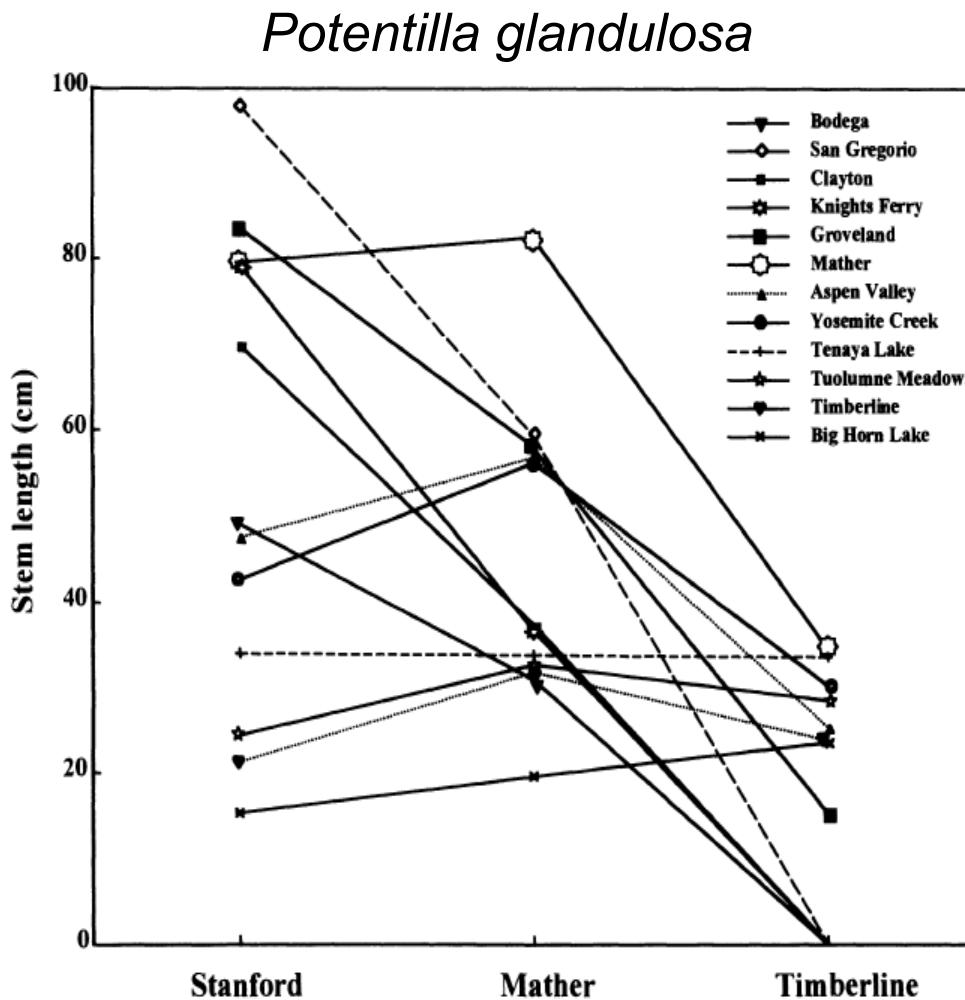


Mechanisms of response to novel and changing environments in two contrasting coastal plant systems



Christina Richards
 @ecolepig

Genotype and environment contribute to phenotype



- Replicated clones across environments
- Trait mean AND plasticity are heritable
- Subject to selection-> adaptation
- Genetics AND environment

Clausen, Keck and Hiesey, 1948 reprinted in NúñezFarfán and Schlichting 2001 QRB

Review

Genotype–phenotype mapping and the end of the ‘genes as blueprint’ metaphor

Massimo Pigliucci*

Department of Philosophy, City University of New York-Lehman, NY, USA

Integrative and Comparative Biology, volume 51, number 4, pp. 514–527
doi:10.1093/icb/icr049

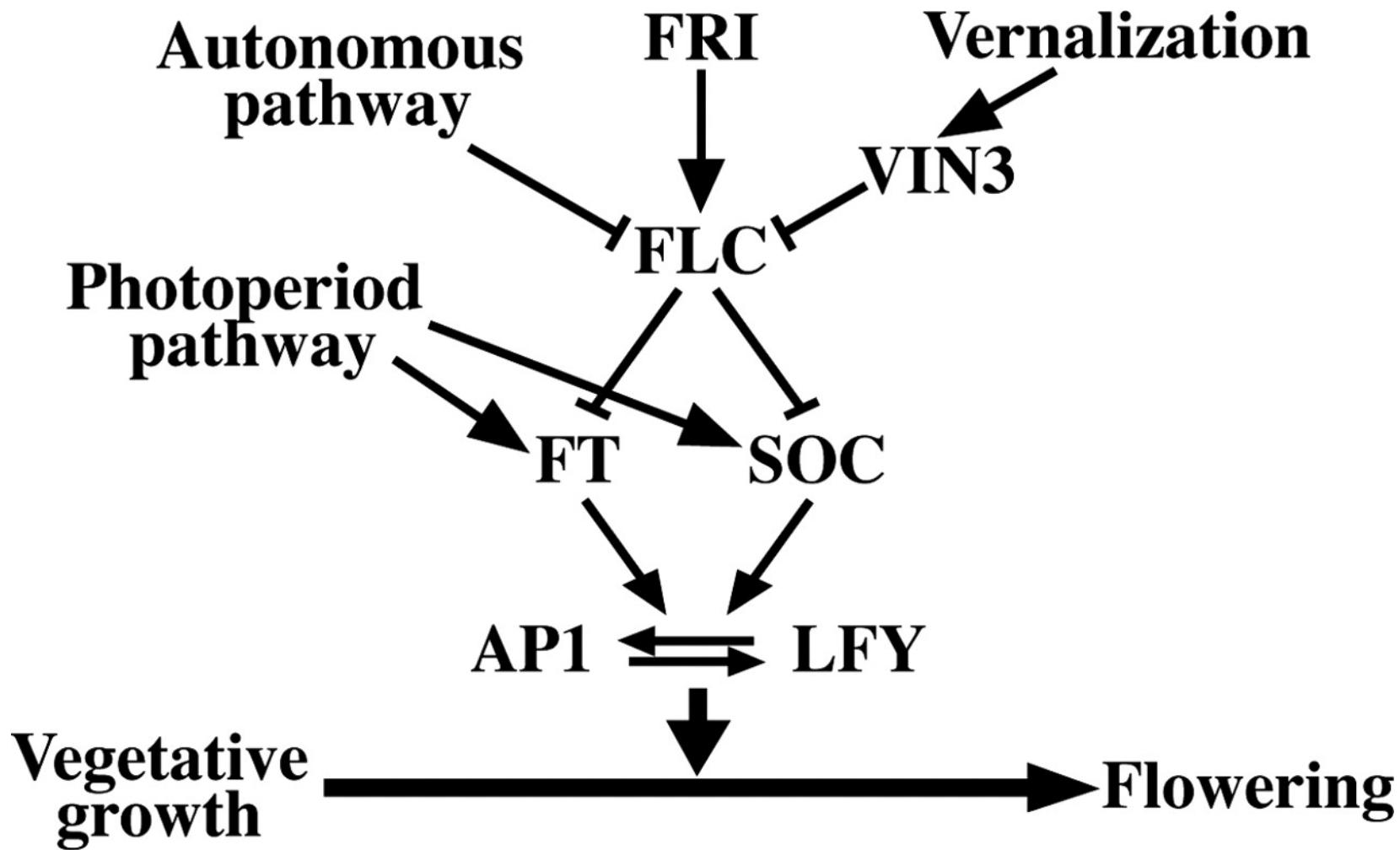


SYMPOSIUM

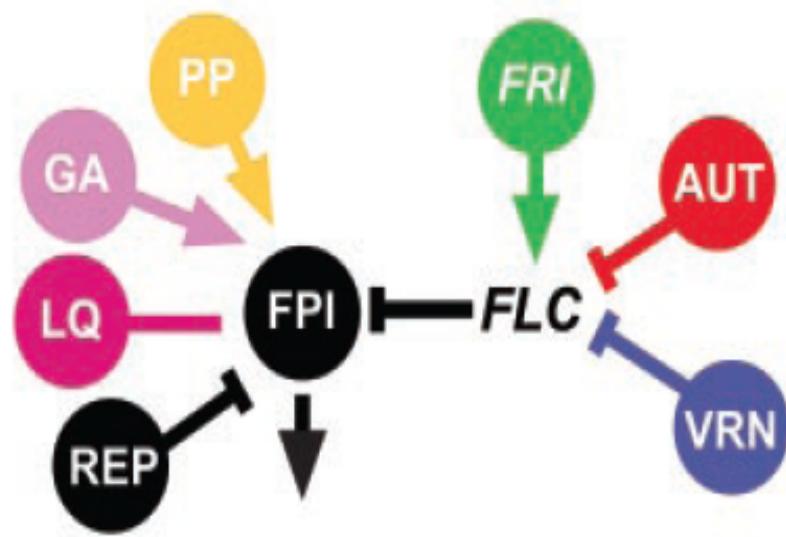
Integrator Networks: Illuminating the Black Box Linking Genotype and Phenotype

Lynn B. Martin,^{1,*} Andrea L. Liebl,^{*} Justin H. Trotter,[†] Christina L. Richards,^{*} Krista McCoy^{*,‡}
and Michael W. McCoy^{§,¶}

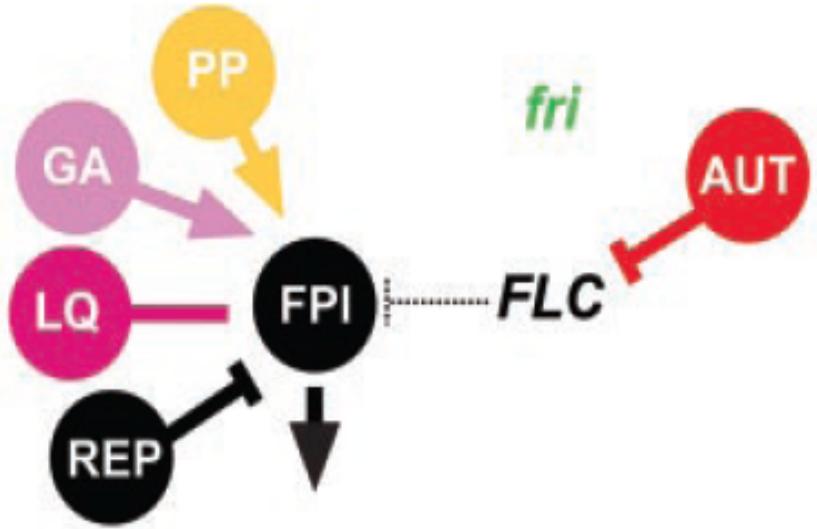
Schematic representation of genetic pathways of flowering time in Arabidopsis.



Winter annual

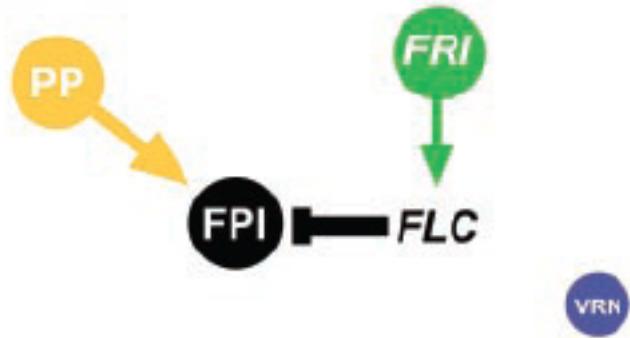


Rapid cycler

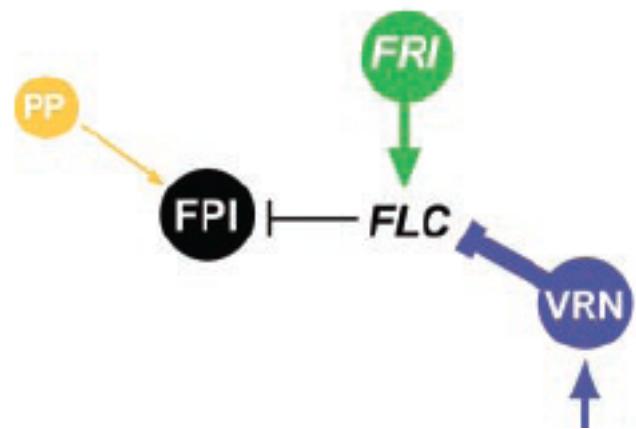


Simpson & Dean Science 2004

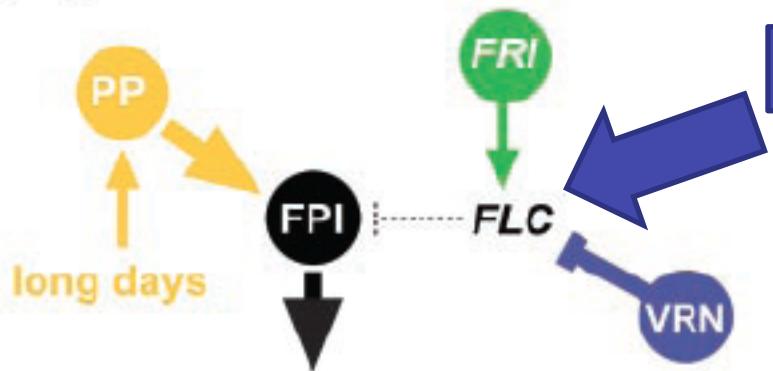
Late summer



Winter



Spring



*Network behavior
is environment
dependent*

VRN mediated Epigenetic Silencing

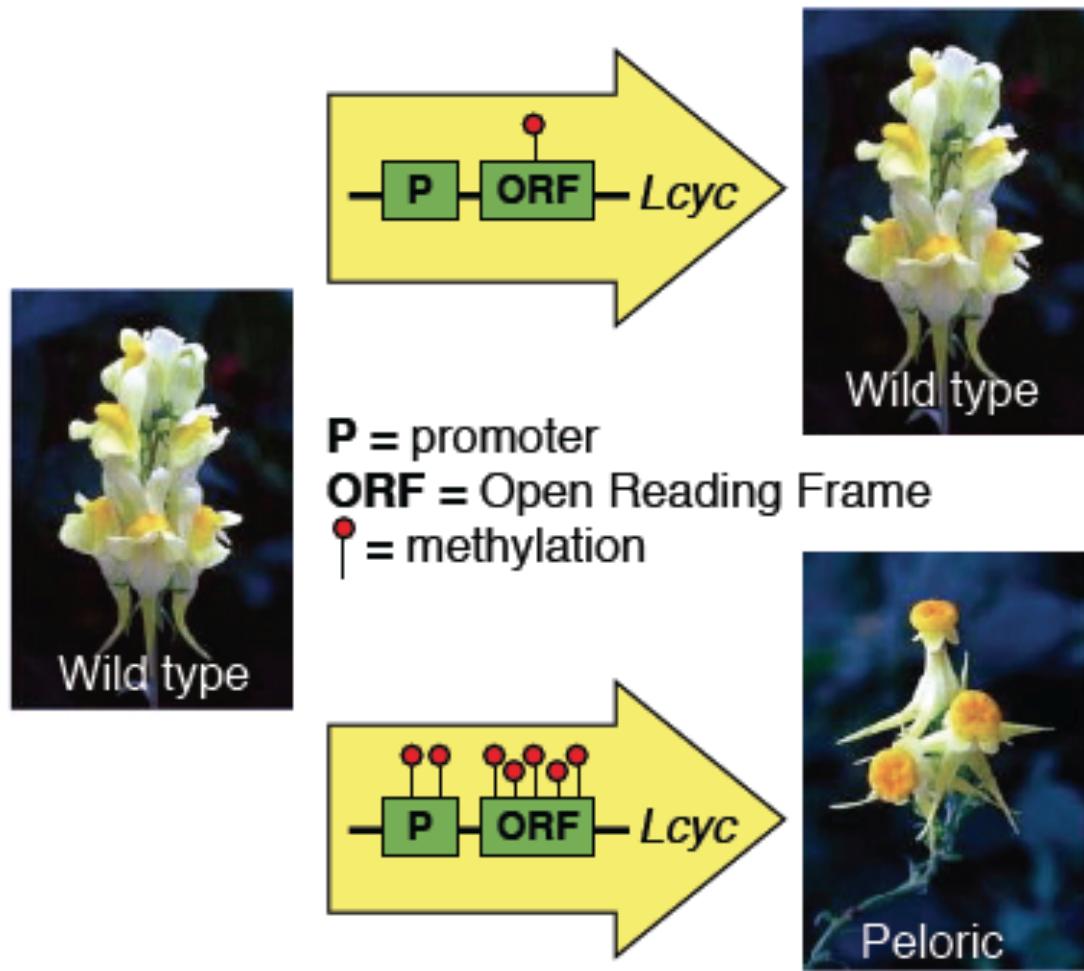
Simpson & Dean Science 2004

Epigenetics- molecular level mechanisms that can alter gene expression and ultimately phenotype without altering DNA sequence.

