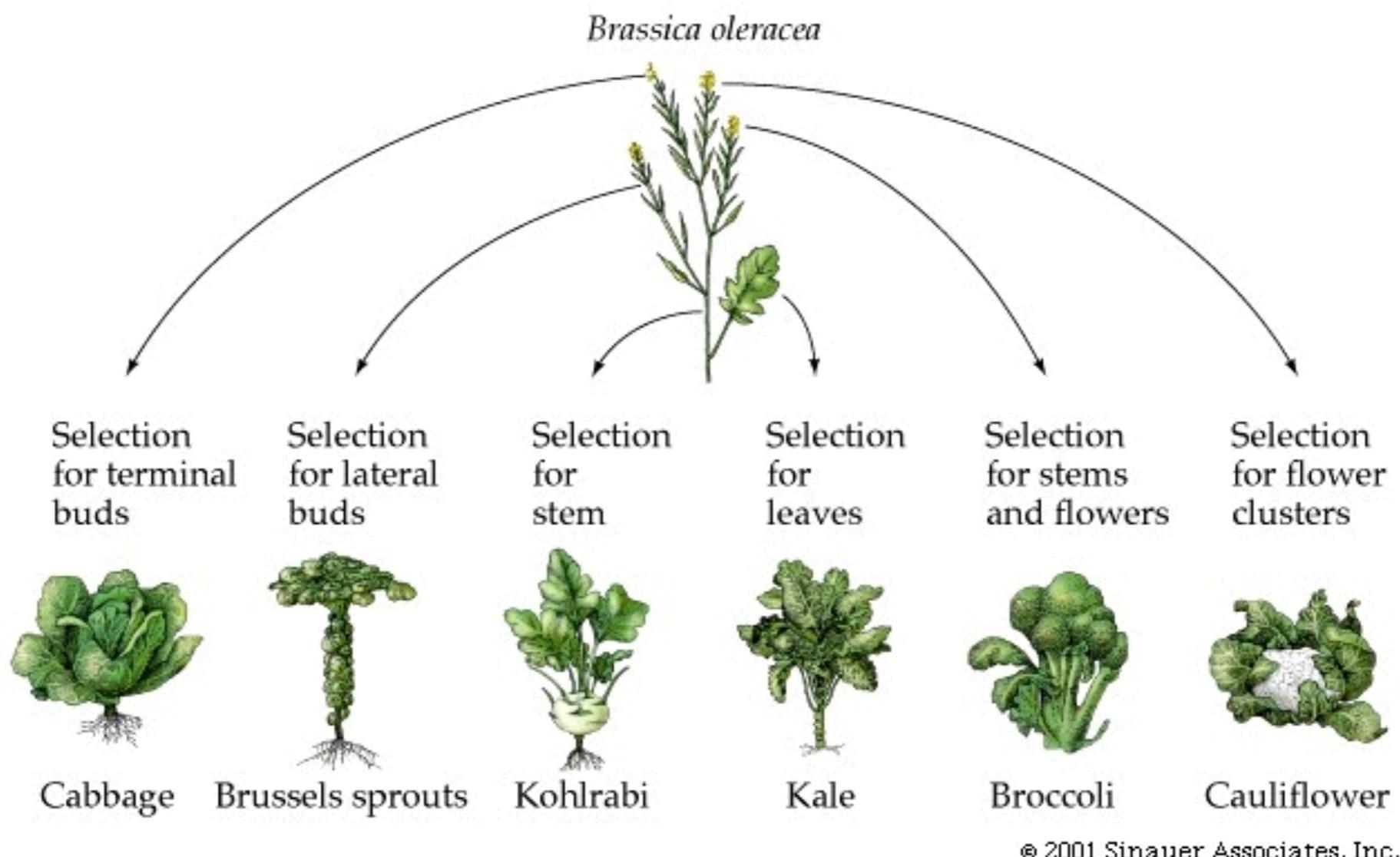
Evolutionary history of phenotypes



Canis familiaris



Evolutionary history of phenotypes



Darwin's Analogue for Natural Selection:
Artificial selection (selective breeding)

Is My Dog a Wolf?

- Geographic location and timing of dog domestication continues to be debated
- “Domestication genes” linked to morphology and behavior
- Relationship of dog breeds
- Trait and disease mapping in dogs (e.g. pigmentation, curly tail, skeletal variation, dwarfism, OCD, narcolepsy)
- But what about epigenetics?!

Comparative RRBS of wild and domestic canids



**Gray wolf
(n=35)**



**AKC dog
(n=49)**



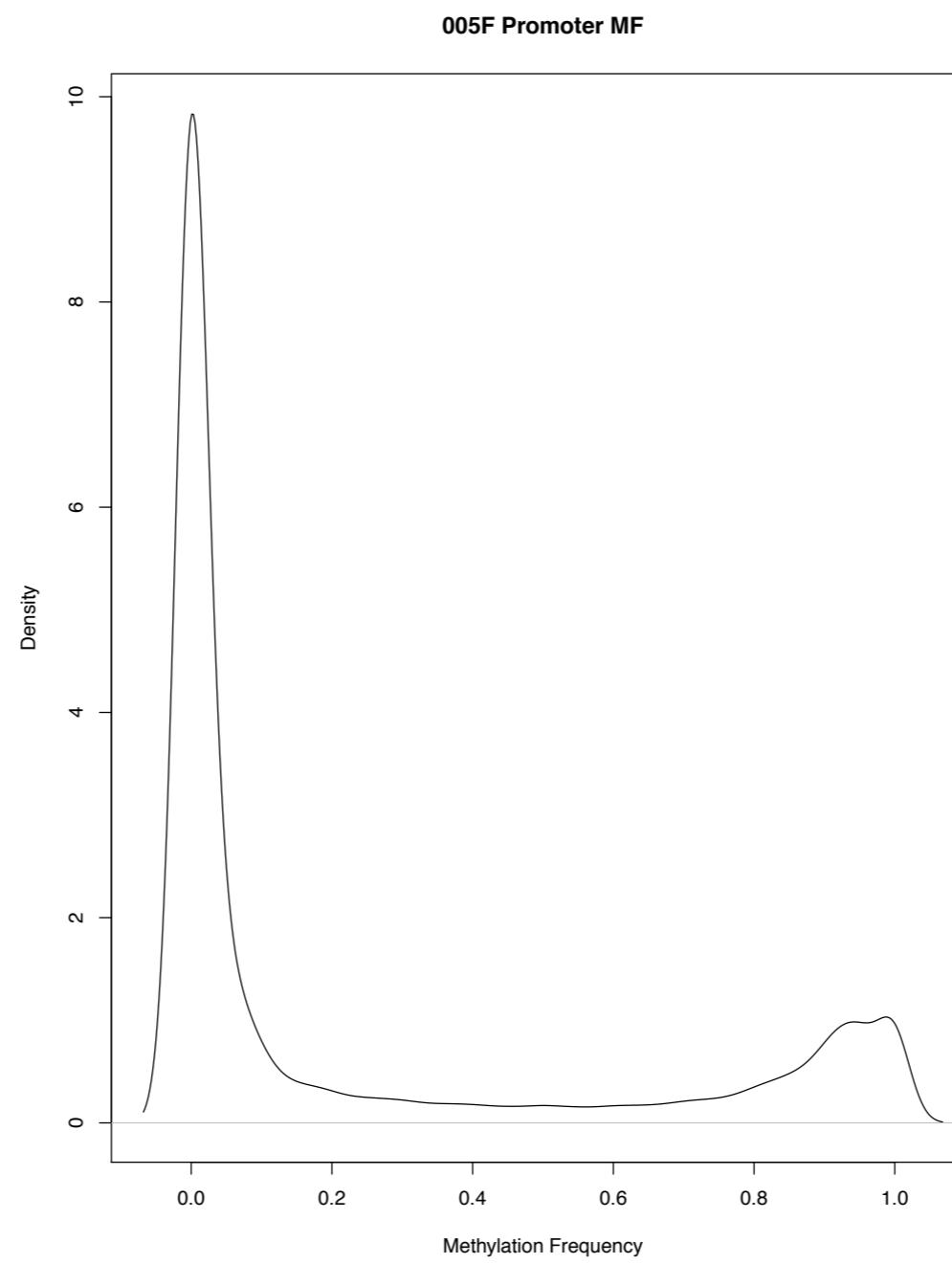
ngsd (n=4)

