



# Why study plants?

Studying plants helps keeping us fed, healthy, sheltered, clothed, and happy.



Production must increase by 70% the next 40y

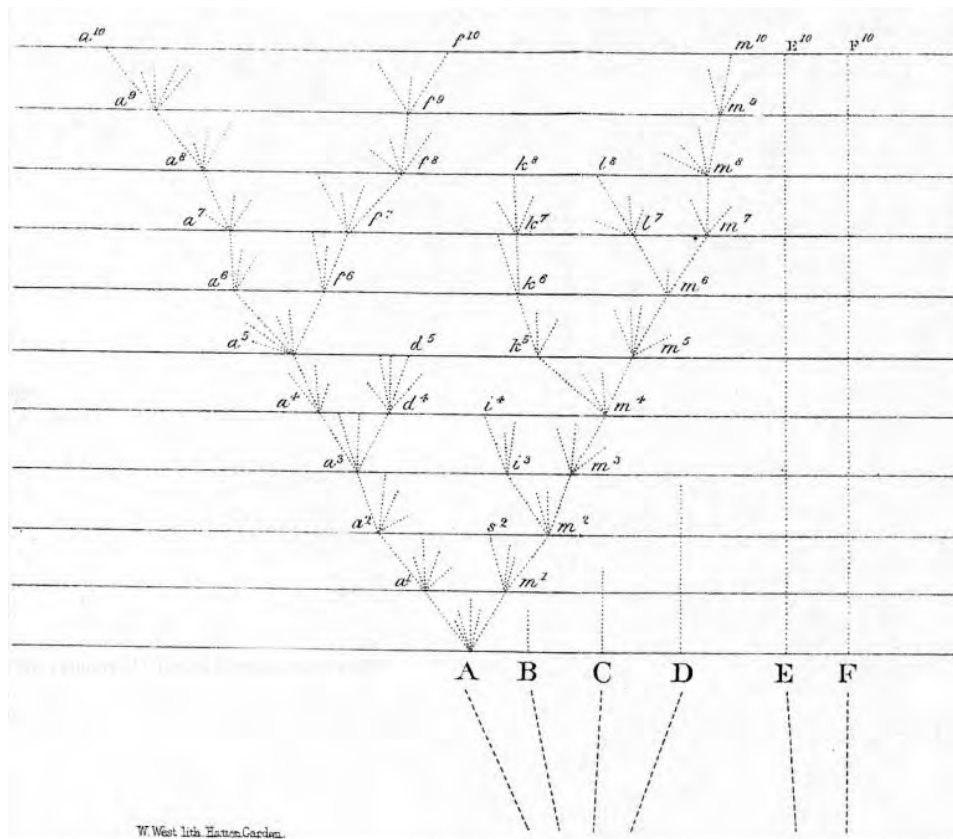


Yield increasingly challenged by global warming (drought)



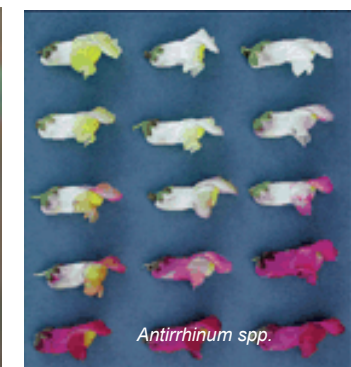
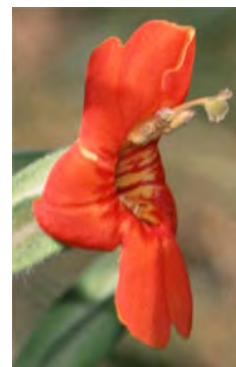
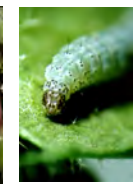
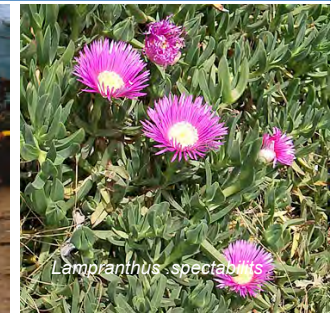
TTPB, Plant Cell