- DNA methylation
- histone modification
- micro and siRNA
- cellular location

- involved in process of differentiation
- can also be triggered by environment
- changes can be inherited

Heritable epigenetic effects

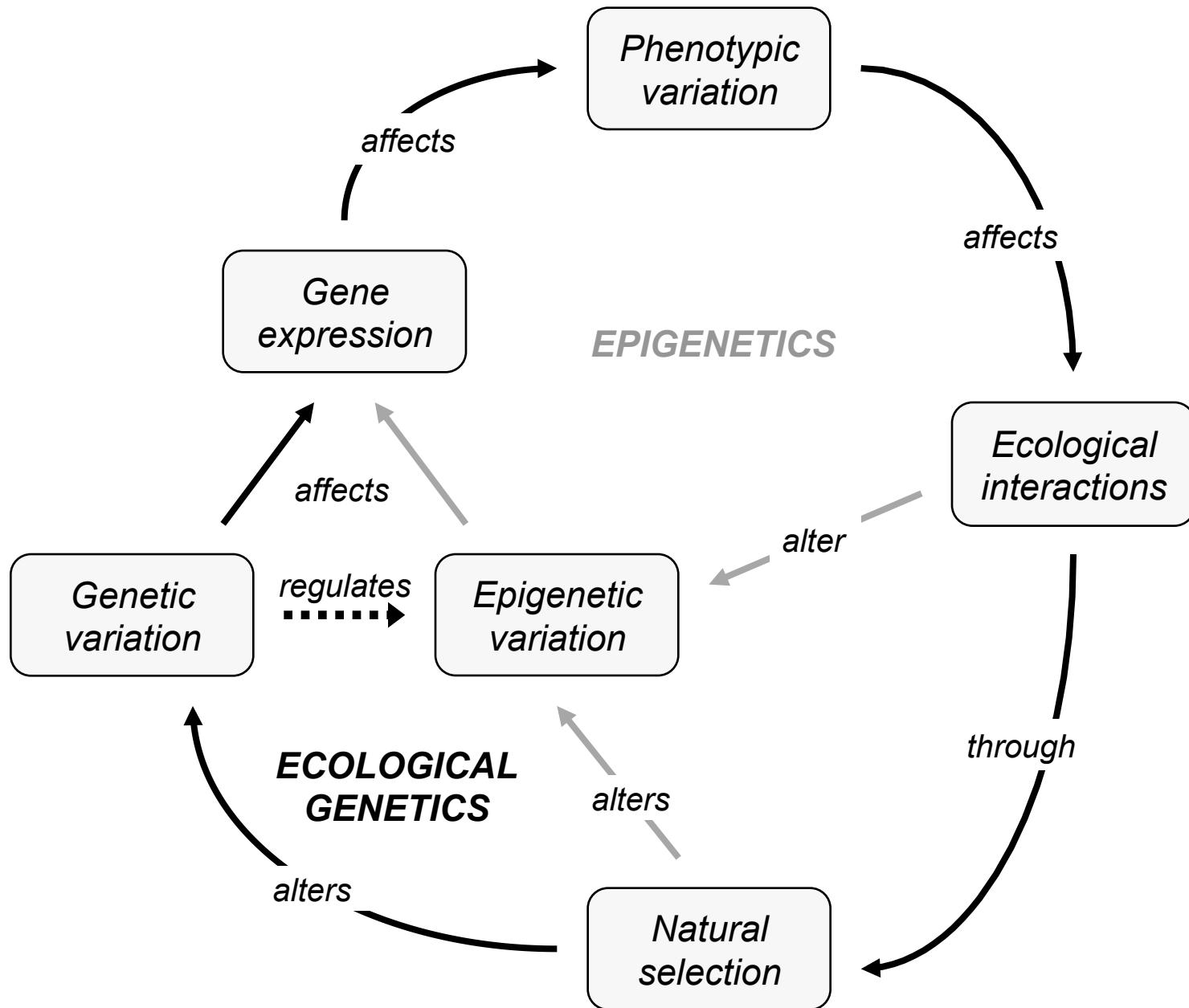


- *Lcyc* in *Linaria vulgaris*
= bilateral symmetry

- *Lcyc* is methylated
= radial symmetry

Cubas, et al. 1999 *Nature*.

Figure credit S. Deban



Bossdorf, Richards & Pigliucci. 2008 *Ecology Letters.*

Epigenetic variation in natural habitats- habitat differences and herbivory in *Viola*



Viola cazorlensis (Herrera & Bazaga 2010 *New Phyt* & 2011 *Mol Ecol*)

Epigenetic variation in natural habitats- river versus salt marsh habitat



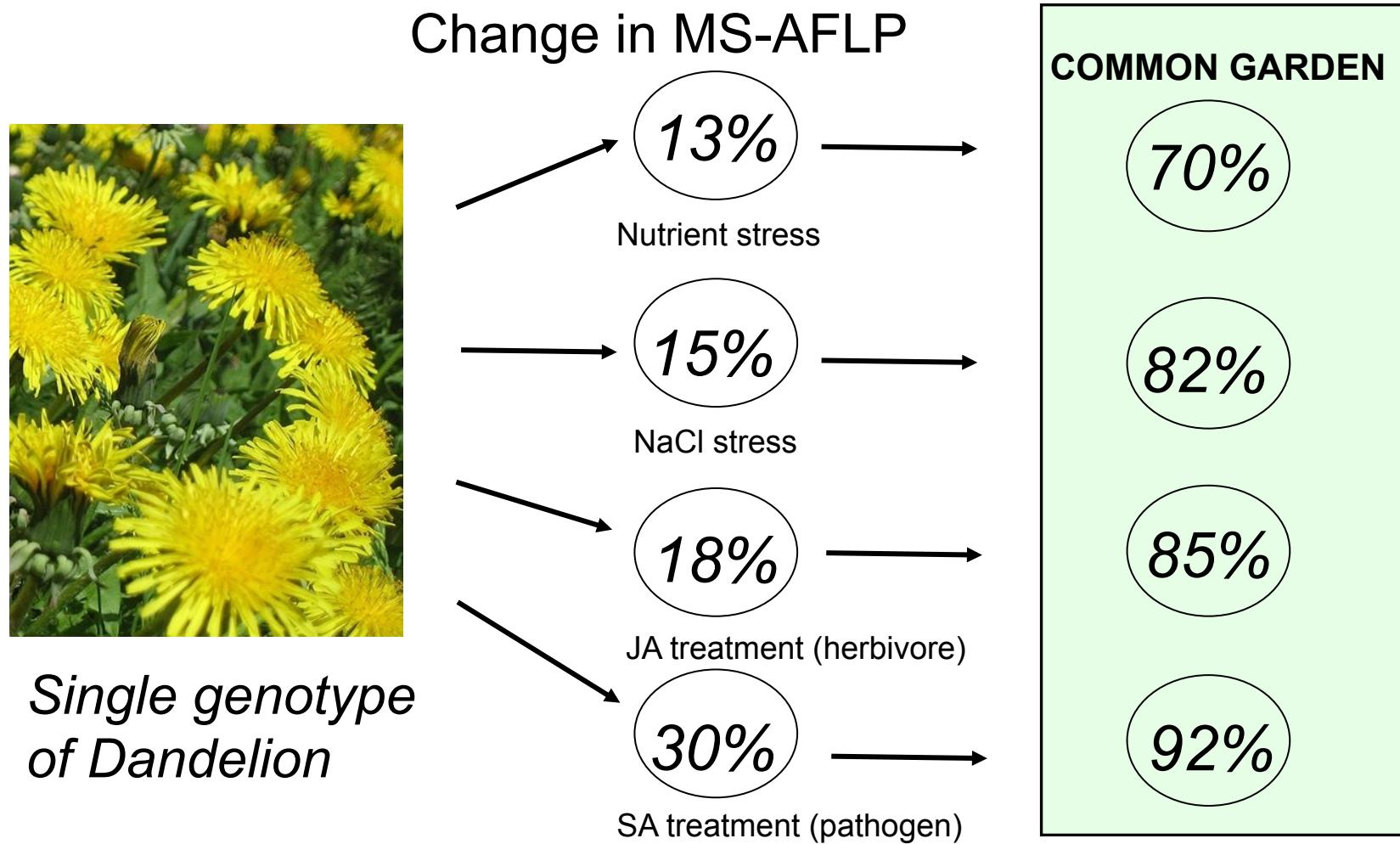
Mangrove Laguncularia racemosa (Lira-Medeiros et al. 2010 PLoS One)

Epigenetic variation in natural habitats- speciation in orchids

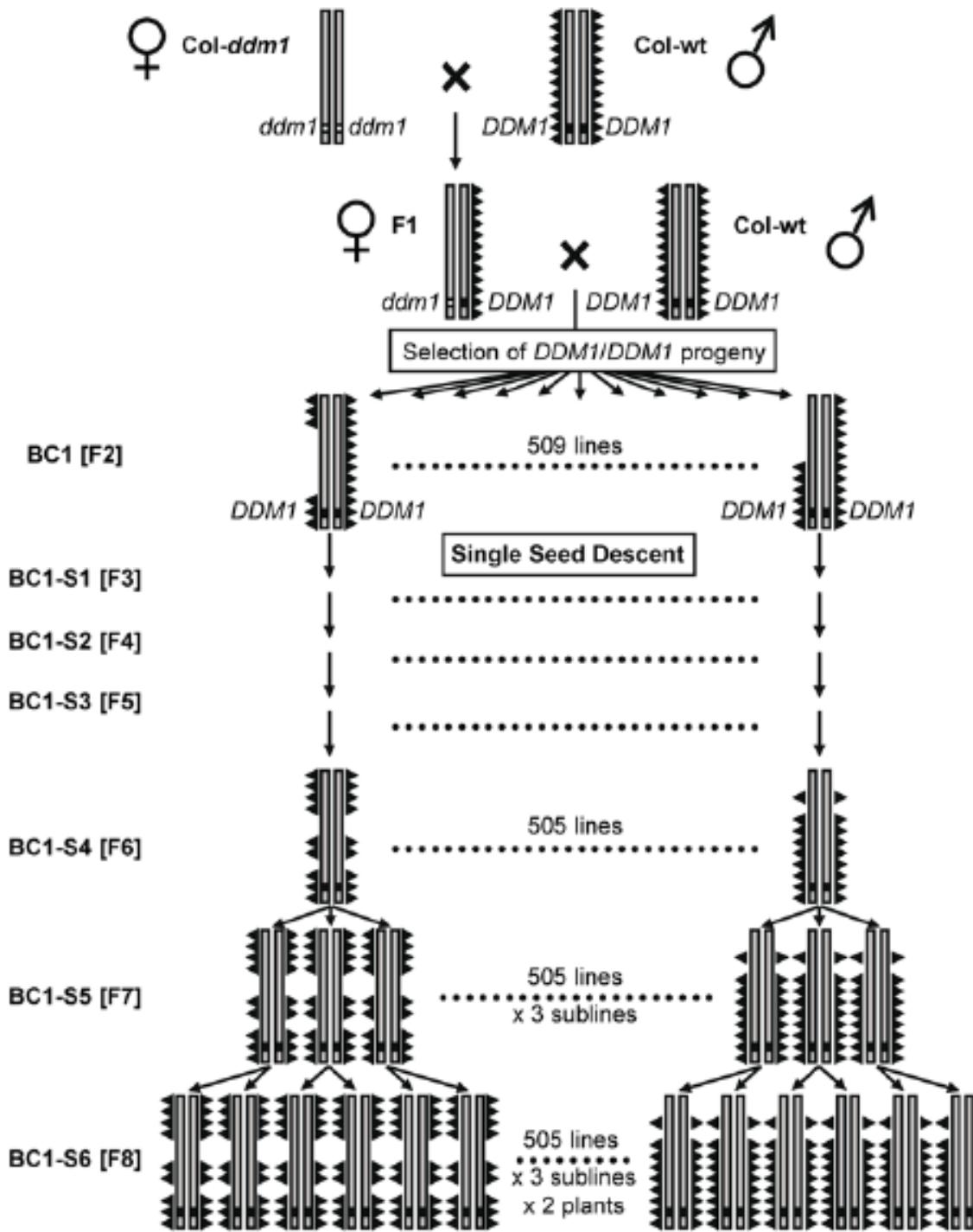


Orchid Dactylorhiza (Paun et al. 2010 *Mol Bio Evol*)