- Canine genome size 2.2Gb, 38 autosomes, 1 sex chromosome
- RRBS targets {CCGG} islands (<5% of genome)
- Pool 4 libraries/lane = 15-20x coverage

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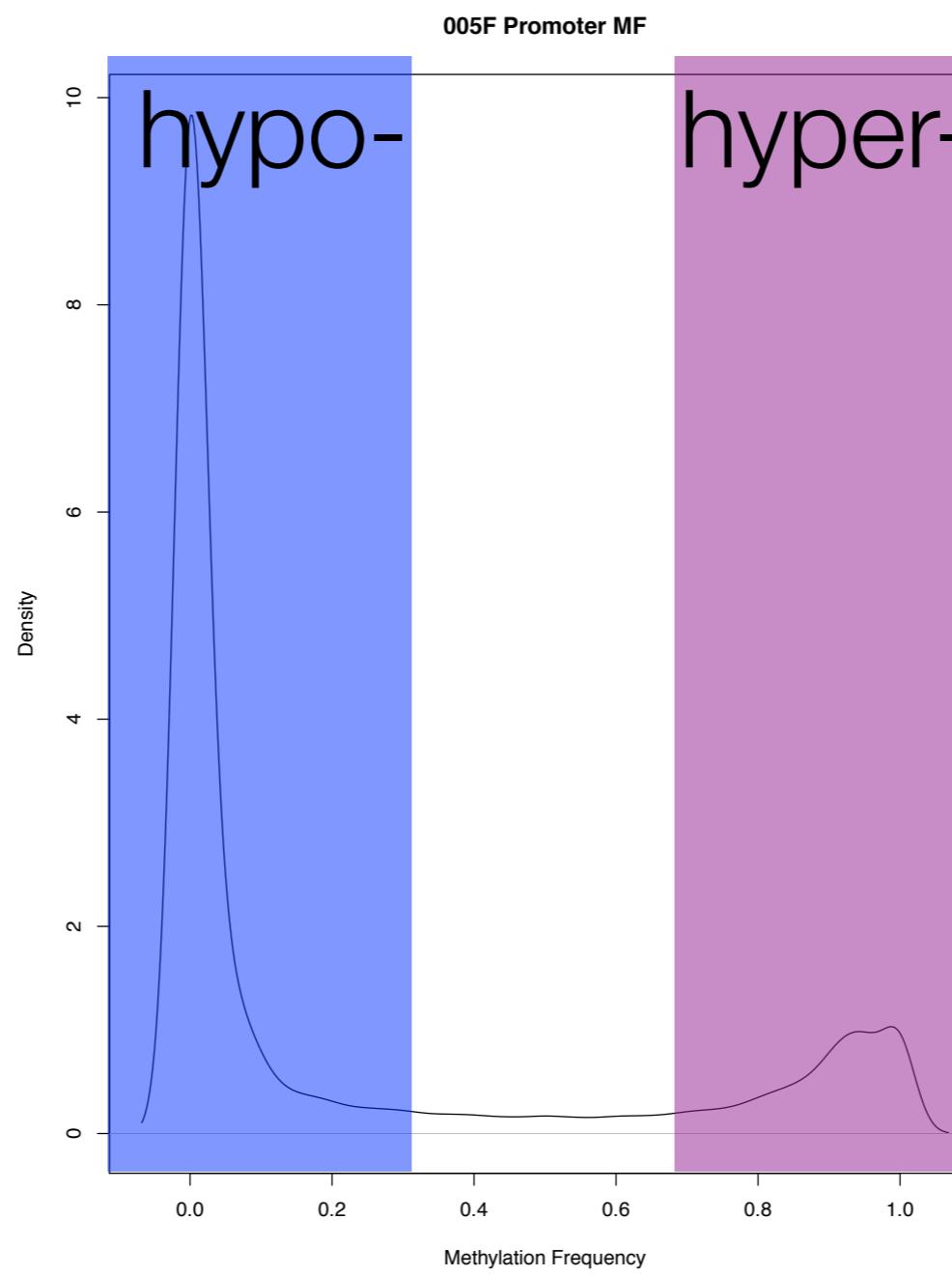
Classification of methylation marks

- Conversion of methylation frequency into “alleles” that retain some amount of methylation information



Classification of methylation marks

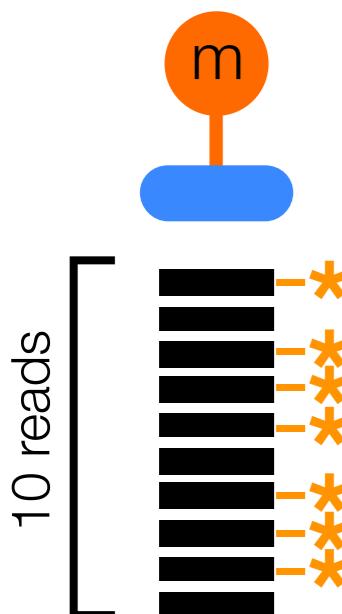
- Conversion of methylation frequency into “alleles” that retain some amount of methylation information



Classification of methylation marks

- Conversion of methylation frequency into “alleles” that retain some amount of methylation information

Ind1



Methylation frequency

$$MF = 7 / 10 = 0.7$$

Conversion thresholds:

$MF < 0.33$ = AA (hypomethylated)

$0.33 < MF < 0.66$ = AB

$MF > 0.66$ = BB (hypermethylated)

SMP genotype:
BB

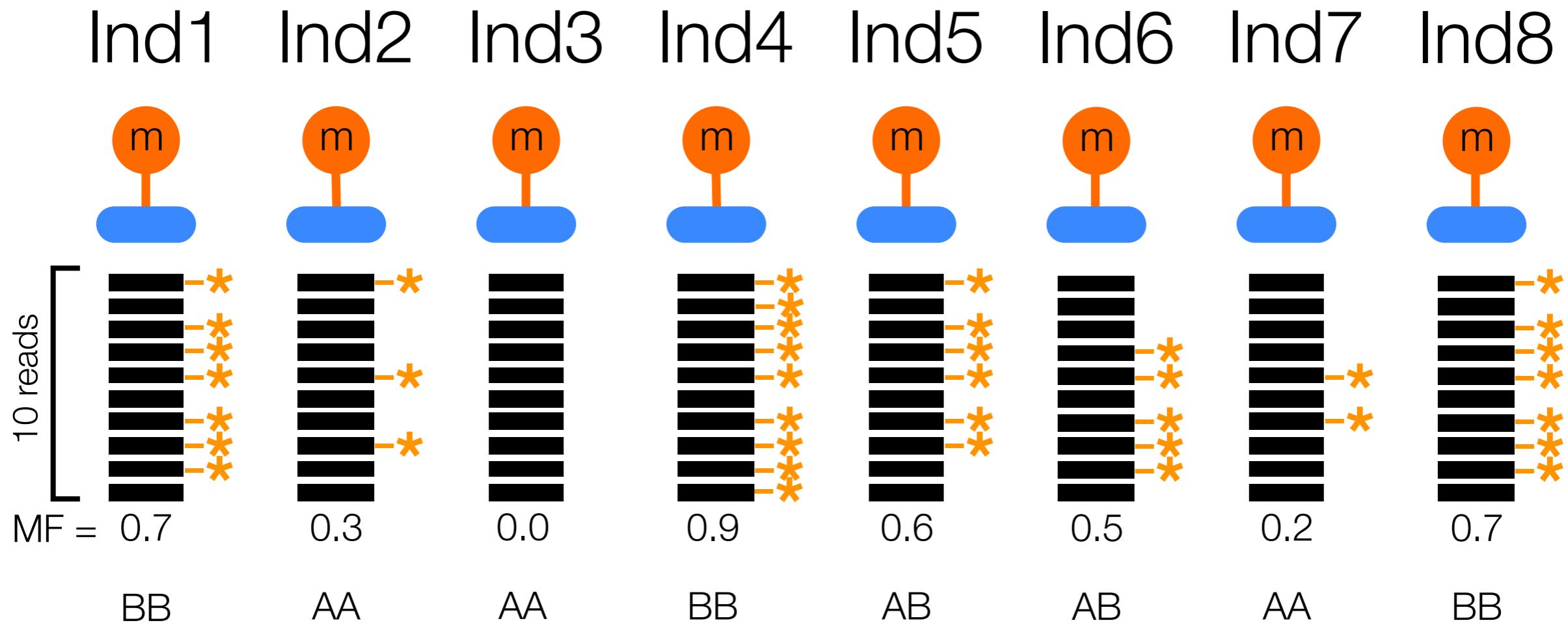
Key:

unmethylated

methylated

Classification of methylation marks

- Conversion of methylation frequency into “alleles” that retain some amount of methylation information