# Lineage birth-and-death



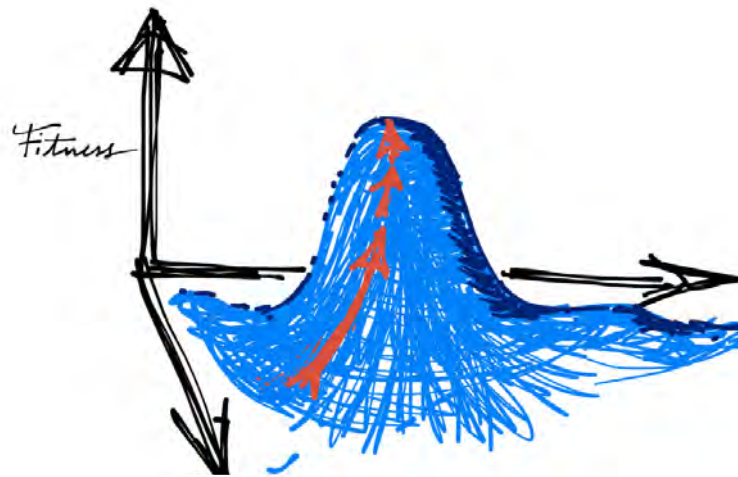
85 lineages  
3 survived

The Origin of species, 1859



# Which molecular changes are important for adaptative evolution?

**NATURAL SELECTION ....**



and/or ...



**...DRIFT**

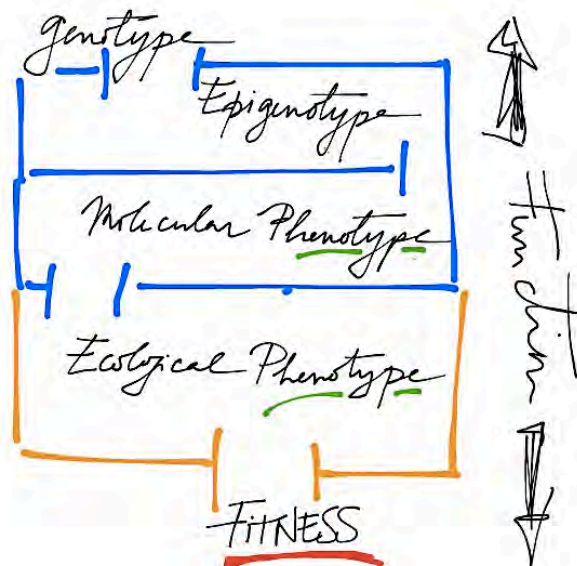
# How to elucidate adaptation at the molecular level ?

Single-gene approaches



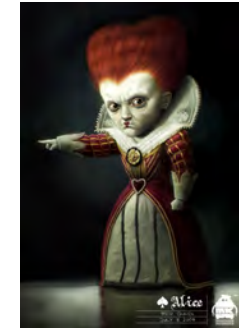
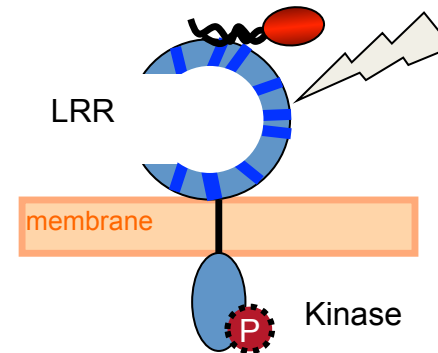
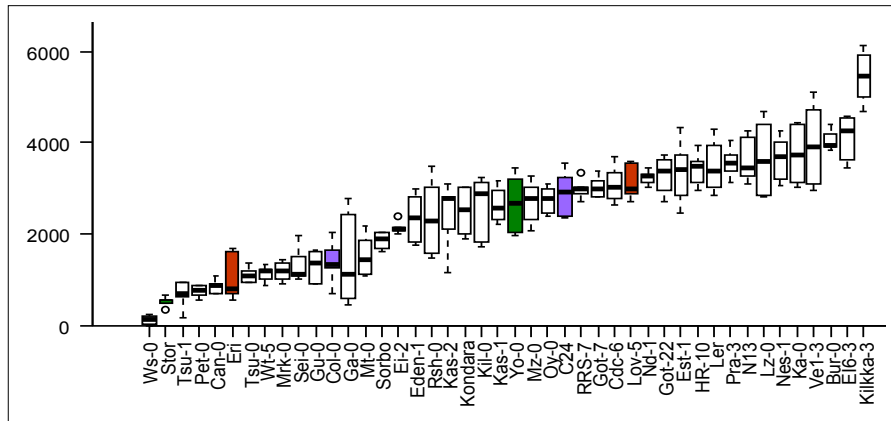
molecular genetics  
ecological genetics  
population genetics  
phylogeography  
evol. genomics