DNA methylation changes induced by stress – some are inherited

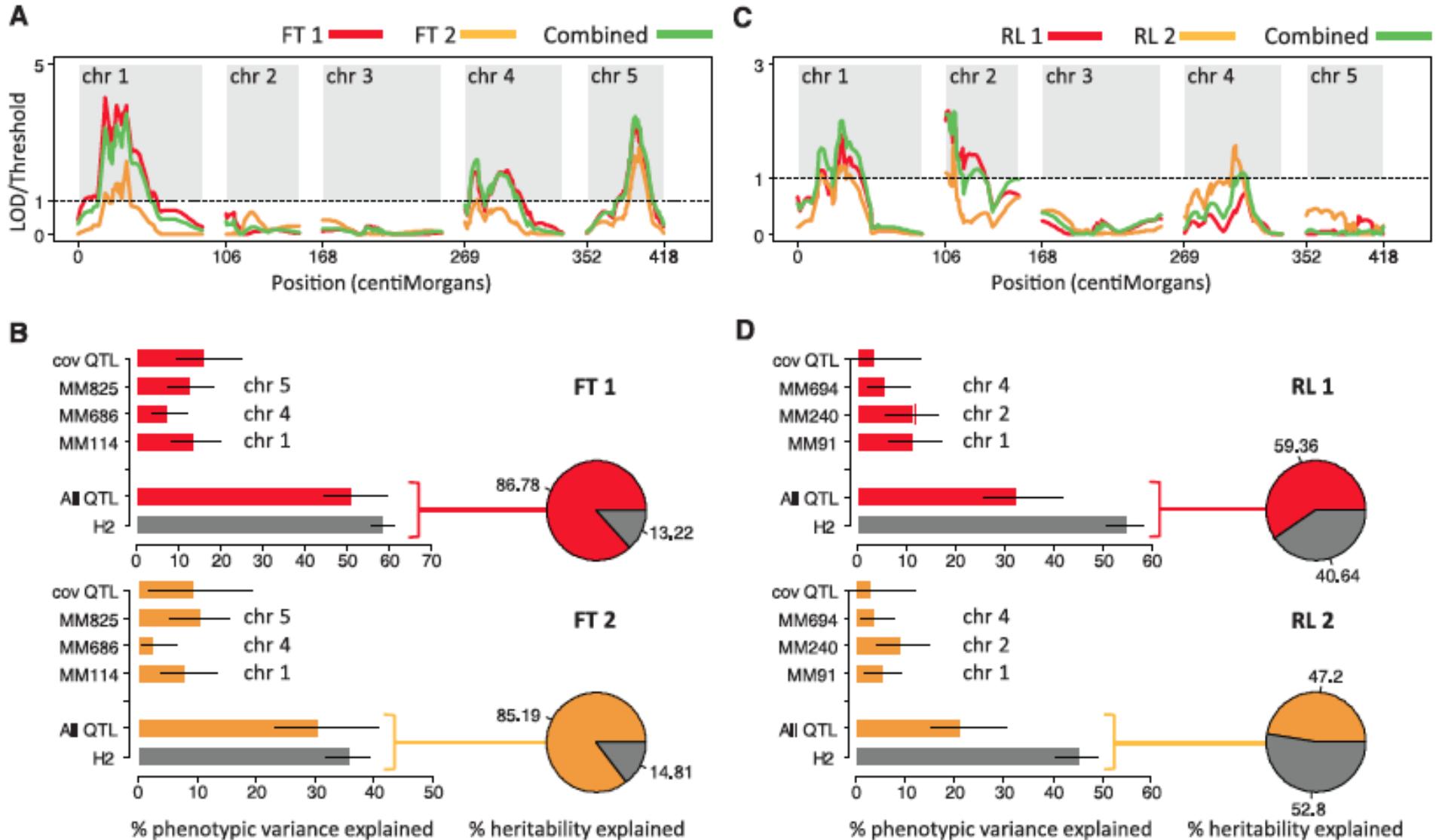


Arabidopsis EpiRILs



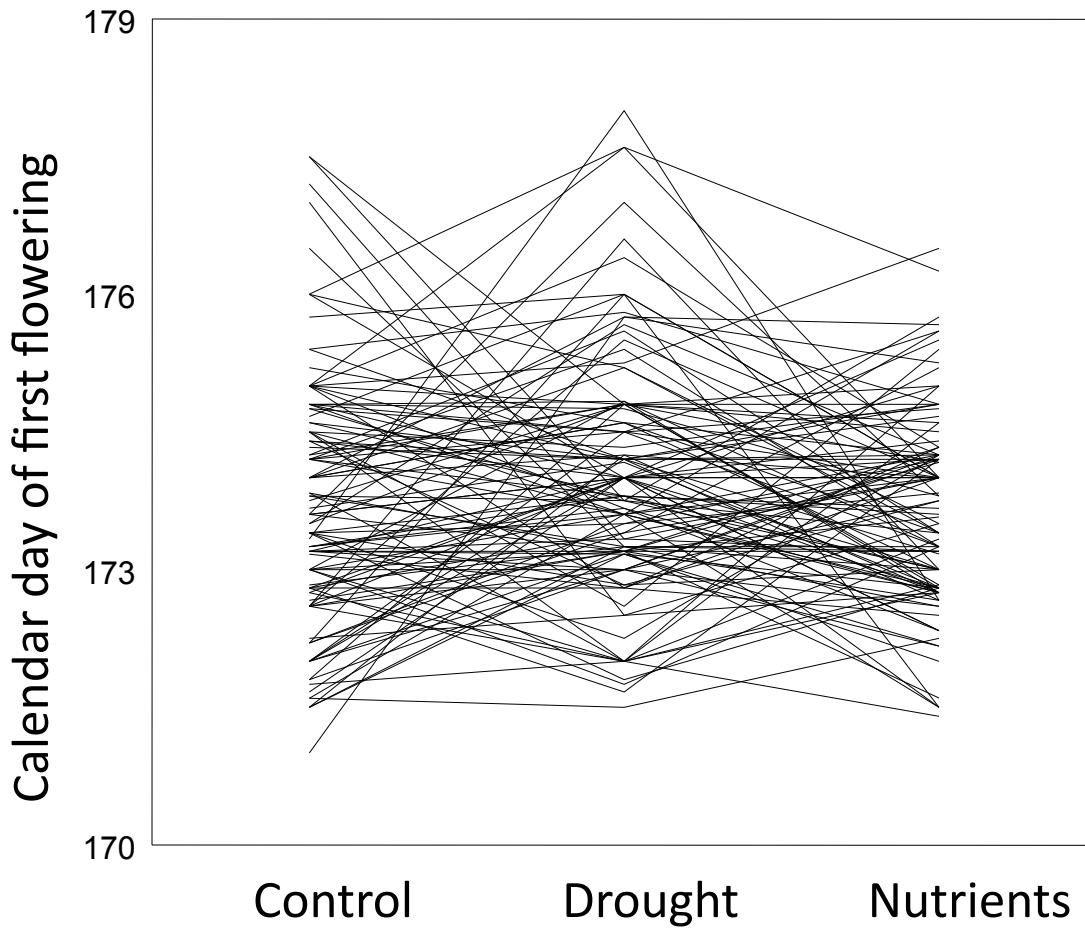
Johannes et al. 2009 PLoS Genetics

In *Arabidopsis* EpiRILs: Methylation accounts for heritability of traits



Cortijo et al. 2014 Science

Arabidopsis EpiRILs: Significant variation in phenotypic plasticity



- All same genotype*
- epiRIL x treatment effect ($P=0.0077$)

Zhang et al. 2013 *New Phytologist*

Agouti coat color and obesity in genetically identical mice



Agouti

A – wild type brown

aa – black (loss of function)

4 alleles with retro TEs:

Avy, *Aiapy*, *Aiy* and *Ahvy*

RTE drives expression of agouti

= *pseudo-agouti*

Yellow

Slightly
mottled

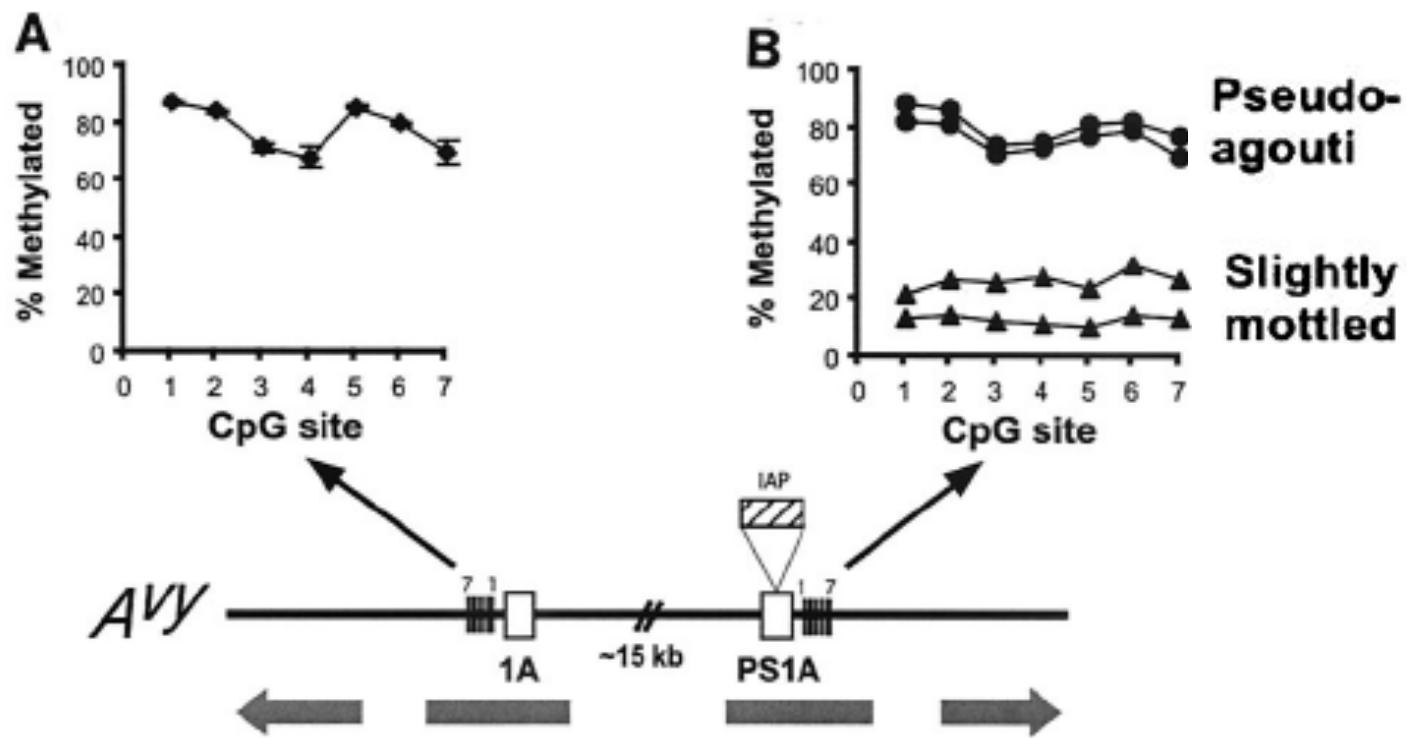
Mottled

Heavily
mottled

Pseudo-
agouti

Morgan et al. 1999 Nature Genetics
Waterland & Jirtle 2003 Mol and Cell Biol

Epigenetic inheritance in genetically identical mice

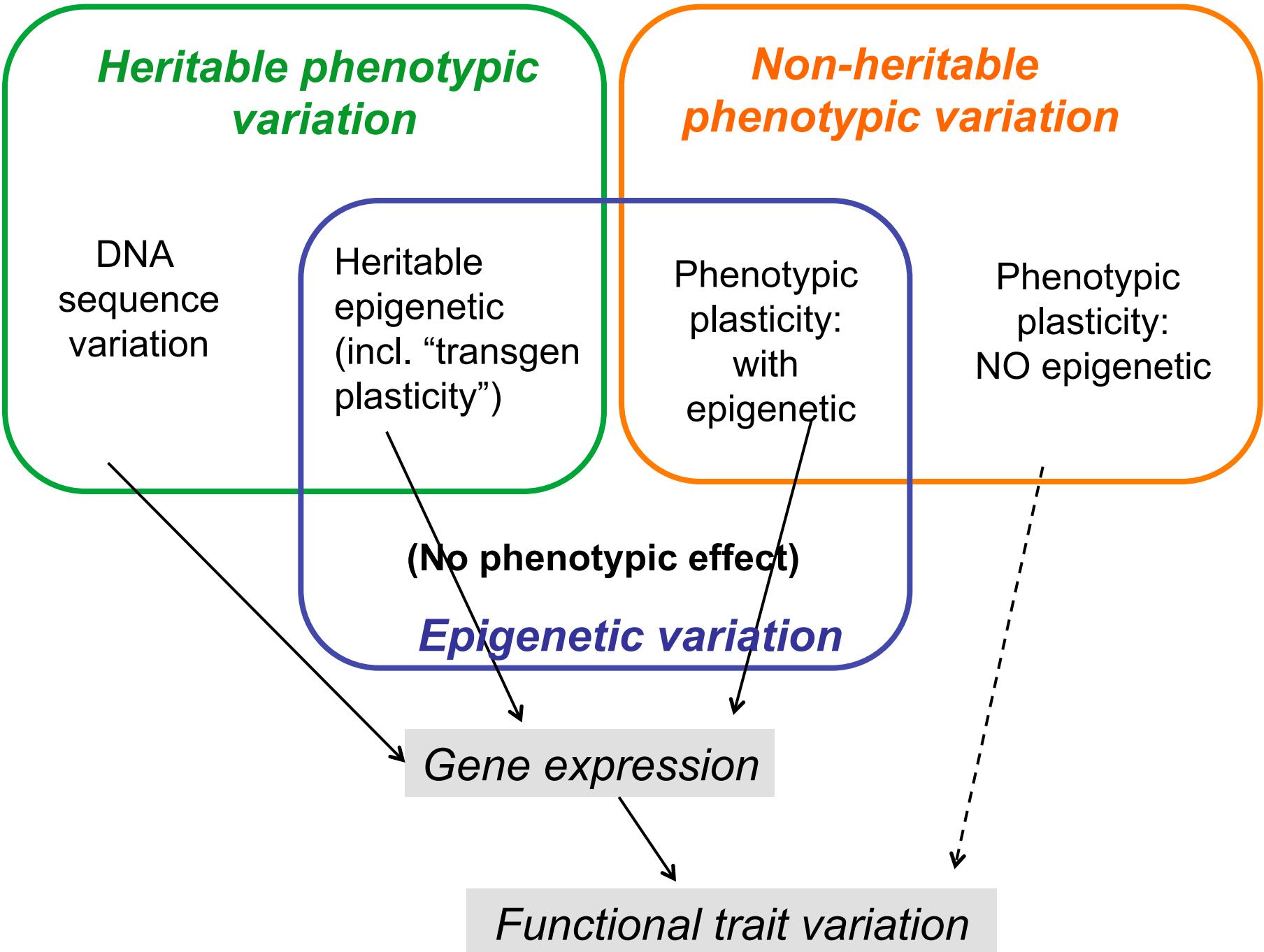


Phenotype can be dramatically altered by diet

Phenotype of mom and grandmom effects offspring phenotype

Incomplete erasure of methylation in the germ line!

Morgan et al. 1999 Nature Genetics
Waterland & Jirtle 2003 Mol and Cell Biol



Response to complex environments



- Model plant *Arabidopsis* (proof of concept): How do diverse genotypes vary in gene expression in response to environment?



- How do epigenetic effects contribute to plant invasion (*Fallopia*)?



- Are there genetic, epigenetic and gene expression signatures of response to a novel stressor?

The geographic range of *A. thaliana*



Genome wide expression differences: Genotype x development

Bay



Shah

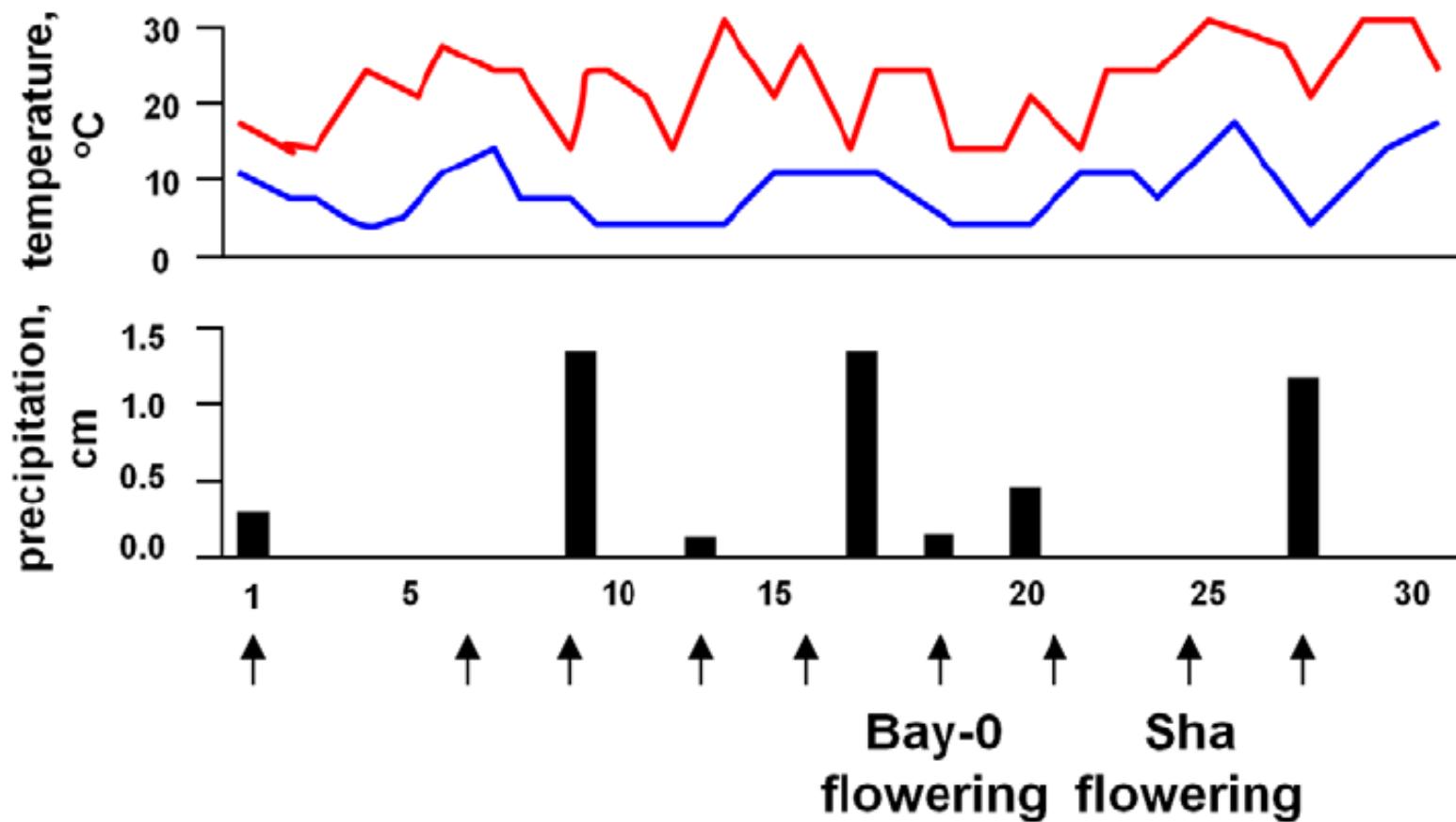


4-6 Leaf

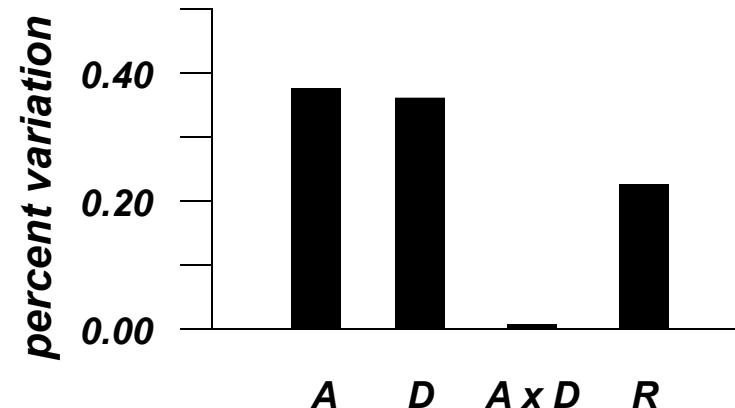
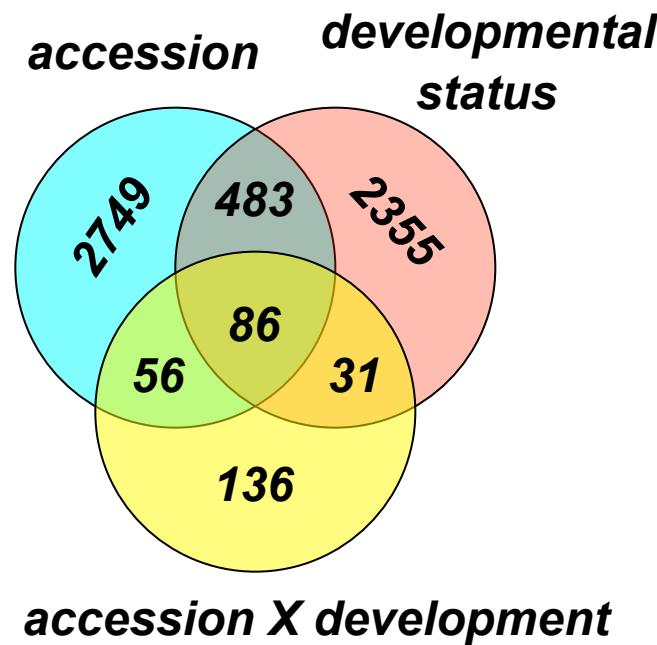
Bolting