Key:

■ unmethylated

■ —★ methylated

Classification of methylation marks

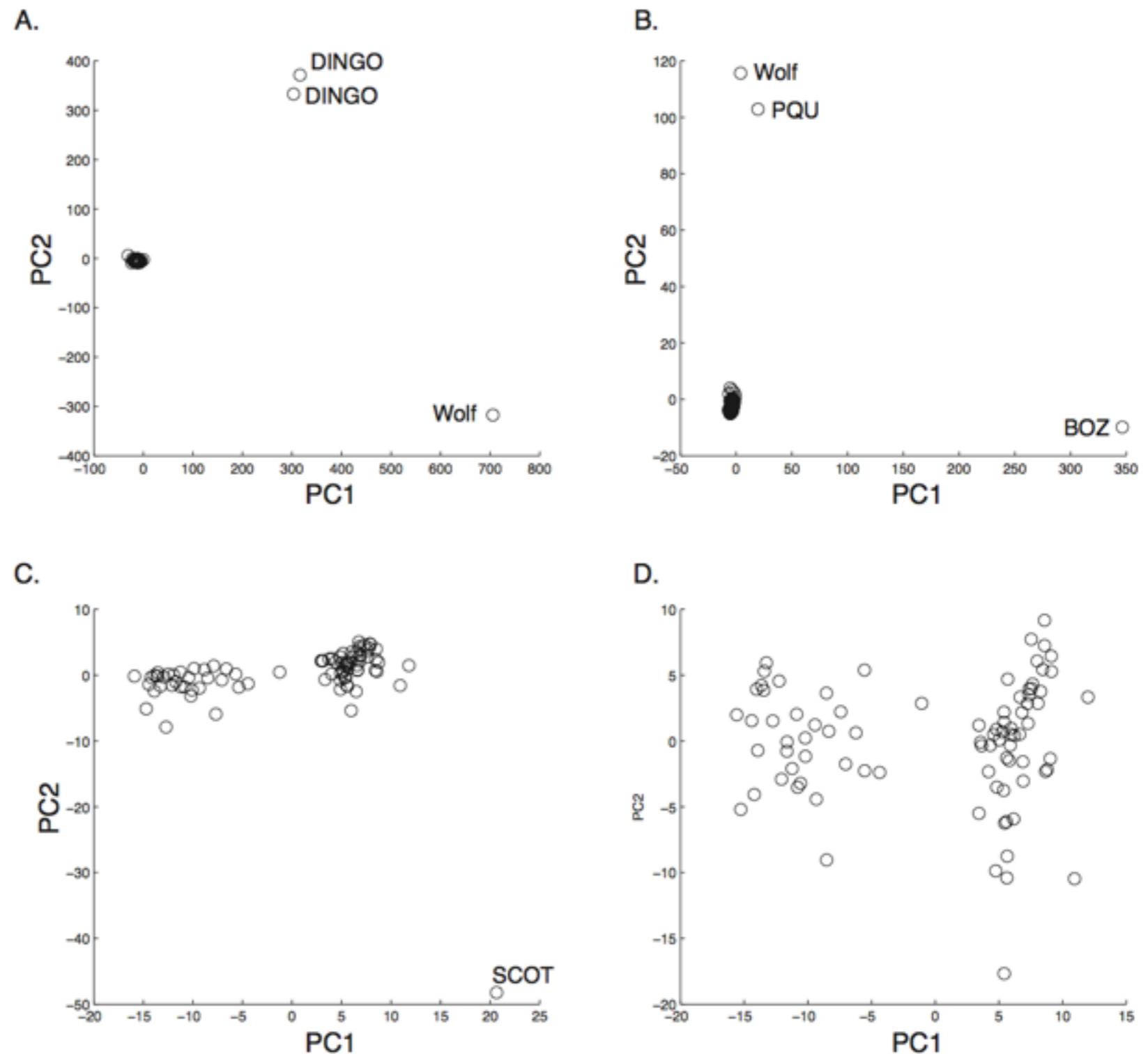
- Each individual has a diploid genotype that preserves some degree of methylation information
 - AA hypomethylation
 - BB hypermethylation (common in the mammalian genome)

Classification of methylation marks

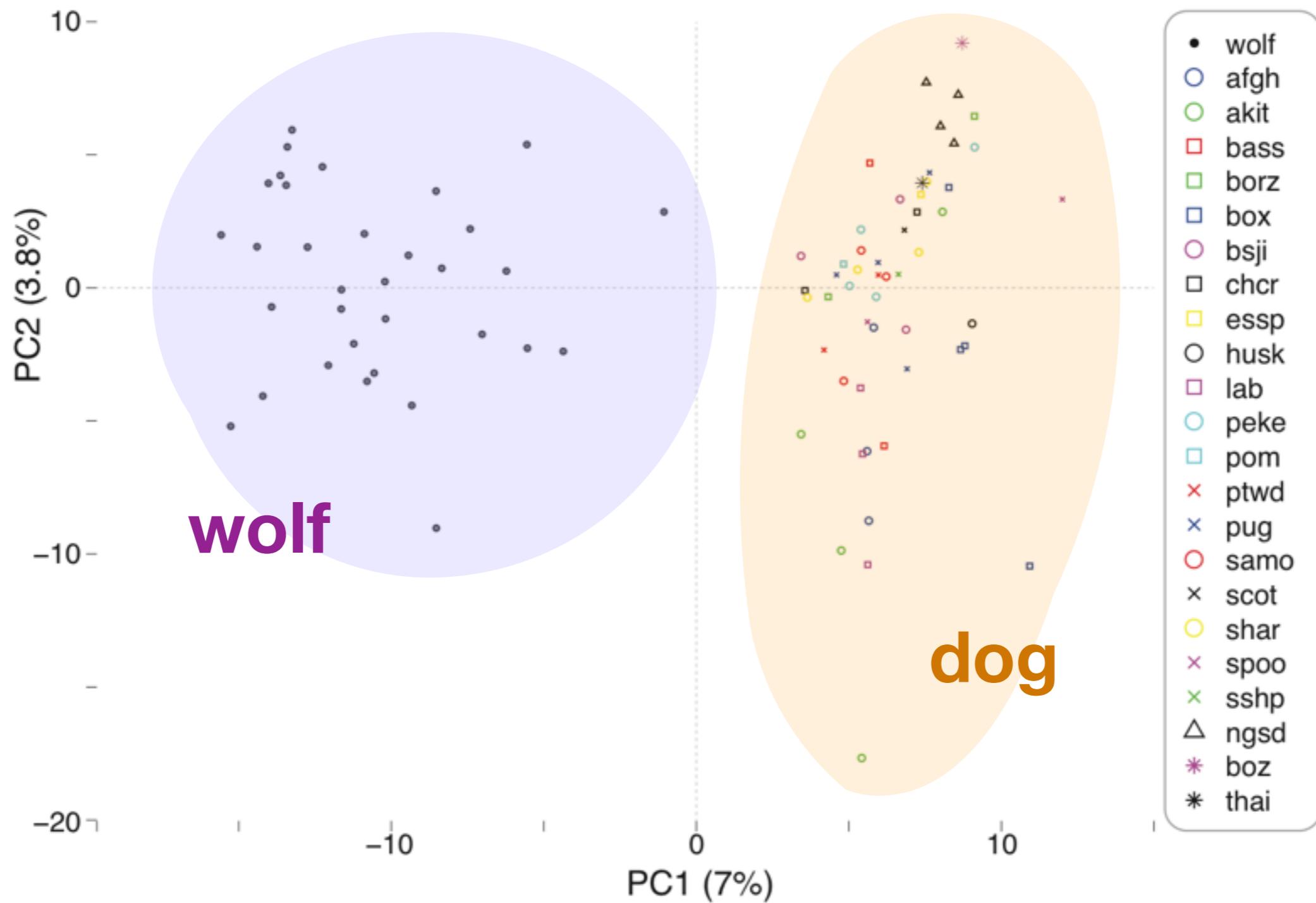
- Each individual has a diploid genotype that preserves some degree of methylation information
 - Analyze loci just like you would SNPs
 - PCA
 - Dendrogram
 - Epigenetic structure analysis
 - Case/control or quantitative association in PLINK
 - Tajima's Dm scan
 - Use PLINK to manage data in bed/map or tped/tfam formats