Define Ecological function



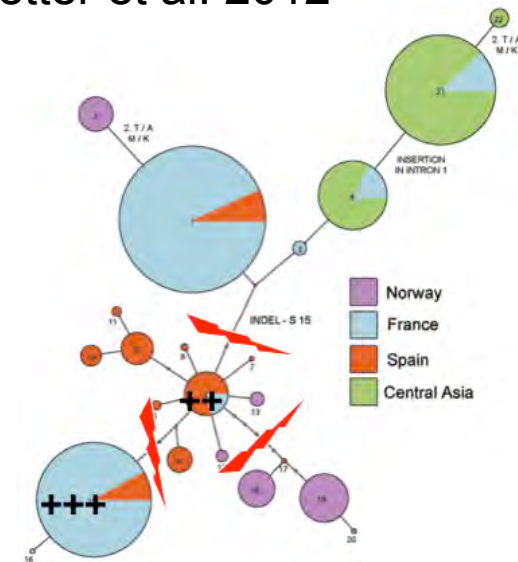
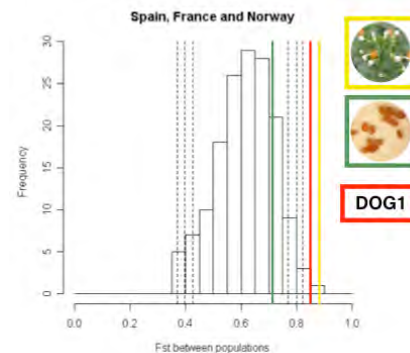
# Single genes can explain adaptive dynamics

**Basal immunity:**  
selective sweep and variation at the *FLS2* locus



Vetter et al. 2012

**Dormancy:**  
*DOG1* multiple alleles involved in local adaptation



Kronholm et al. 2012

# How to elucidate adaptation at the molecular level ?

Single-gene approaches

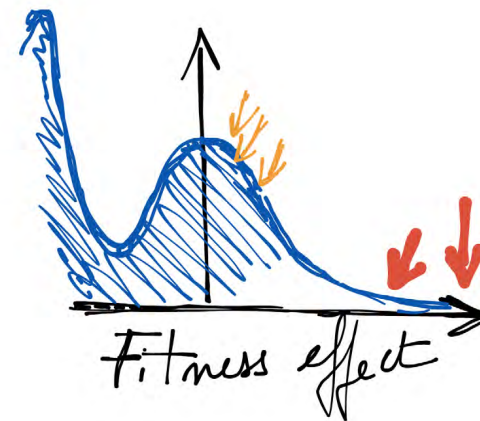


molecular genetics  
ecological genetics  
population genetics  
phylogeography  
evol. genomics