Cold Spring Harbor
2 genotypes x
8-10 growth times x
3 individuals x
22,000 genes
(ATH1 microarray
from Affymetrix)

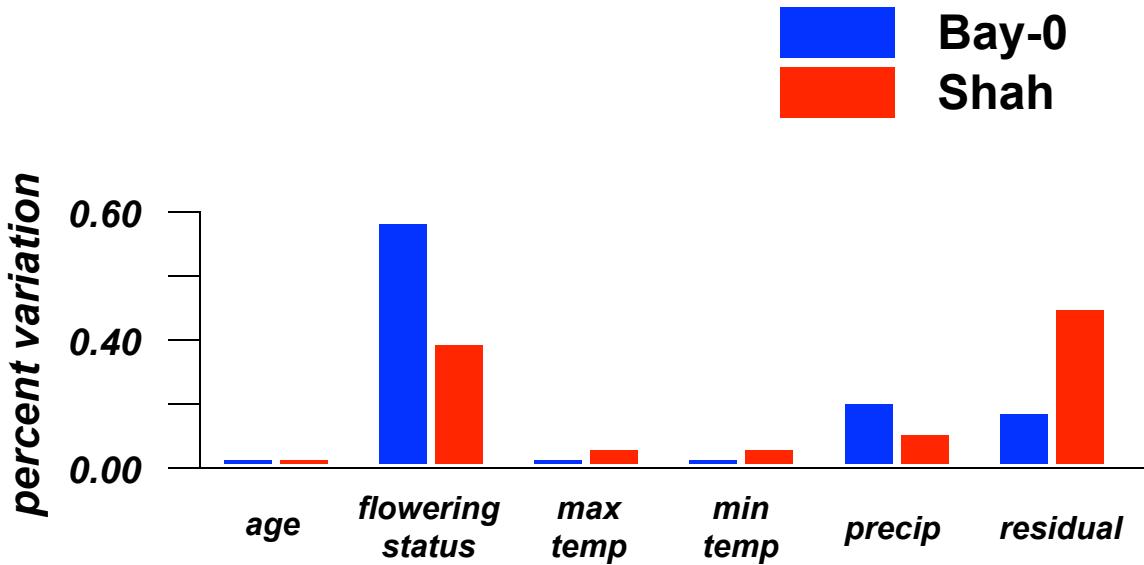
Field conditions during sampling

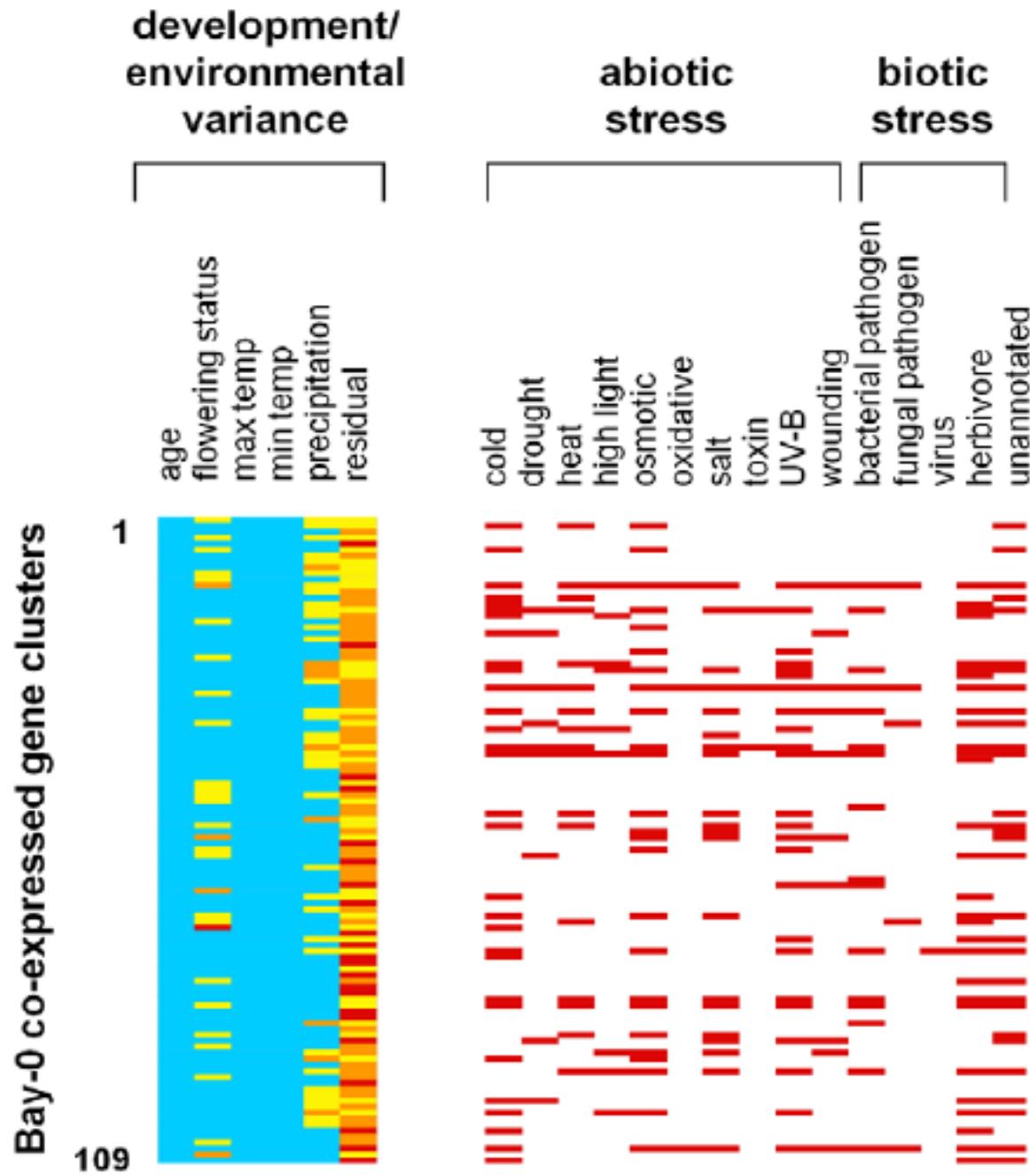


Genotype and development explain equal amount of expression variance!



Effect of development and environmental factors on expression varies by genotype



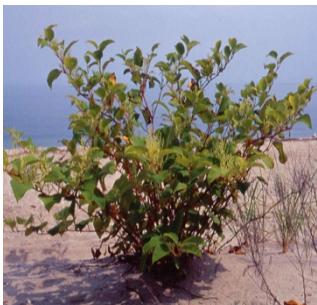


Clusters identified across developmental sequence-could reveal novel relationships between genes in response to stress

Response to complex environments



- Model plant *Arabidopsis* (proof of concept): How do diverse genotypes vary in gene expression in response to environment?

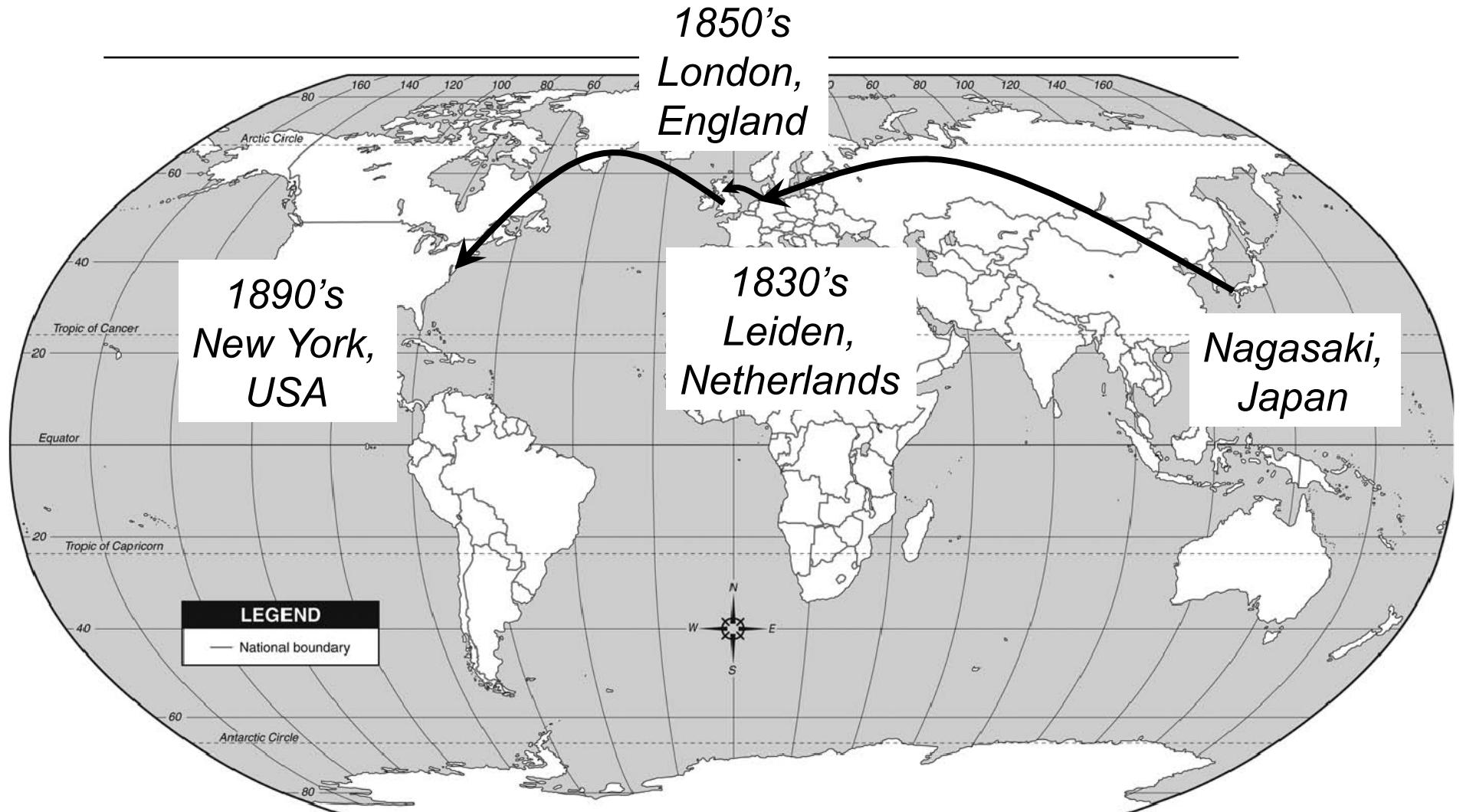


- How do epigenetic effects contribute to plant invasion (*Fallopia*)?

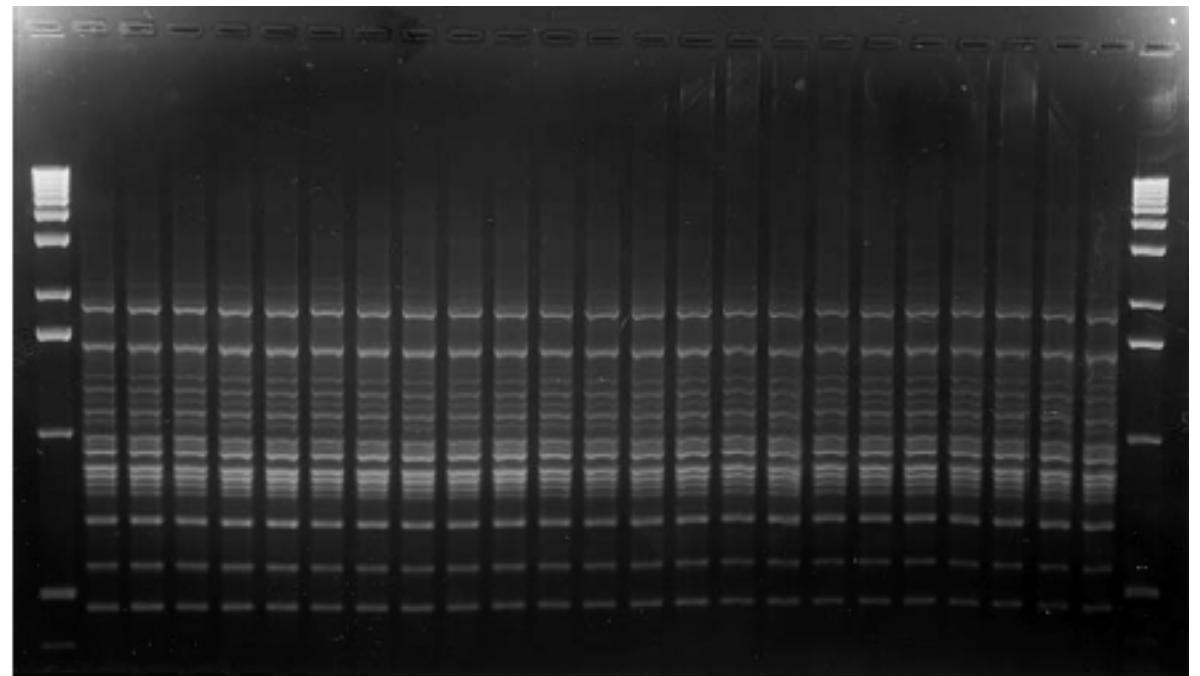
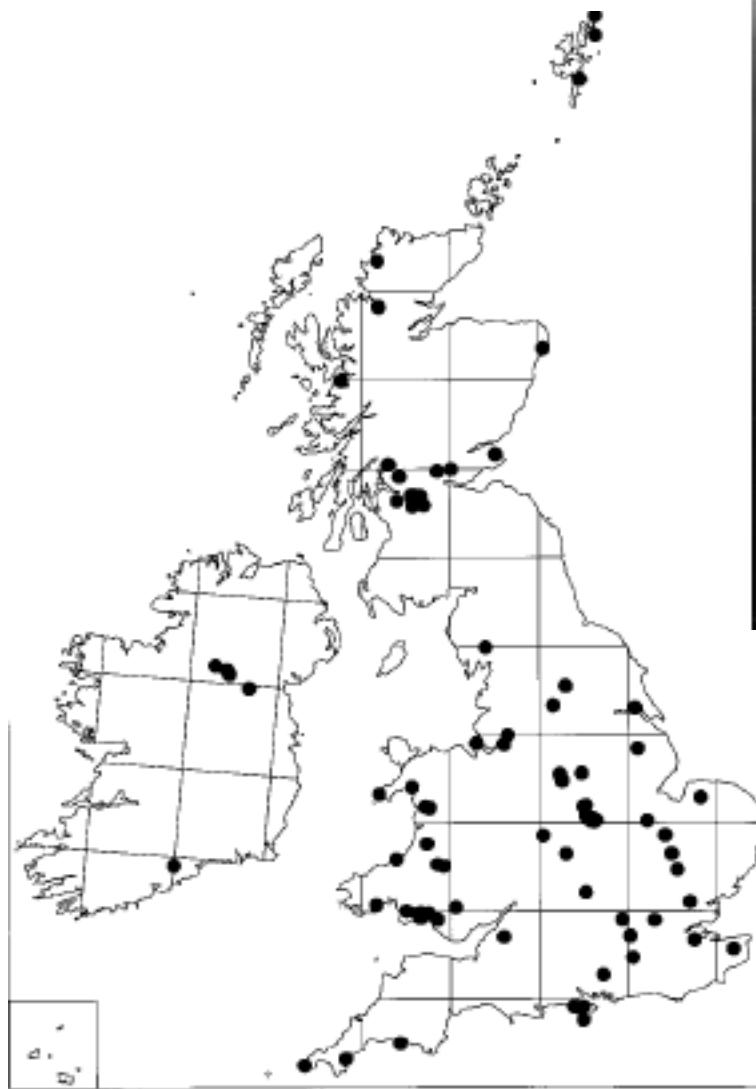


- Are there genetic, epigenetic and gene expression signatures of response to a novel stressor?

Invasive species paradox- Genetic bottleneck



No genetic variation in invasive *F. japonica*



Hollingsworth and Bailey 2000.
Botanical Journal of the Linnean Society.