Classification of methylation marks

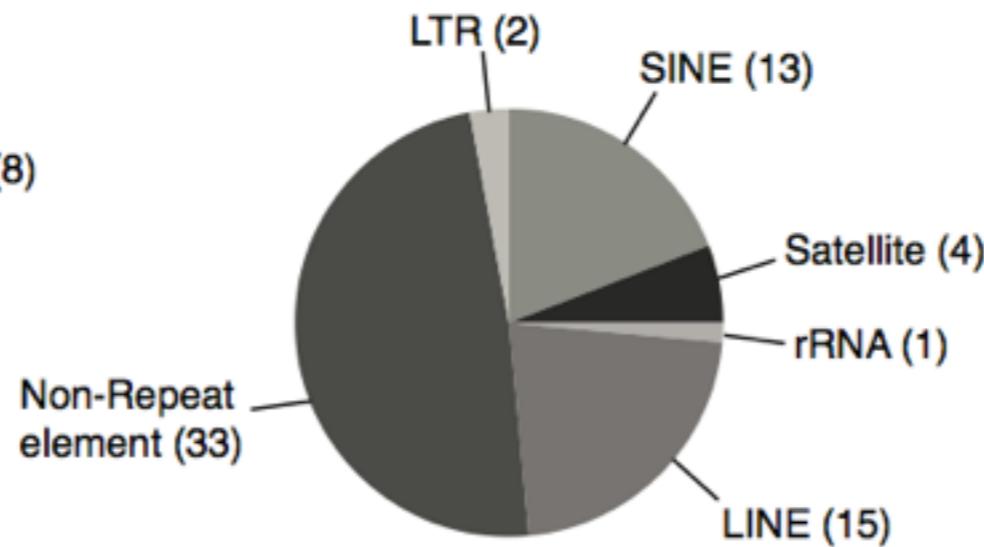
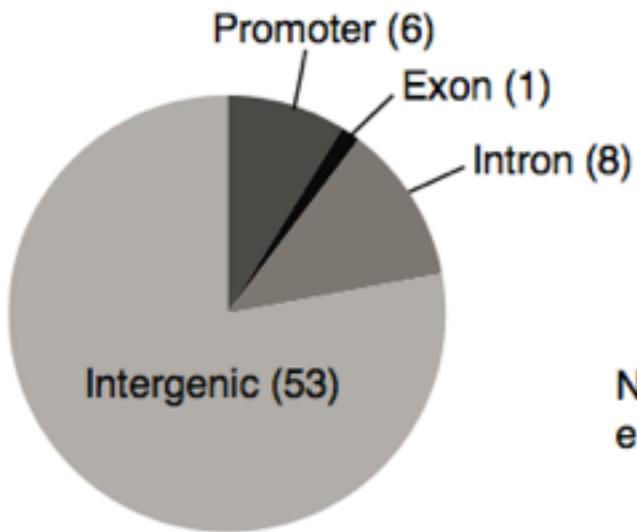
- PCA in *flashpca* to identify outliers for removal before analysis



Methyl clustering: Dogs and wolves

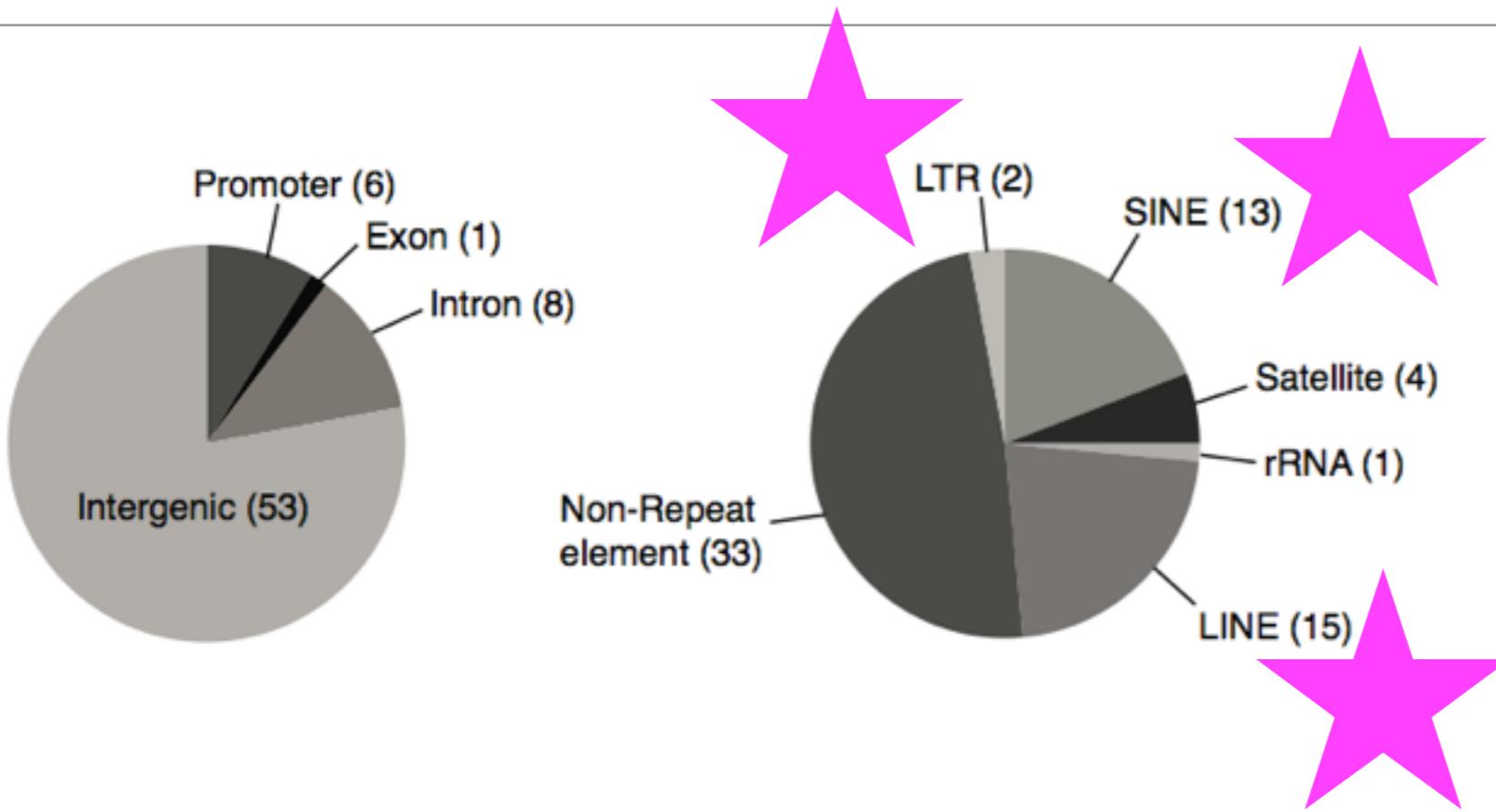


EWAS: Dogs and wolves



- Of 962 sites, 68 were differentially methylated ($p<0.05$)

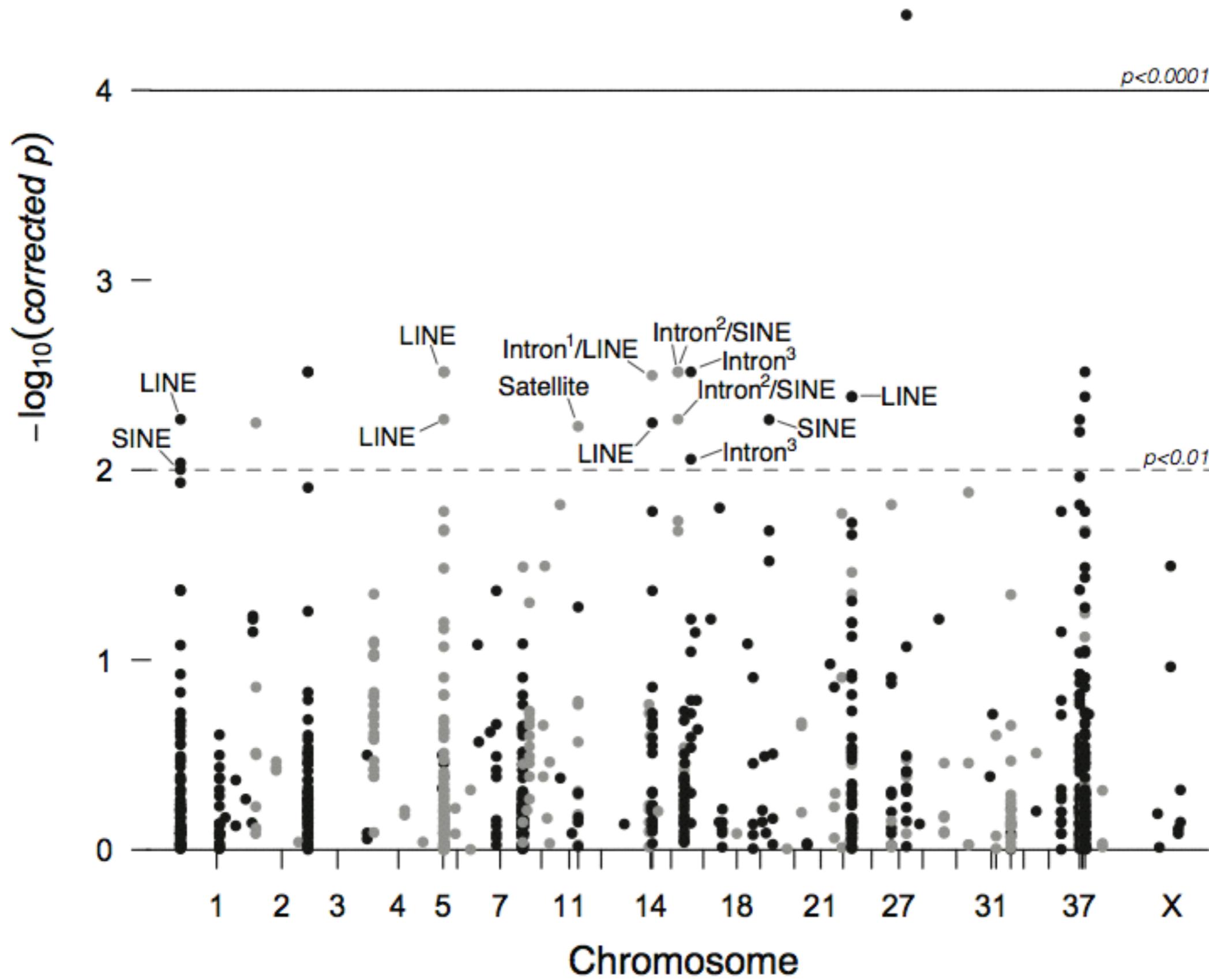
EWAS: Dogs and wolves



- Of 962 sites, 68 were differentially methylated ($p<0.05$)
- 30/68 LTR-RT

18 of 30 transposons: hyper-methylated in dogs

Of the 68 sites, 14 were outlier loci ($p < 0.005$; $-\log_{10} p > 2.3$)



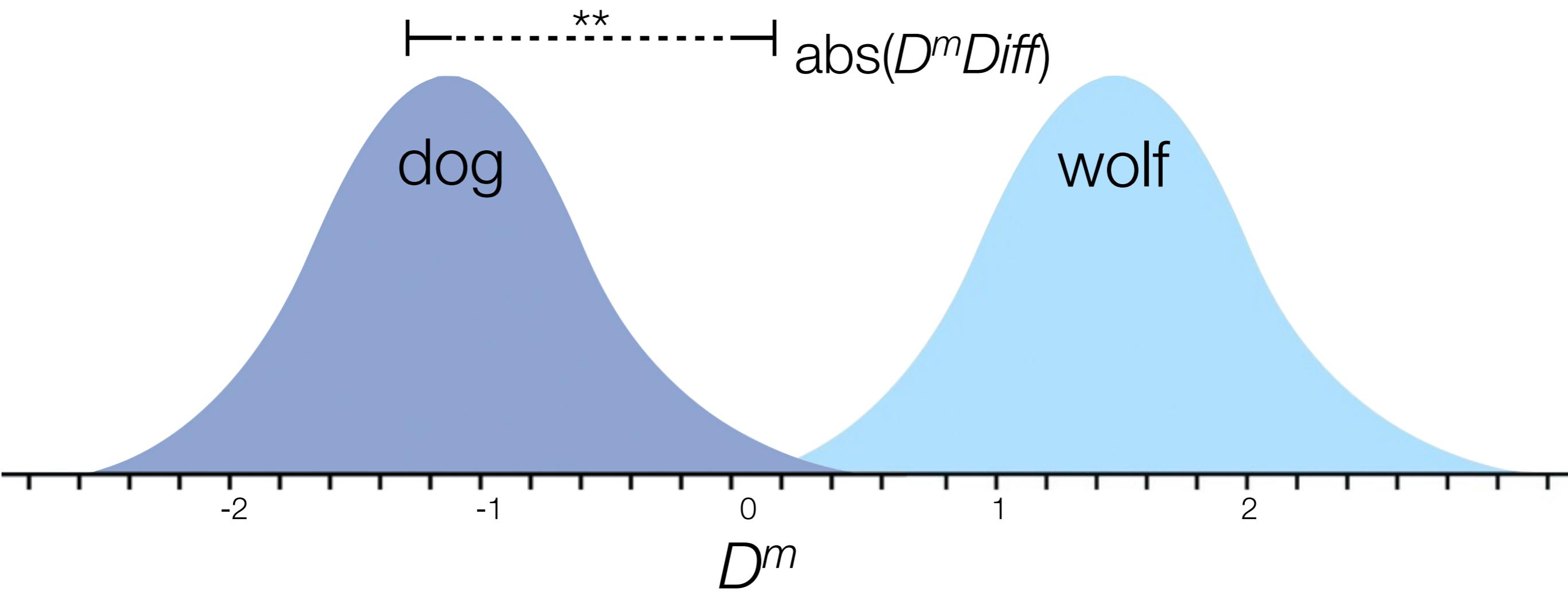
EWAS: Dogs and wolves

CFA	Position	Corrected <i>p</i> -value	Rank	MF _{wolf} , MF _{purebred}	MF _{wolf} – MF _{purebred}	In repeat element?	Annotation	Gene name
3	69553	0.003	5	0.74, 0.78	0.04		Intergenic	
3	69592	0.003	4	0.74, 0.78	0.04		Intergenic	
6	168151	0.003	3	0.33, 0.28	0.05		Intergenic	
6	172430	0.003	2	0.73, 0.58	0.15	LINE	Intergenic	
6	178713	0.003	8	0.33, 0.27	0.06	LINE	Intergenic	
13	43138016	0.003	11	0.61, 0.53	0.08	LINE	Intron	GABRB1
15	39451104	0.003	6	0.48, 0.66	0.18	SINE	Intron	SLC17A8
15	39451120	0.003	7	0.52, 0.70	0.18	SINE	Intron	SLC17A8
16	10475592	0.003	9	0.54, 0.19	0.35		Intron	Novel transcript
22	7972	0.004	13	0.76, 0.79	0.03	LINE	Intergenic	
22	44061	0.004	14	0.50, 0.61	0.11		Intergenic	
25	72494	0.00004	1	0.51, 0.59	0.08		Intergenic	
37	105637	0.003	10	0.78, 0.80	0.02		Intergenic	
37	105647	0.004	12	0.59, 0.63	0.04		Intergenic	

Average of 10% methylation difference between dogs and wolves (*p*<0.01)

Neutrality estimates

- Adaptation of Tajima's D for epi-allele mutations (D^m) for 121 gene bodies
- Estimated mutation rate 10^{-4}



9 genes deviated from $D^m \sim 0$ and were divergent between species ($p < 0.05$)