Long Island knotweed populations

Roadside

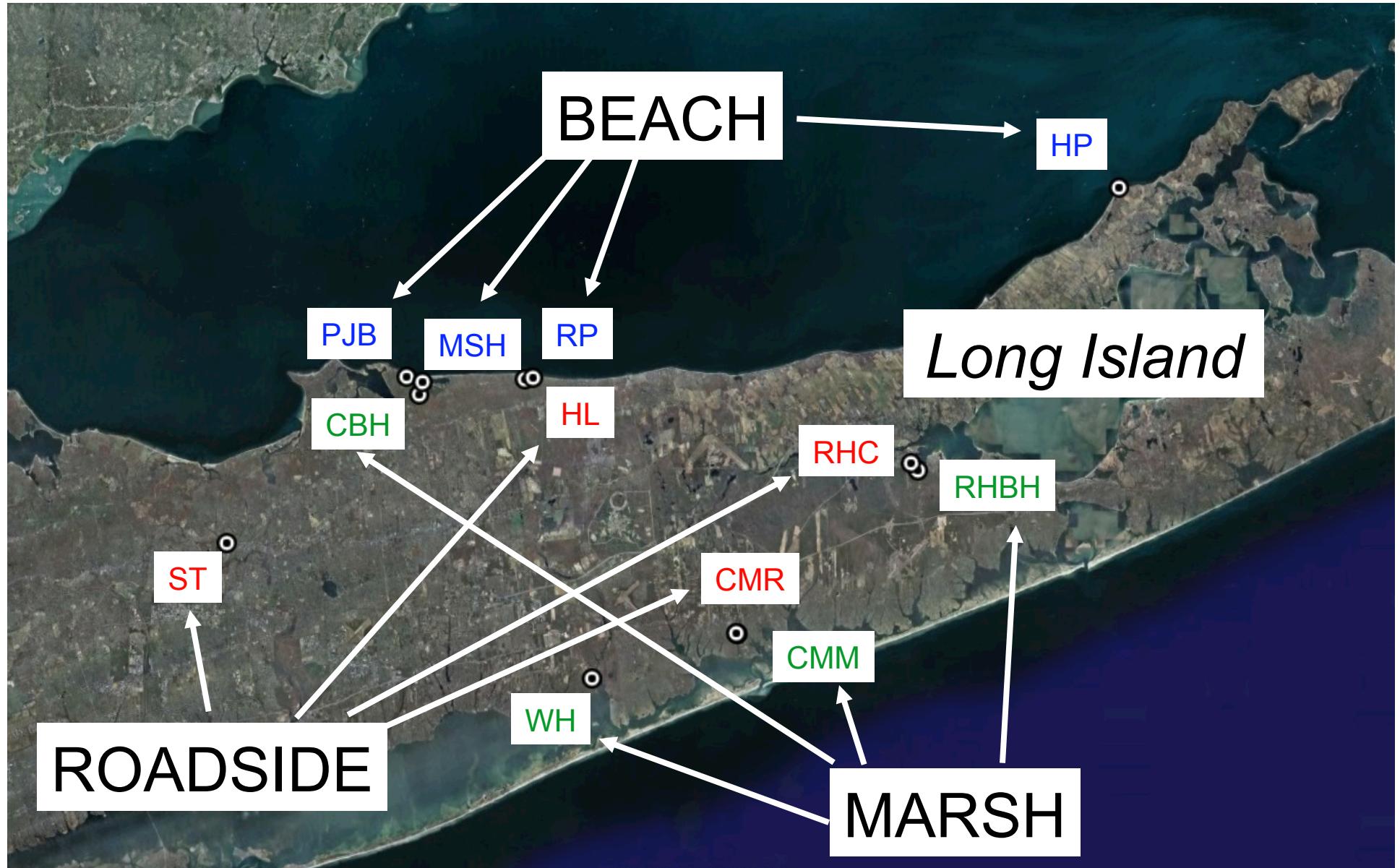


Beach

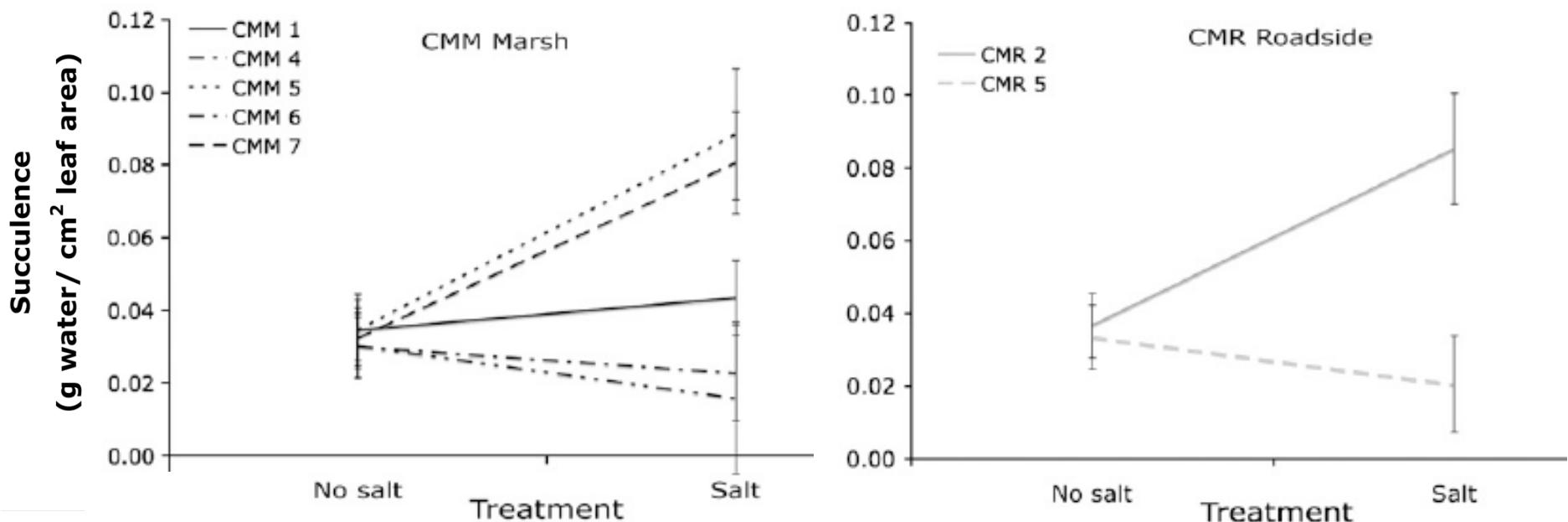


Saltmarsh





Greenhouse salt tolerance results



Same “D” genotype of *F. japonica* from both sites

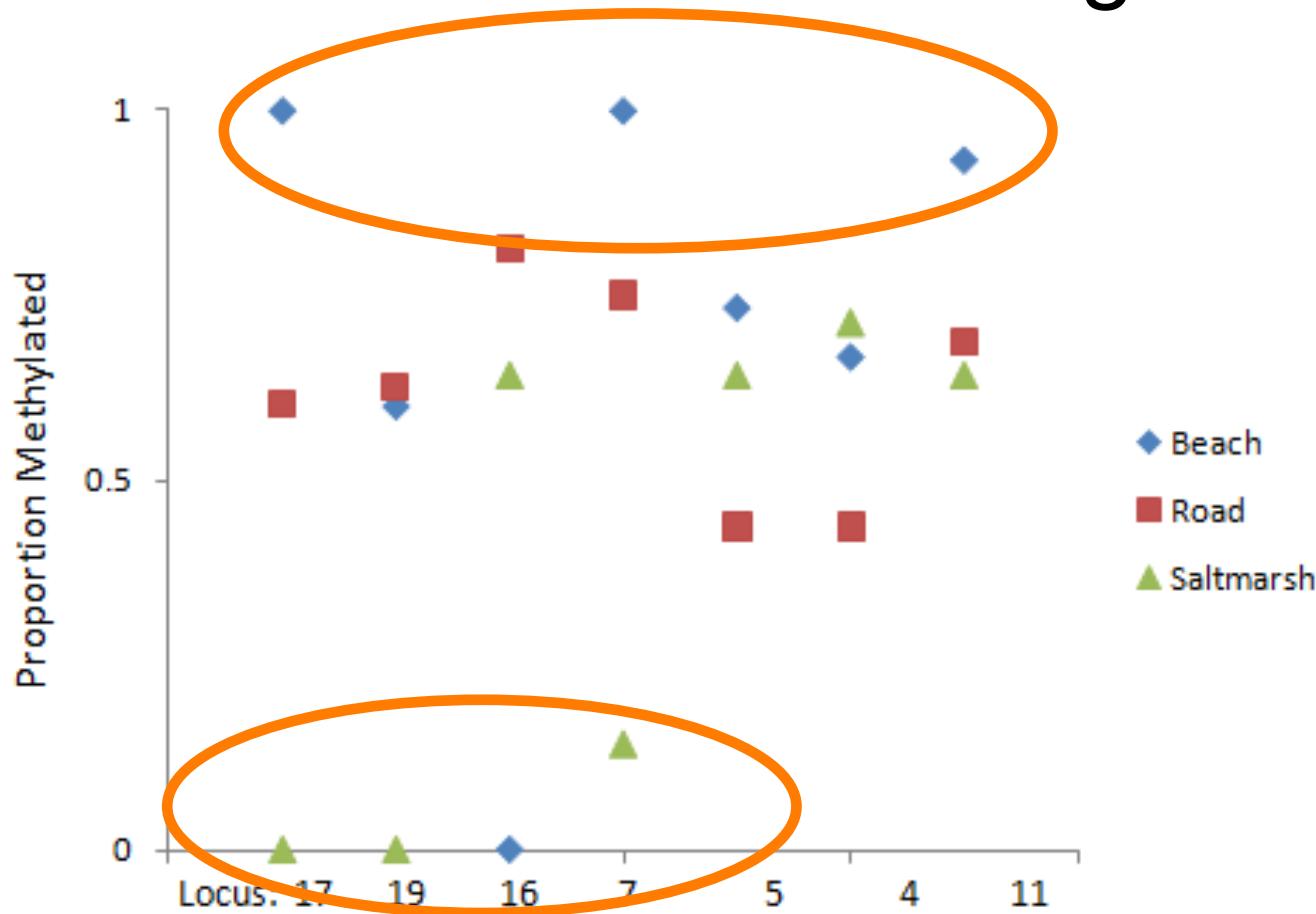
Richards et al. 2008 AJB.

Genetic and epigenetic variation in common garden *Fallopia*

- AFLP (200 markers)
 - 4 / 200 loci are polymorphic
 - 8 haplotypes, all but 1 haplotype occurred in multiple sites
 - 10 sites had only one haplotype
 - All *F. japonica* were one haplotype
- MS-AFLP (180 markers) in *F. japonica*
 - 19 / 180 loci are polymorphic
 - 34 epigenotypes
 - All but one site had multiple epigenotypes
 - Correlated with habitat!

Richards et al. 2012 Ecology Letters

MS-AFLP in *F. japonica* from common garden differentiated among habitats

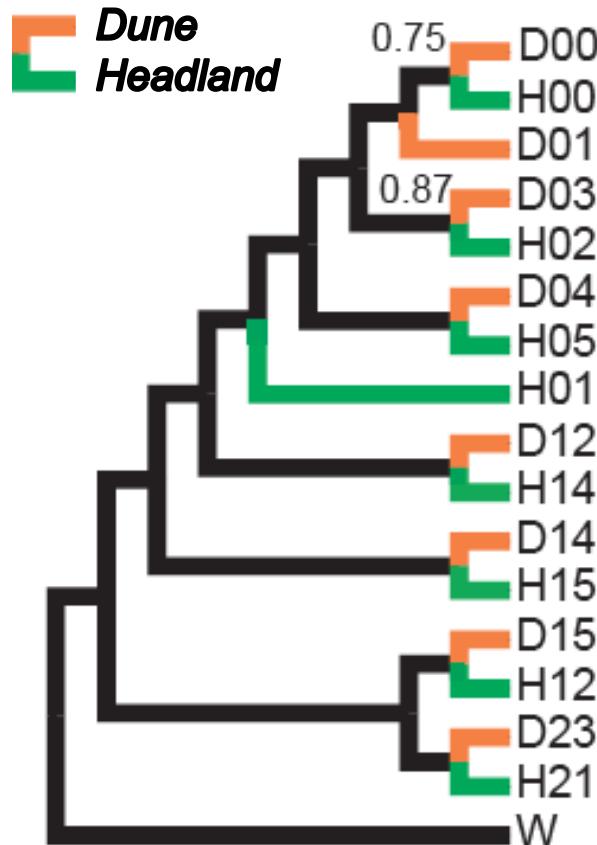


PROBLEMS:

- 1) What is AFLP?
- 2) No direct connection to phenotypic effects.

Richards et al. 2012 Ecology Letters

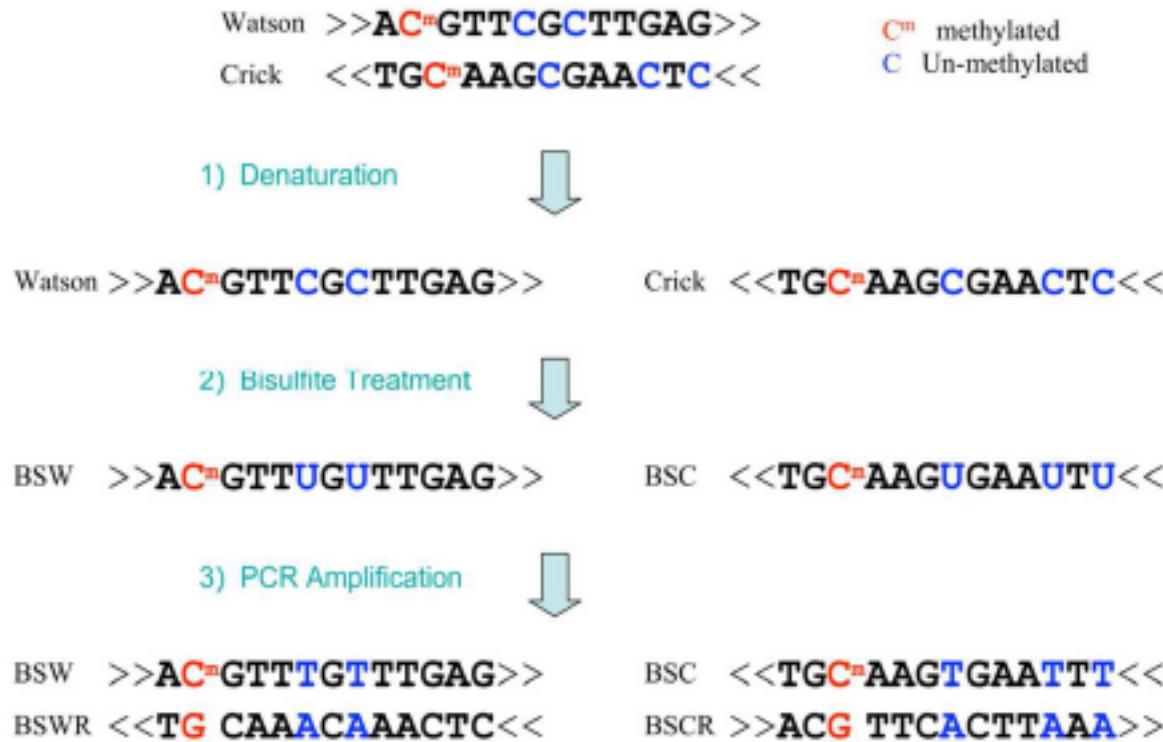
Genotyping By Sequencing (Illumina) detects sequence divergence to habitats



- 29,000 GBS markers in *Senecio lautens*
- Parallel divergence to habitats

Roda et al. 2013 Molecular Ecology

Epi-GBS in *Fallopia* among habitats



<http://www.biomedcentral.com/>



EAGER: Developing epigenetic genotyping-by-sequencing in the non-model invasive Japanese knotweed with Van Gurp and Verhoeven, Netherlands Institute of Ecology (NIOO-KNAW)