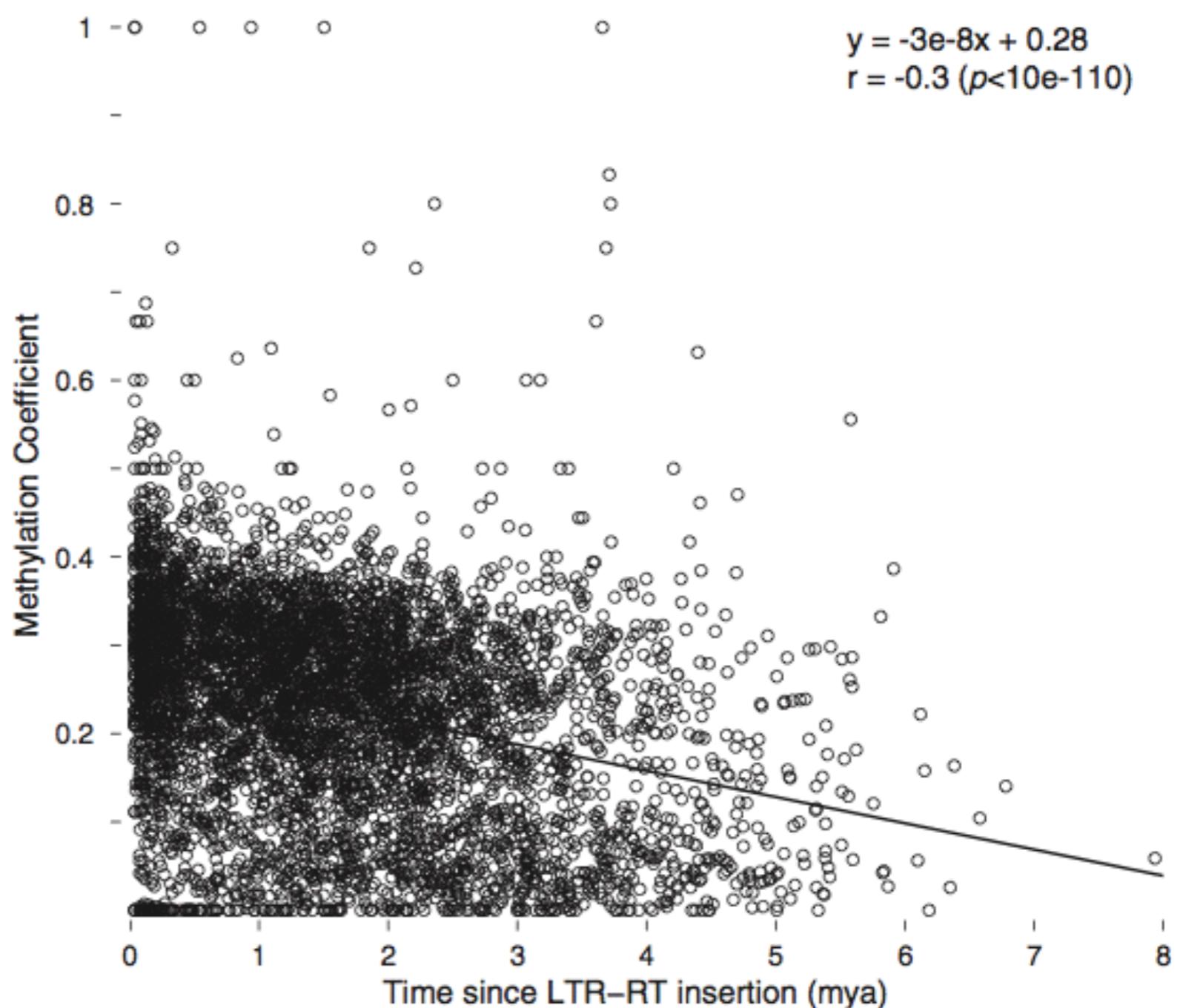
Outlier loci between dogs and wolves

- Non-neutral, differentially methylated sites found in functionally enriched regions
 - GRBRB1: GABA-A receptor (excitatory neurotransmitter)
 - SLC17A8: glutamate transport gene with a role in cognition (inhibitory neurotransmitter)
 - ADCY1: role in memory acquisition and learning
- **TRANSPOSONS!**



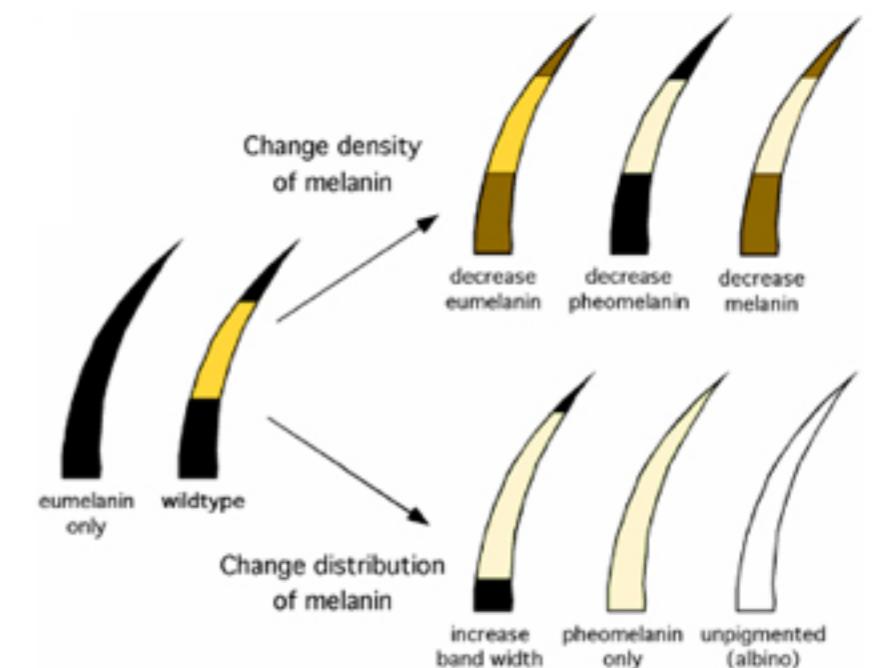
Transposons and methylation

- TEs are highly methylated when younger in age and/or proximal to genes
- Purged from genome over time
- Methylation loss with age
- Does that mean this is all just genotype-dependent???



Outlier loci between dogs and wolves

- Non-neutral, differentially methylated sites found in functionally enriched regions
 - GRBRB1: GABA-A receptor (excitatory neurotransmitter)
 - SLC17A8: glutamate transport gene with a role in cognition (inhibitory neurotransmitter)
 - ADCY1: role in memory acquisition and learning
 - Others with putative impact on phenotype
 - NEK1: polydactyly
 - MLPH: hair shaft pigmentation



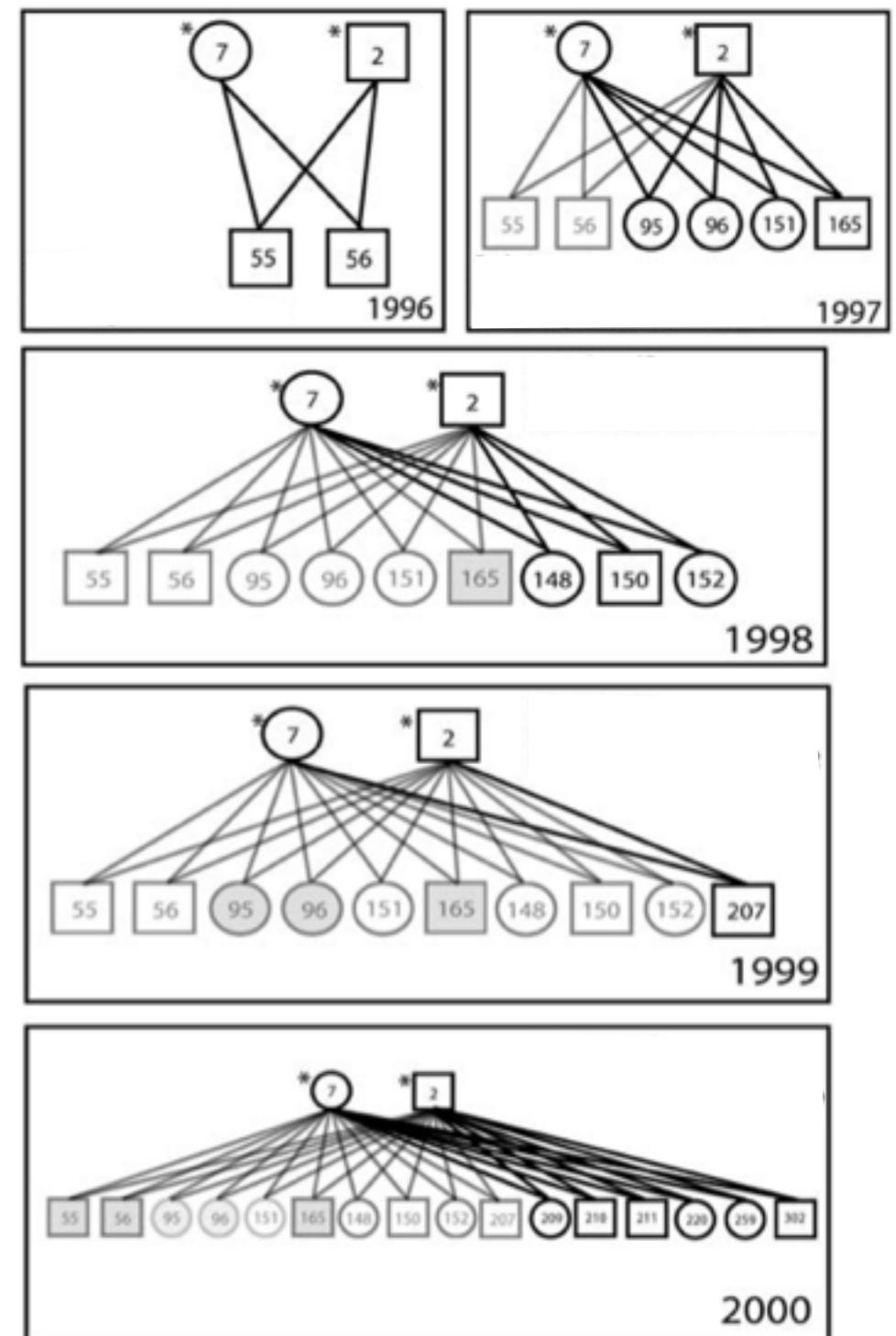
Caveats? Concerns?

- Purely environmental influence?
- Are environments recreated the molecular phenotype of is this a heritable process?
- Can/when is epigenetic variation genotype-dependent?
- Adaptation? Fitness measures?

Epigenetics and Heritability – Are methyl marks stably transmitted across generations?

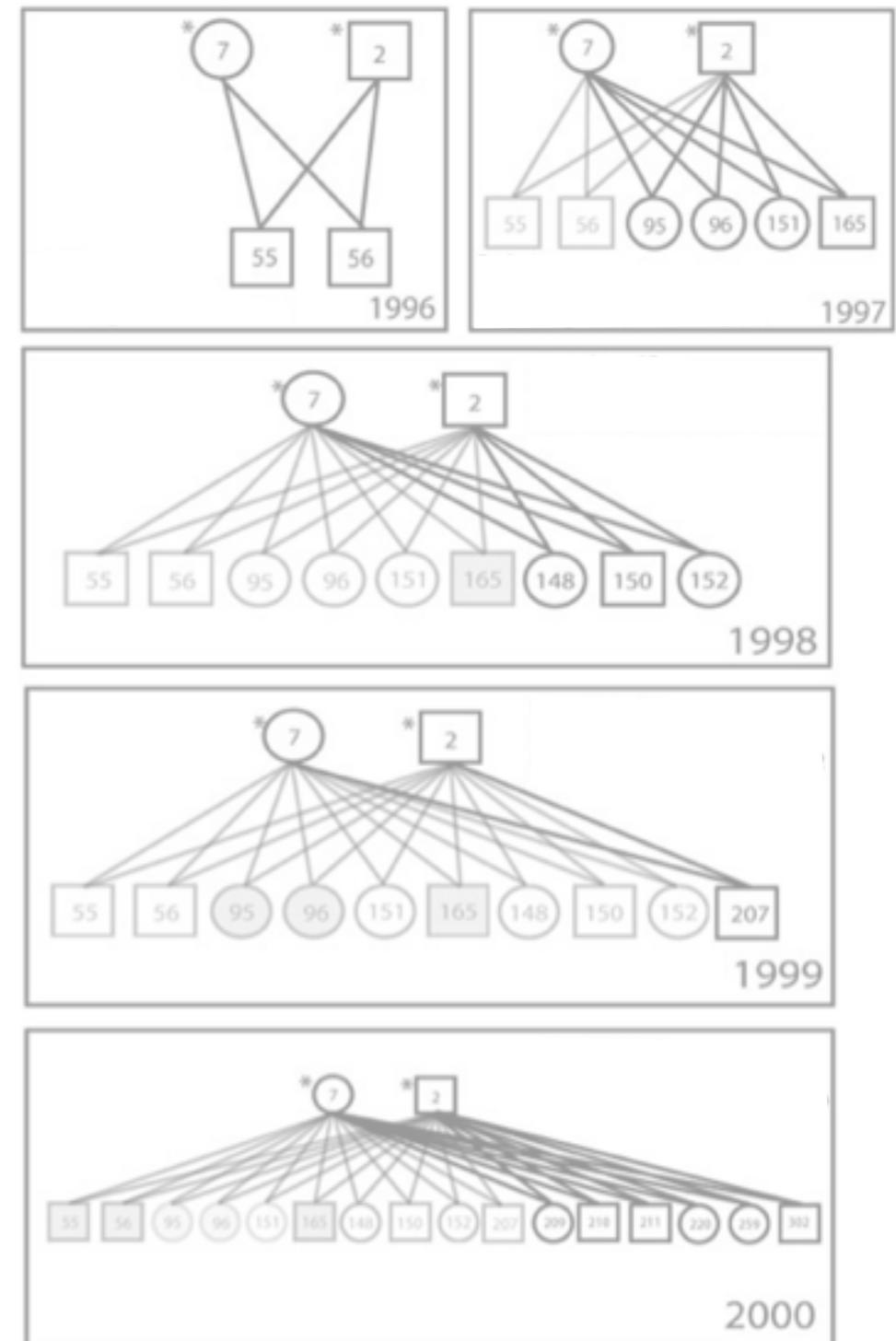
Pedigreed wild populations

- 35 wolves with both parents known
- 24,000 cytosines with methylation data
 - 81% with no narrow sense heritability ($h^2 < 0.01$)
 - 9% with high narrow sense heritability ($h^2 > 0.99$)