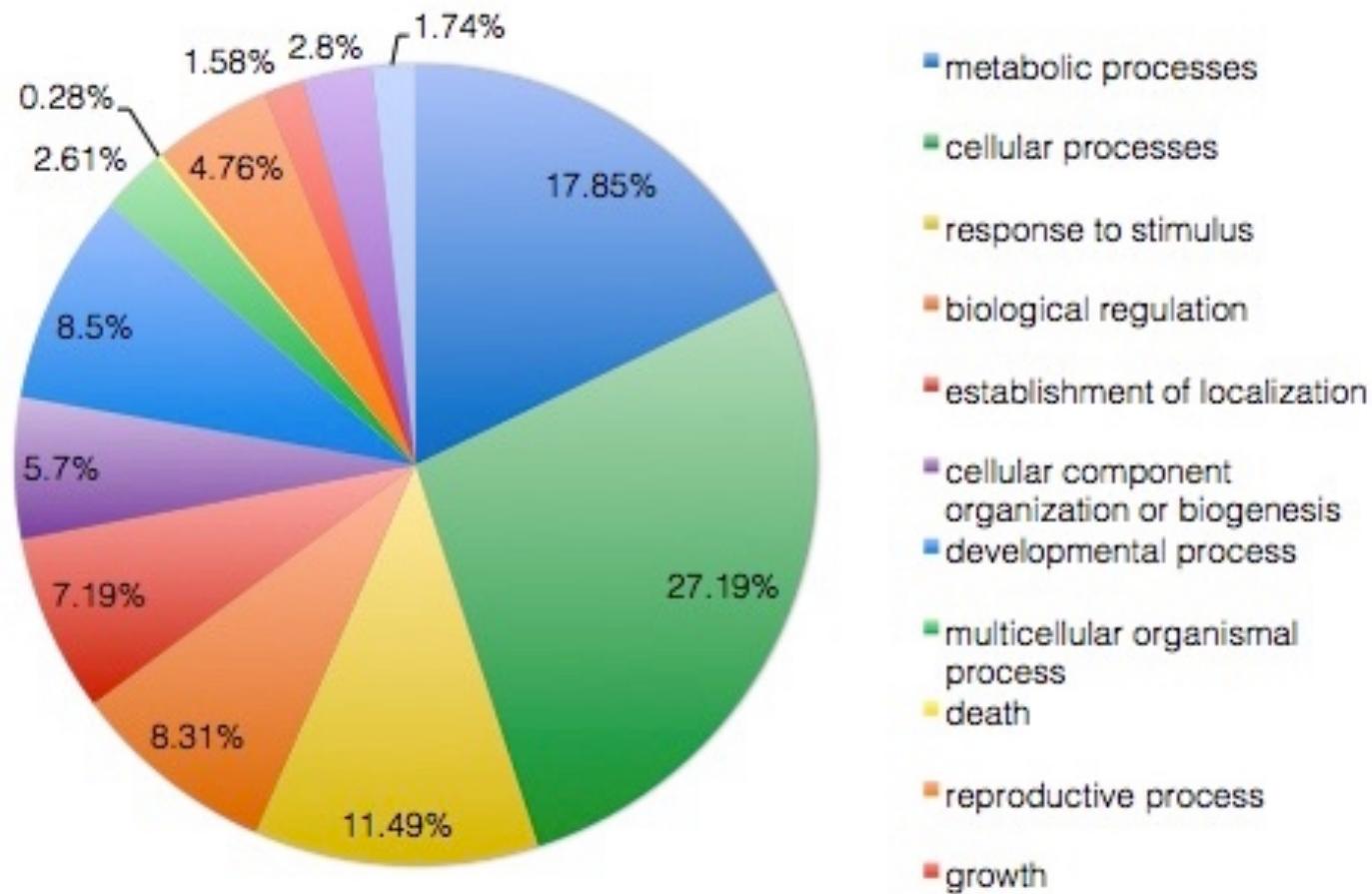
Epi-GBS in *Fallopia* among habitats

- 7,924 contigs
 - based on van Gurp et al 2016 *Nature Methods*
- 2,522 functional genes
 - Align to PacBio transcriptome
 - Includes stress response genes (ABI2) and chromatin remodelers (BSH, CHR1, CHR5)
- 325,333 methylation polymorphisms
 - 20% variation explained by population
- separate by habitat? (in progress)



EAGER: Developing epigenetic genotyping-by-sequencing in the non-model invasive Japanese knotweed with Van Gurp and Verhoeven, Netherlands Institute of Ecology (NIOO-KNAW)

Epi-GBS in *Fallopia* among habitats-Gene ontology



EAGER: Developing epigenetic genotyping-by-sequencing in the non-model invasive Japanese knotweed with Van Gorp and Verhoeven, Netherlands Institute of Ecology (NIOO-KNAW)

Iso-Seq™ Method: PacBio® Sequencing for Isoform Analysis

- Single-molecule observation
 - One read = one transcript
- Sequence transcript in full length
 - Most transcripts 1-5 kb
 - No assembly required
- No systematic bias
 - GC-rich, AT-rich, tandem repeats
- Knotweed
 - 50,435 annotated transcripts
 - 357 unannotated transcripts
 - High ploidy!



EAGER: Developing epigenetic genotyping-by-sequencing in the non-model invasive Japanese knotweed with Van Gurp and Verhoeven, Netherlands Institute of Ecology (NIOO-KNAW)

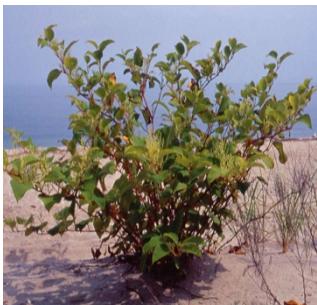
Genome function in *Fallopia*

- Are there genomic “hotspots” of variation that we miss with AFLP?
 - What are the functional targets of DNA methylation variation?
 - How does DNA methylation respond to environment? Is it functional?
-
- Increase power with epi-GBS protocol
→ Function by mapping to PacBio transcriptome
→ Reciprocal transplants in natural environments and genomics tools to monitor genome response

Response to complex environments



- Model plant *Arabidopsis* (proof of concept): How do diverse genotypes vary in gene expression in response to environment?



- How do epigenetic effects contribute to plant invasion (*Fallopia*)?



- Are there genetic, epigenetic and gene expression signatures of response to a novel stressor?



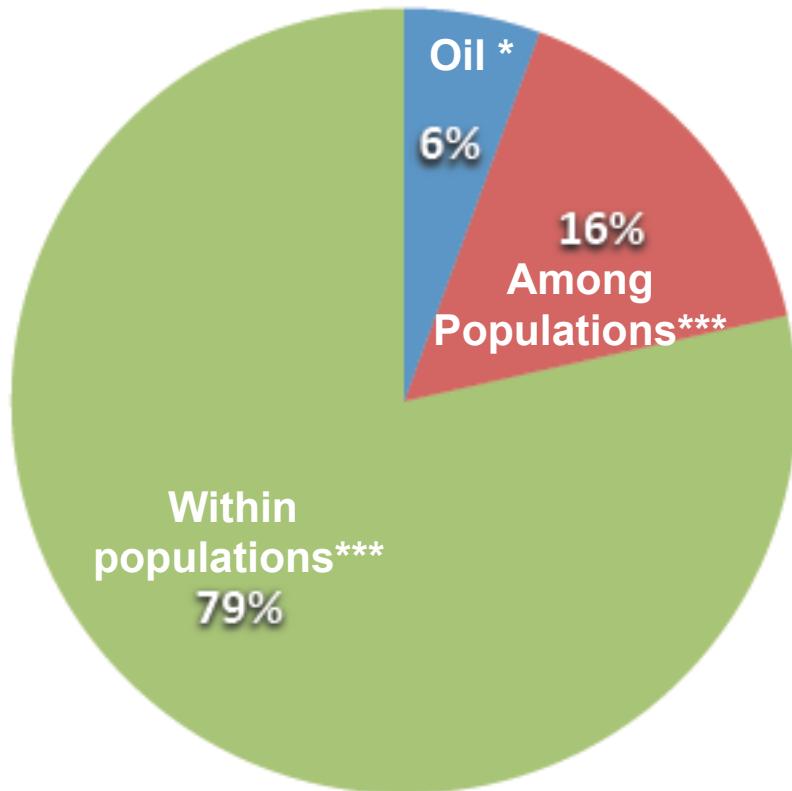
Genetic structure (AFLP) in oil exposed populations



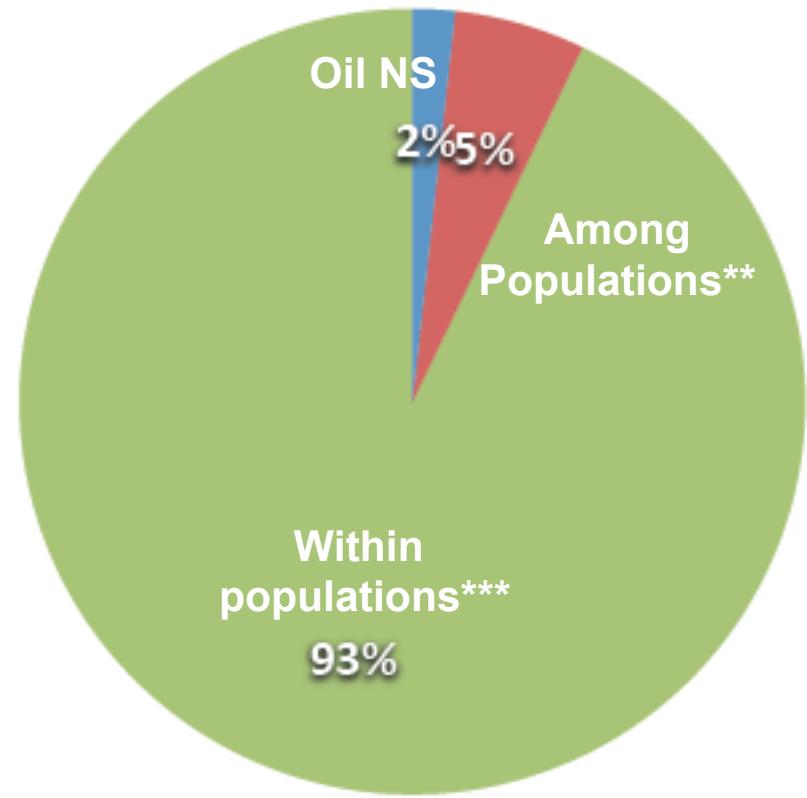
*3 populations
x 2 habitats (oil or no)
x 10 ramets*

$$K=2$$

Genetic & Epigenetic variation (AFLP) in response to oil contamination



AFLP (Genetic, 71 loci)

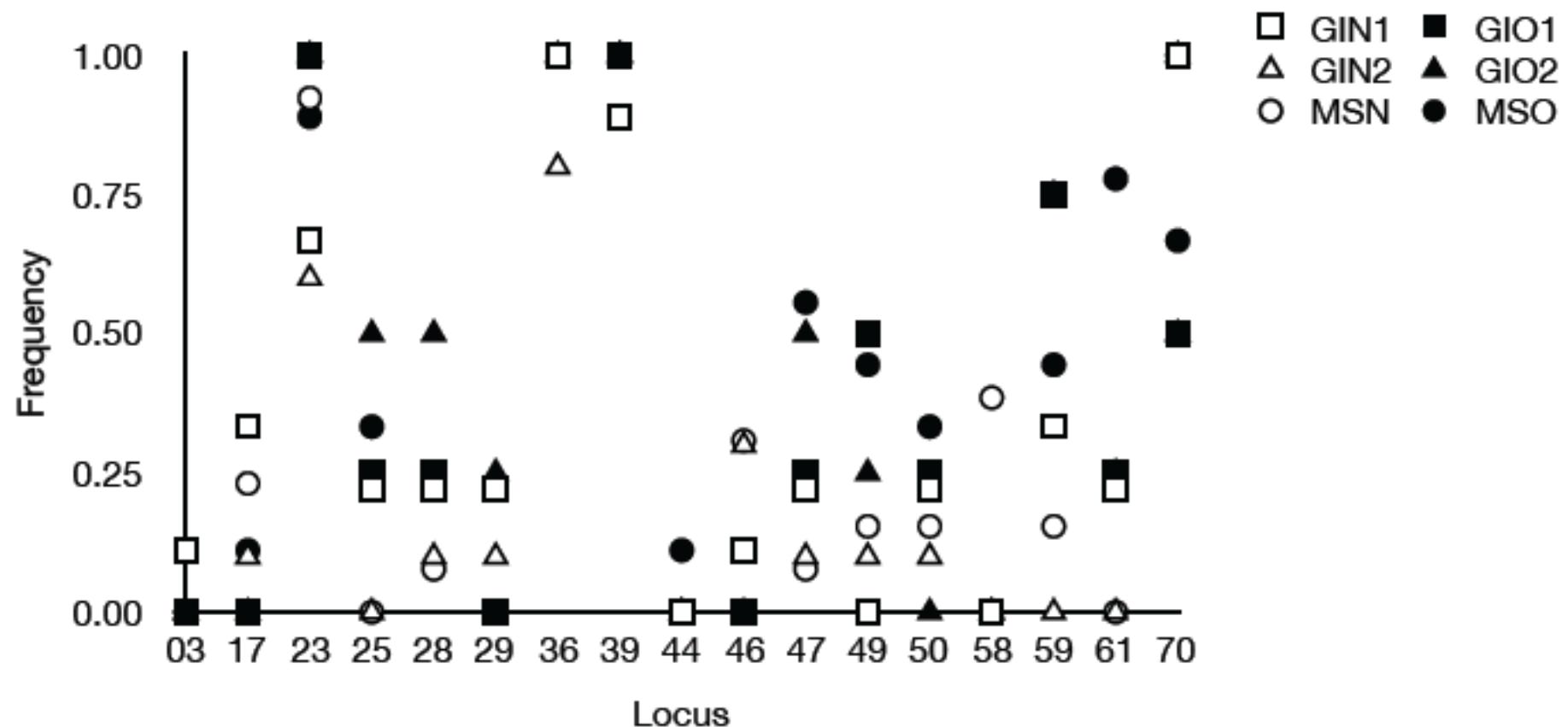


MS-AFLP (Epi-genetic, 39 loci)

3 populations x 2 habitats (oil or no) x 10 ramets

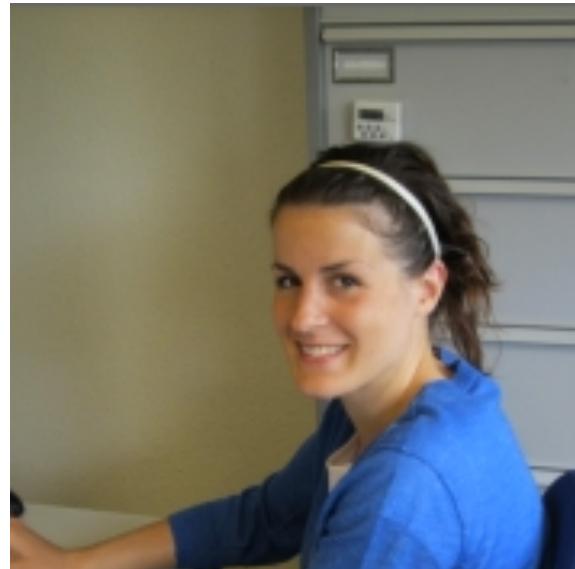
Robertson et al. in review

Habitat differentiation at epigenetic loci in response to oil





Malika Ainouche



Julie Ferreira de Carvalho



Armel Salmon

4x 44k Agilent microarray custom design (A. Salmon)

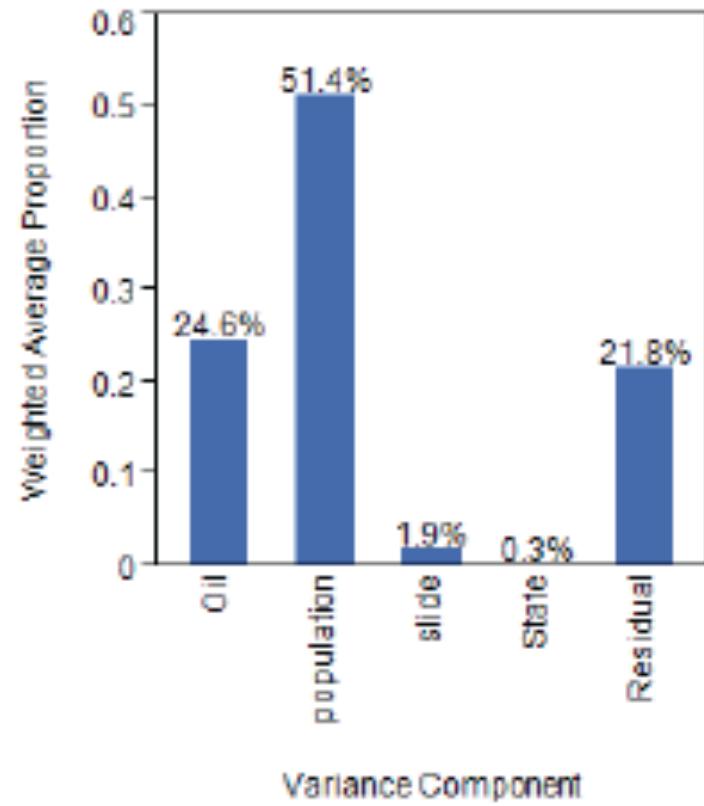
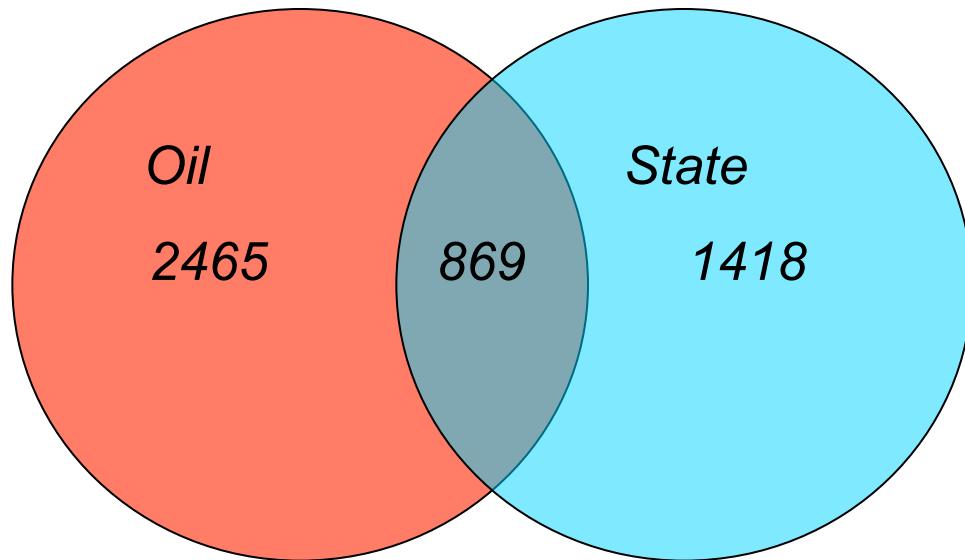


17049 60-mer probes (unigenes-16608 have a functional annotation- *Arabidopsis* & *Oryza*)

Designed from

- 9356 from *S. alterniflora* 454 EST assemblies
- 7170 from 5 *Spartina* species 454 EST co-assemblies
- 523 from *S. maritima* 454 EST assemblies

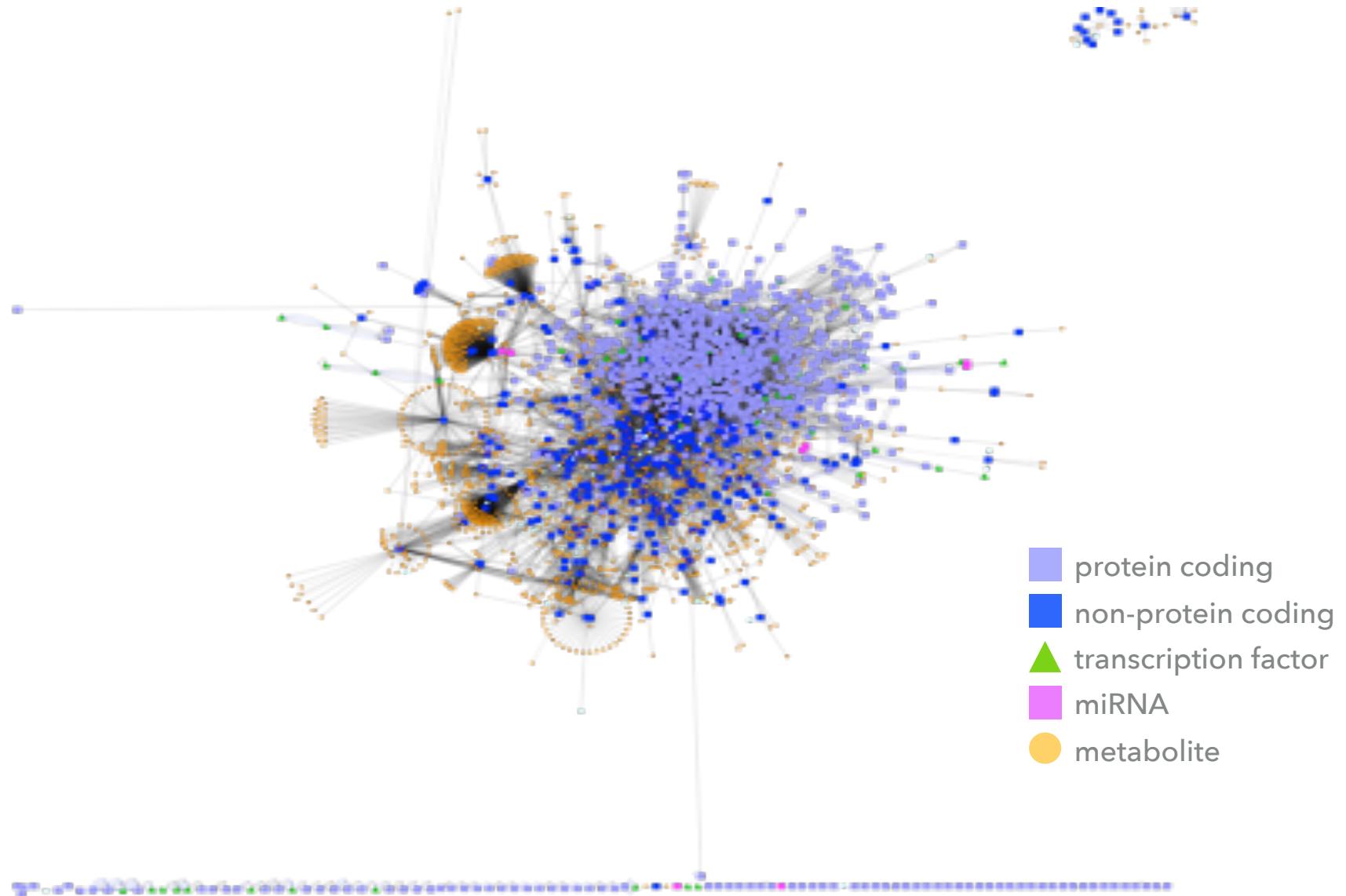
Spartina gene expression response to DWH oil spill



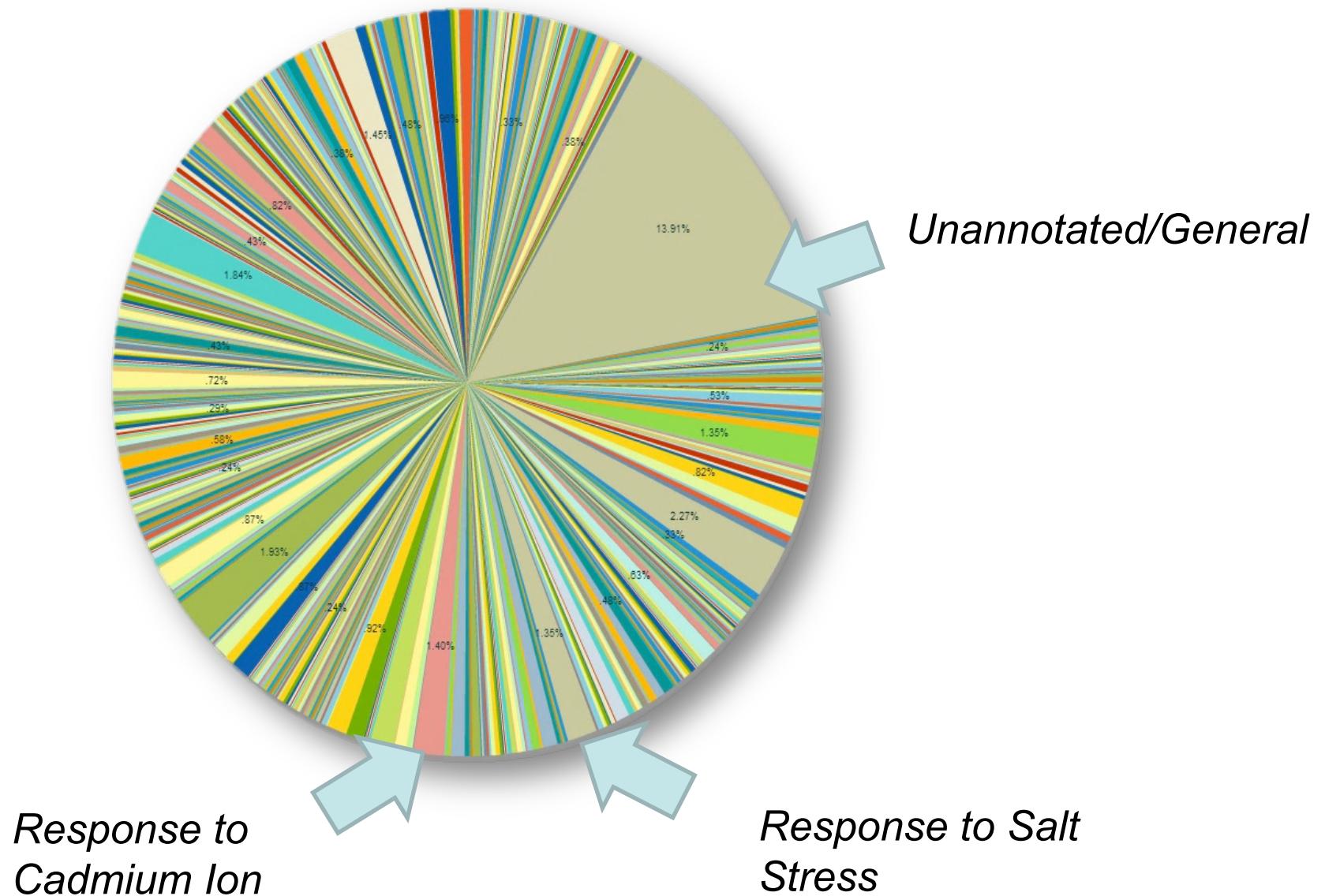
3 populations x 2 habitats (oil or no) x 3 pools of 3 ramets (leaf)

Alvarez et al. in progress

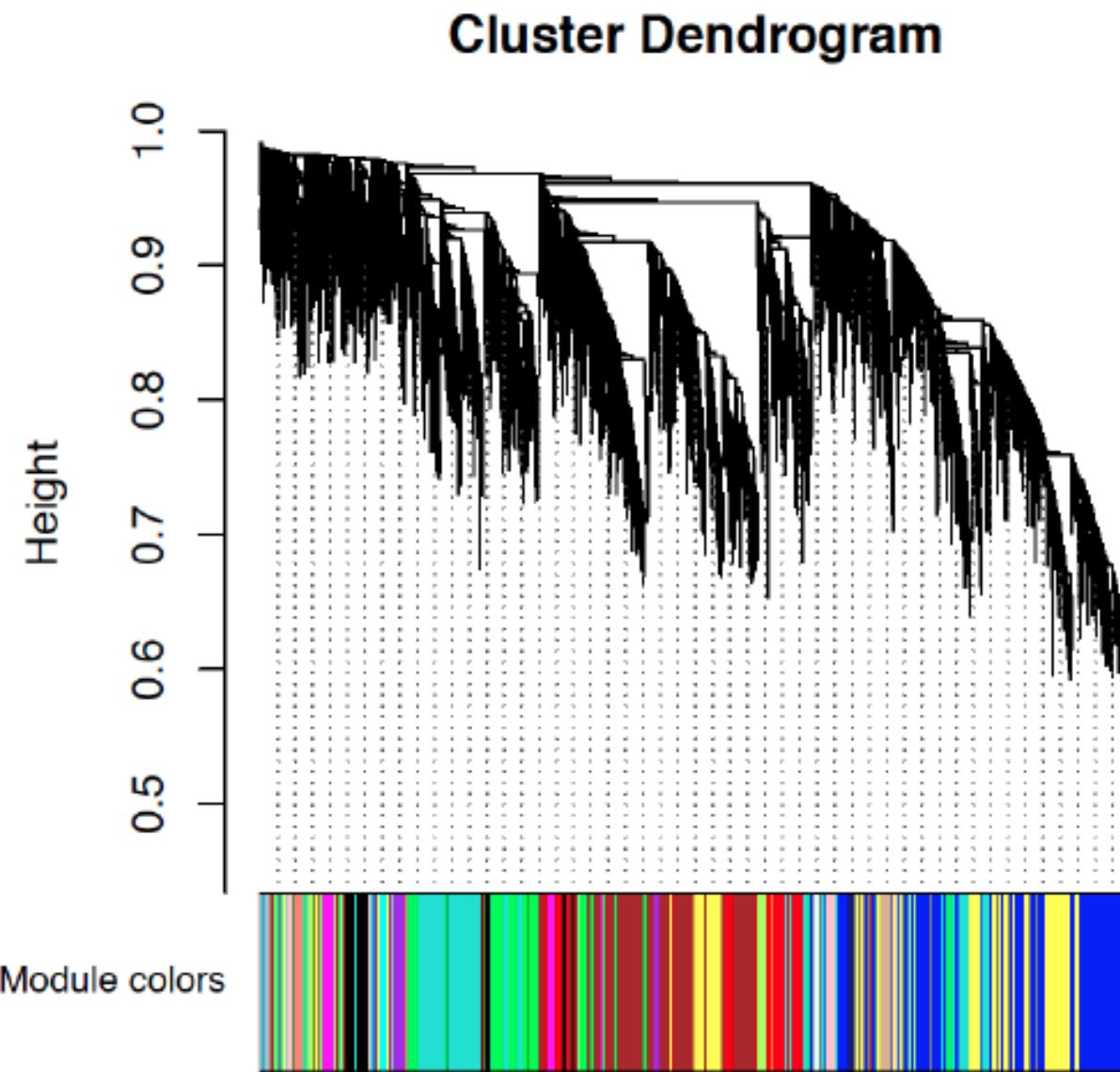
Oil responsive genes - Virtual Plant (Katari et al. 2010)



Gene Ontology – Biological Processes in oil responsive genes



Expression networks– WGCNA

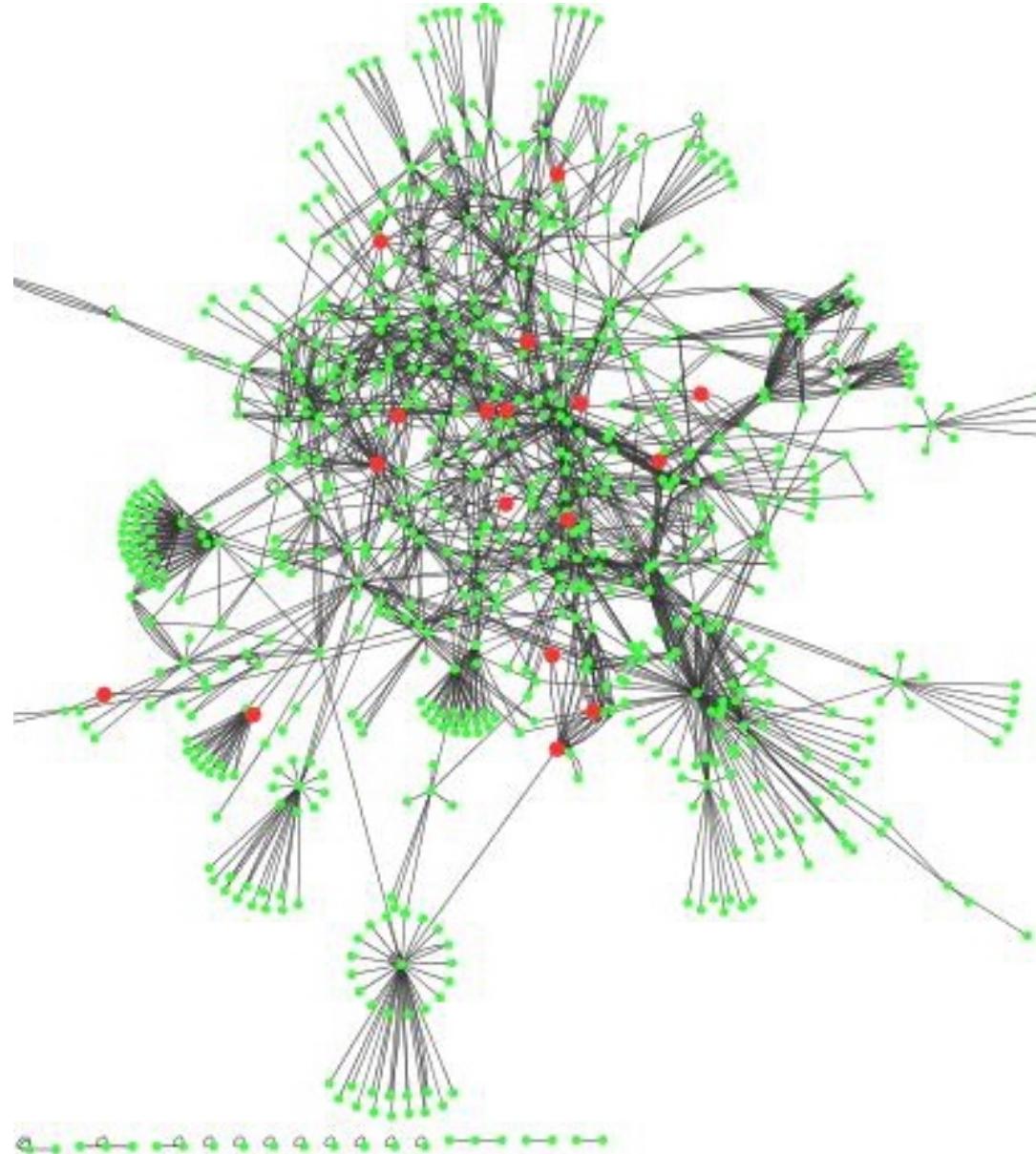


Total Gene Network – Response to Cadmium Ion

Shown in Cytoscape

Green shows genes known to respond to cadmium ion in *A. thaliana* (330)