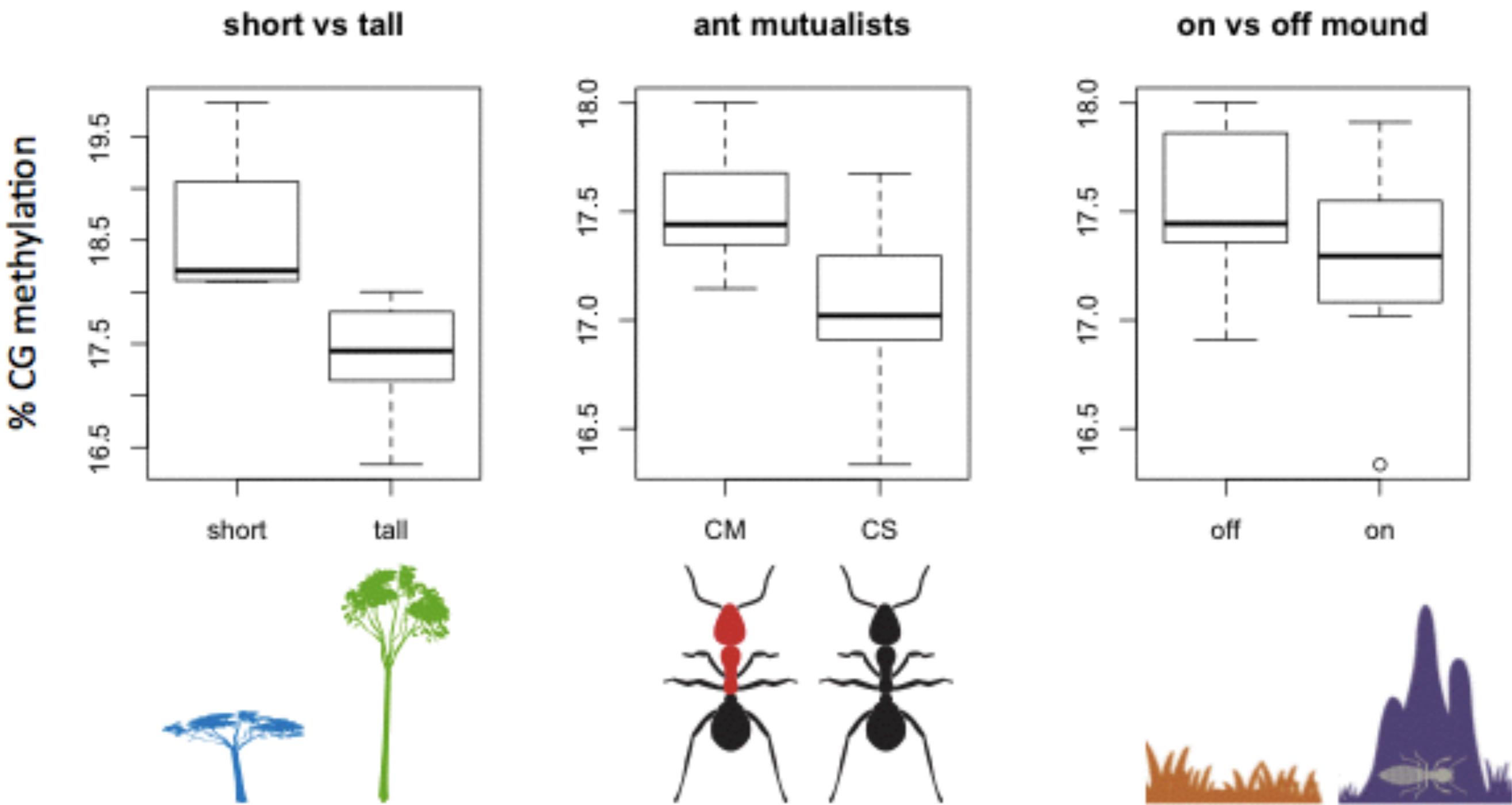
Pedigreed wild populations

- 35 wolves with both parents known
- 24,000 cytosines with methylation data
 - 81% with no narrow sense heritability ($h^2 < 0.01$)
 - 9% with high narrow sense heritability ($h^2 > 0.99$)
- Collaboration with Gabi Pinho and Dan Blumstein (UCLA) on their pedigreed marmot population

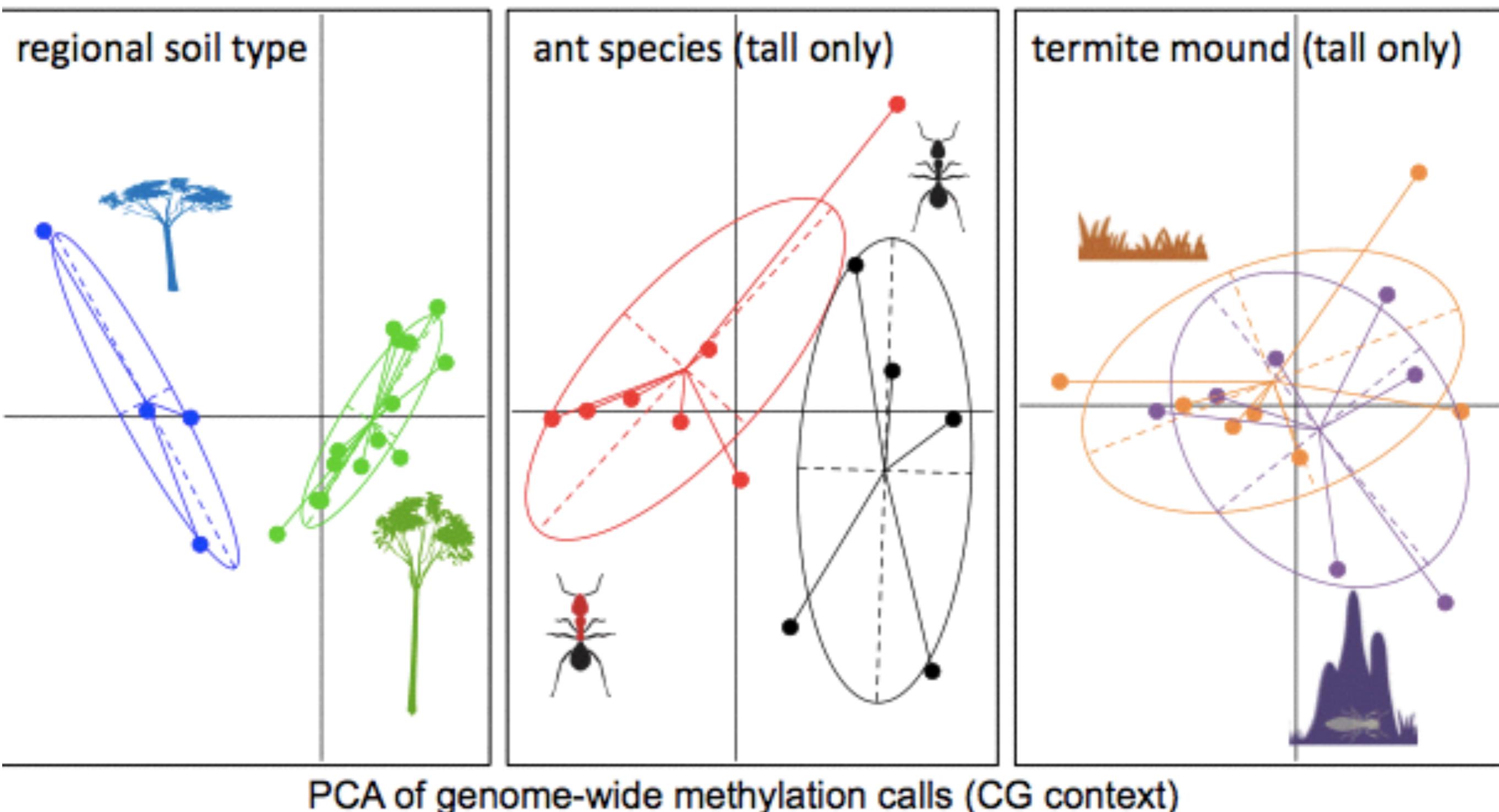


Epigenetic and species interactions – Can species relationships dynamically alter methylation?

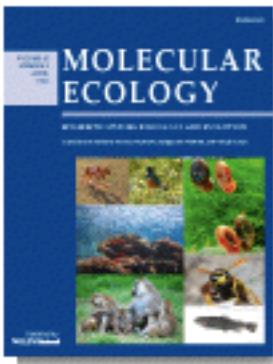
Differences in genome-wide %CG methylation



Regional soil variation + mutualists elicit different methylation patterns



This nascent field is quickly building...



Molecular Ecology Special Issue: Epigenetic studies in ecology and evolution

April 2016, Vol 25, Issue 8

- Environmental epigenetics

[Genetic and epigenetic differences associated with environmental gradients in replicate populations of two salt marsh perennials \(pages 1639–1652\)](#)
C. M. Foust, V. Preite, A. W. Schrey, M. Alvarez, M. H. Robertson, K. J. F. Verhoeven and C. L. Richards

[Resource base influences genome-wide DNA methylation levels in wild baboons](#)

[\(*Papio cynocephalus*\) \(pages 1681–1696\)](#)

Amanda J. Lea, Jeanne Altmann, Susan C. Alberts and Jenny Tung

- Linking epigenetic variation to phenotypes

[Natural epigenetic variation contributes to heritable flowering divergence in a widespread asexual dandelion lineage \(pages 1759–1768\)](#)

Rutger A. Wilschut, Carla Oplaat, L. Basten Snoek, Jan Kirschner and Koen J. F. Verhoeven

- Evolutionary potential of epigenetic variation

[The concerted impact of domestication and transposon insertions on methylation patterns between dogs and grey wolves \(pages 1838–1855\)](#)

Ilana Janowitz Koch, Michelle M. Clark, Michael J. Thompson, Kerry A. Deere-Machemer, Jun Wang, Lionel Duarte, Gitanjali E. Gnanadesikan, Eskender L. McCoy, Liudmilla Rubbi, Daniel R. Stahler, Matteo Pellegrini, Elaine A. Ostrander, Robert K. Wayne, Janet S. Sinsheimer and Bridgett M. vonHoldt

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- * **NIH/NHGRI:** Elaine Ostrander
- * **Yellowstone NP:** Douglas Smith and Daniel Stahler
- * **AKC:** OAK Funding Program



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