Red shows genes that responded to DWH in *S. alterniflora* (29)



Phenotyping insertion lines of *Brachypodium distachyon* under crude oil stress

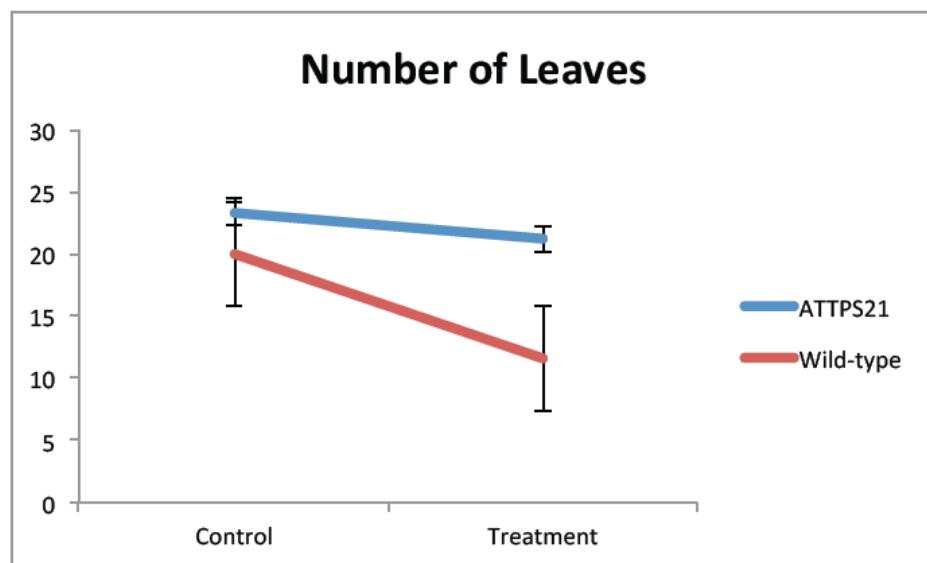


- *Small stature, short lifecycle*
- *Small, tractable genome (270Mbp), diploid*
- *Several knockout lines available*
- *Each knockout line will be exposed to crude oil stress under controlled conditions*
- *Quantifying effects on physiology and fitness-related traits and confirm function in response to oil*
- *Divergence from Spartina ~40my*

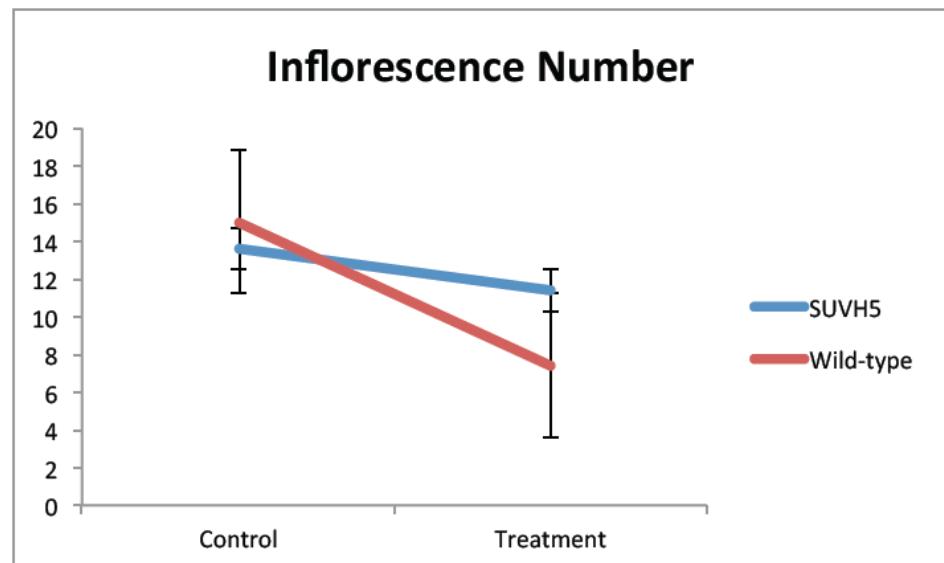
T-DNA insertion lines

<i>Spartina alterniflora</i> gene	<i>Bd</i> gene	Gene Name	<i>S. alterniflora</i> Expression due to Oil	Modification type	Justification
S_5sp_contig45211	Bradi3g07 730	KCS11	Increased	Overexpression	Highly connected gene (107 connections)
S_alt_contig04438_RC	Bradi1g62 540	ATTPS21	Increased	Overexpression	Highly connected gene (44 connections)
S_mar_contig02490_RC	Bradi3g35 330	SUVH5	Decreased	Overexpression	Regulatory gene of interest
S_alt_contig08149	Bradi1g68 290	ATMCB1, MBP1	Decreased	Knockout	Highly connected gene (57 connections)

T-DNA insertion lines

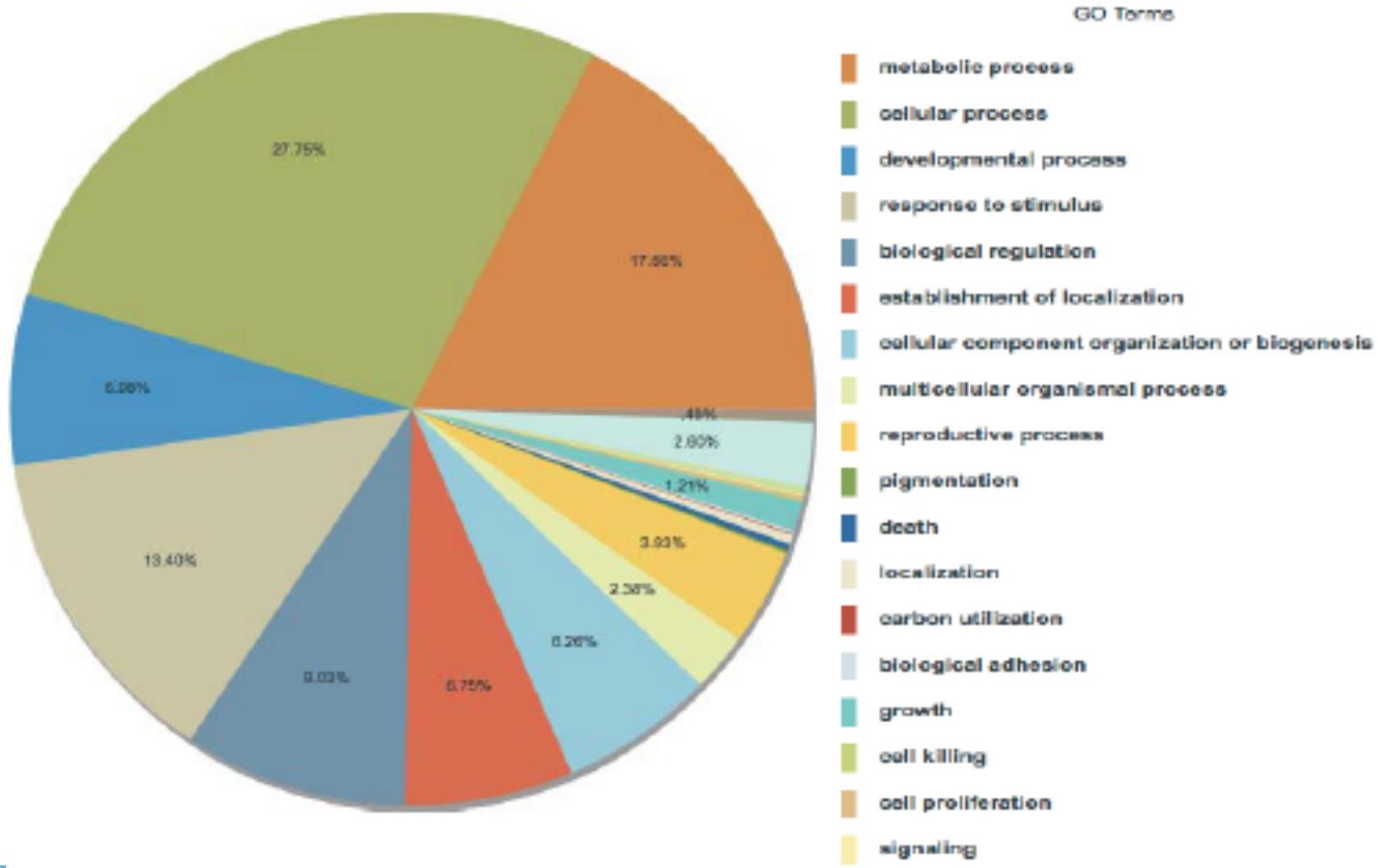


Treatment: * Genotype: NS Treatment by Genotype: *



Treatment: ** Genotype: NS Treatment by Genotype: *

Epi-GBS in *Spartina*- Gene ontology

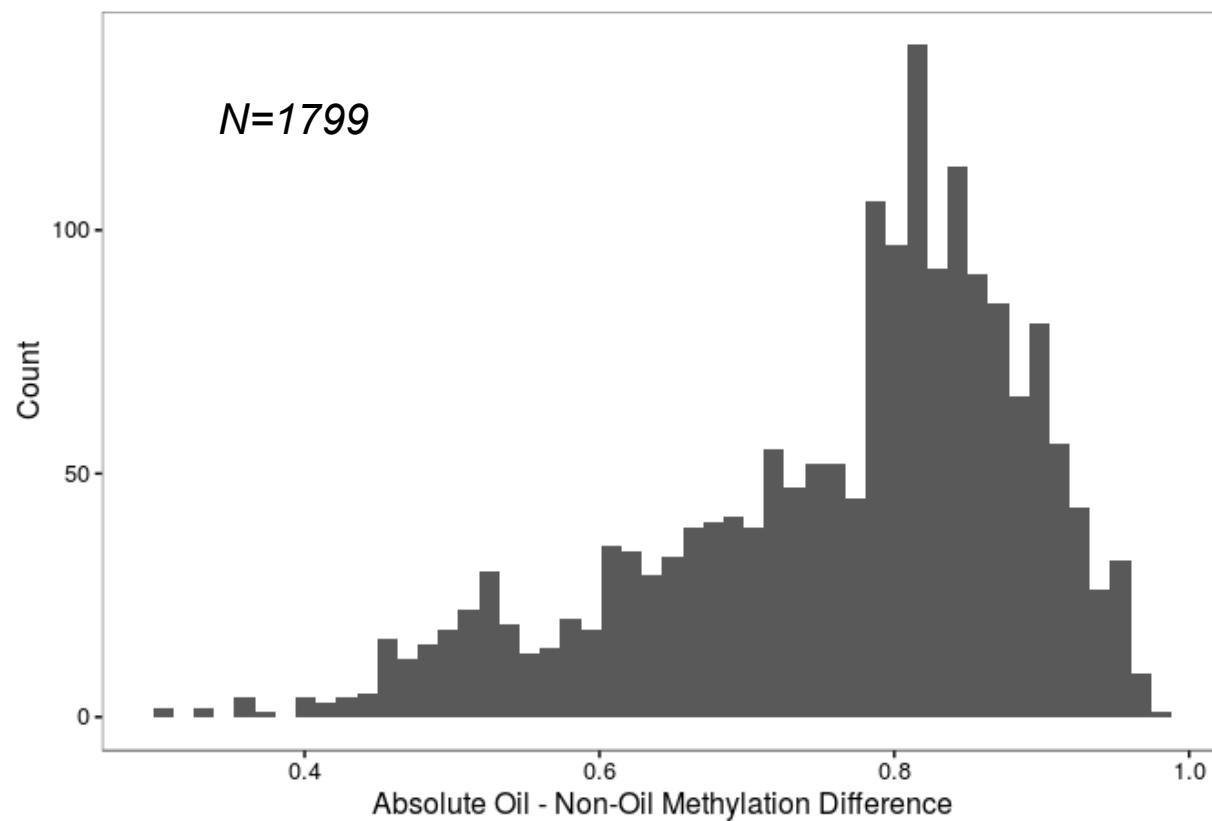


EAGER: Developing epigenetic genotyping-by-sequencing in the non-model invasive Japanese knotweed with Van Gorp and Verhoeven, Netherlands Institute of Ecology (NIOO-KNAW)

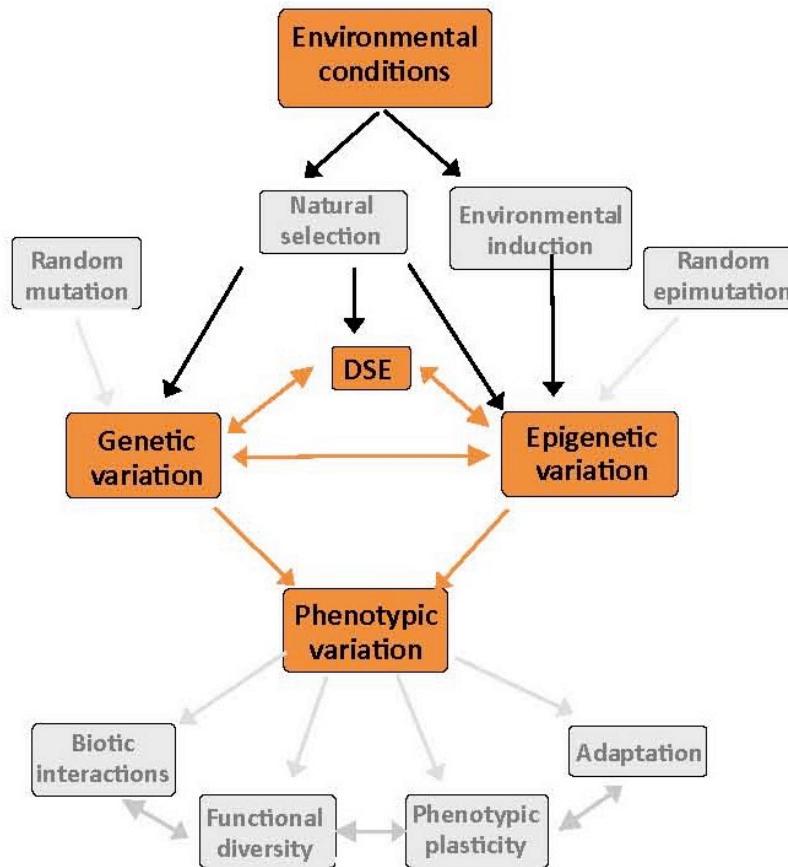
EpiGBS analysis with R package DSS (Bioconductor)

- 2.1 million sites for all contexts (CG, CHH,CHG, 575k after filtering)
- All context DMP = 1799
- CG only = 899
- DMRs = 17
 - Uncharacterized protein in Oryza
 - ARF guanine-nucleotide exchange factor GNOM in Zea mays
 - E3 ubiquitin-protein ligase XB3 in Zea mays

1799 loci differentiate Oil and non-Oil sites



Epi-GBS in non-model plants among habitats



*Collaborative Research: Effects of genetic diversity, epigenetic change, and root-associated fungal colonization on trait variation in the foundation plant *Spartina alterniflora* with Hughes (Northeastern University) & Gehring (N Arizona University)*

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Office of the Chair



Enhancing the biology curriculum at University of South Florida and University of Rennes with ecological epigenetics: Merging ecology and epigenomics approaches to understand plant invasion.



Malika Ainouche



Armel Salmon

Genome function in *Spartina*

- Is there novel functionality (**unannotated genes**/ duplicated genes) within networks that explains ecology?
 - What are the functional targets of epigenetic variation?
 - How do the epigenetic marks respond to environment?
- Increase power with epi-GBS protocol
- 36,000 fragments with 2500 functional genes
- stress response genes (SOS2) and chromatin remodelers (HAC4,5, HAM 1,2, SUVH9, CHR12)
- Reciprocal transplants in natural environments and use genomics tools to monitor genome response

Implications for understanding evolution by natural selection

- Natural selection acts on heritable trait variation
- Problem of translation from genotype to phenotype
- Requires understanding how different components of the genome interact (TF, regulatory, coding, TEs)
- Requires an understanding of response to complex environments
- Combination of studies of model and non-model systems in natural environments will be insightful

Acknowledgements

- Larry Gottschamer and babies Dorothy and John
- Aaron Schrey, Christy Foust, Mariano Alvarez, Marta Roberston, Holly Kilvitis
- Armel Salmon, Julie Ferreira de Carvalho, Malika Ainouche
- Oliver Bossdorf, Adrienne Nicotra, Massimo Pigliucci, Aaron Schrey, Mark van Kleunen, Michael Purugganan, Eric Richards, Koen Verhoeven, Jonathan Wendel for discussions about epigenetics and evolution



Funding:

- NYSea Grant, SUNY Research Foundation, USF

UMR CNRS-Univ. Rennes 1 ECOBIO