Define Ecological function

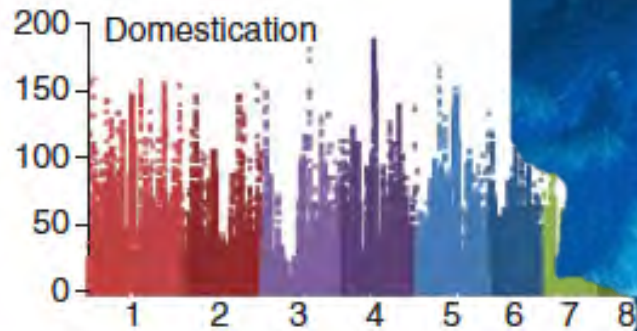


Study the whole spectrum of mutations



# Which adaptive mutations can we see?

Search for Genomic outliers



Hufford et al. 2012

Hard sweeps

Search for adaptive QTLs



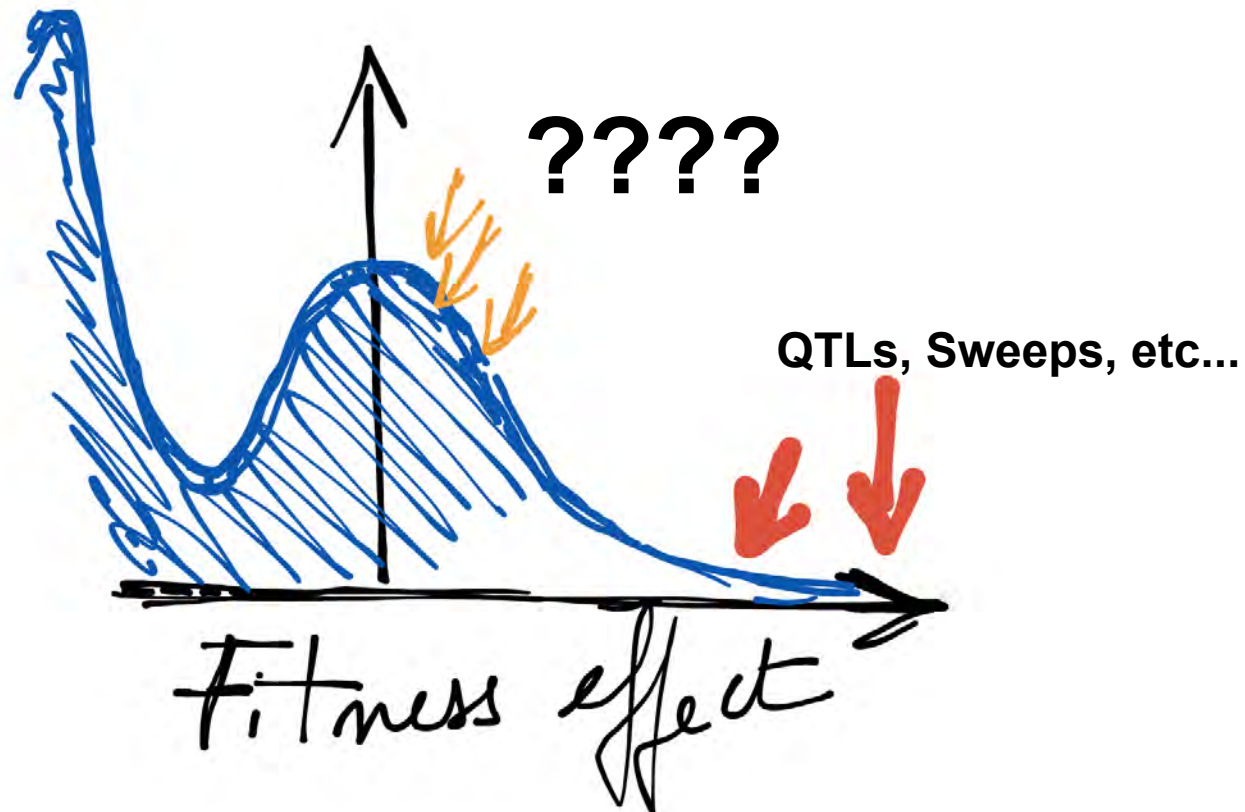
Prasad et al. 2012

Large effect QTLs



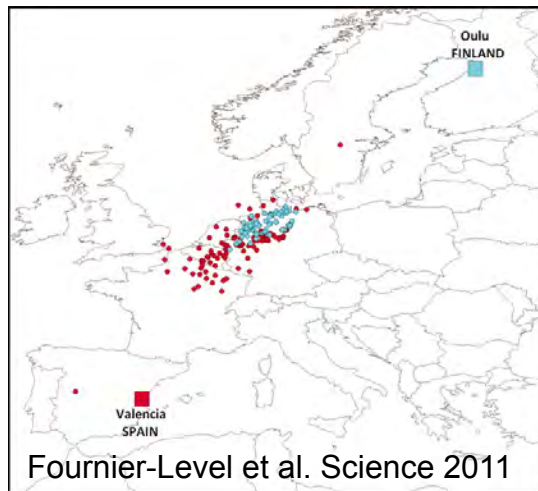
# Small effect mutations predominate in adaptation!

How to study the biology of small effect mutations

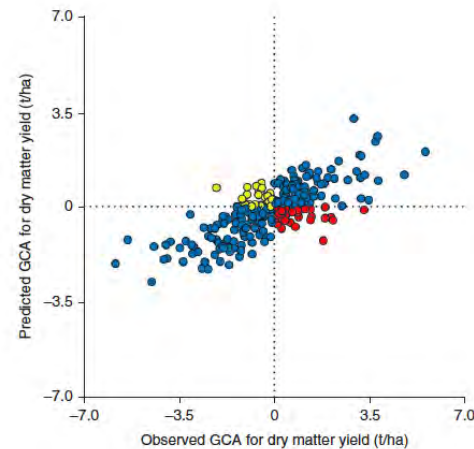


# Small effect mutations matter

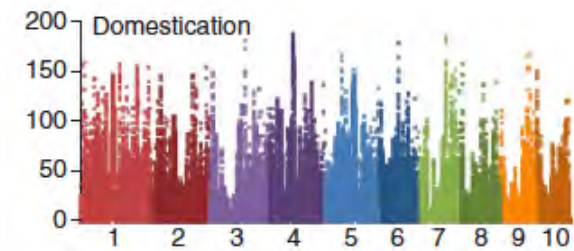
A large number of SNPs associate with fitness



GWAS on fitness



Genome-wide predictions

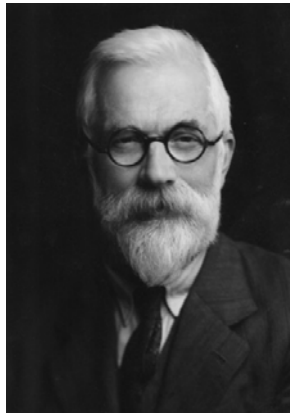


Genome Scans

**Infinitesimal effect alleles add up for adaptation**

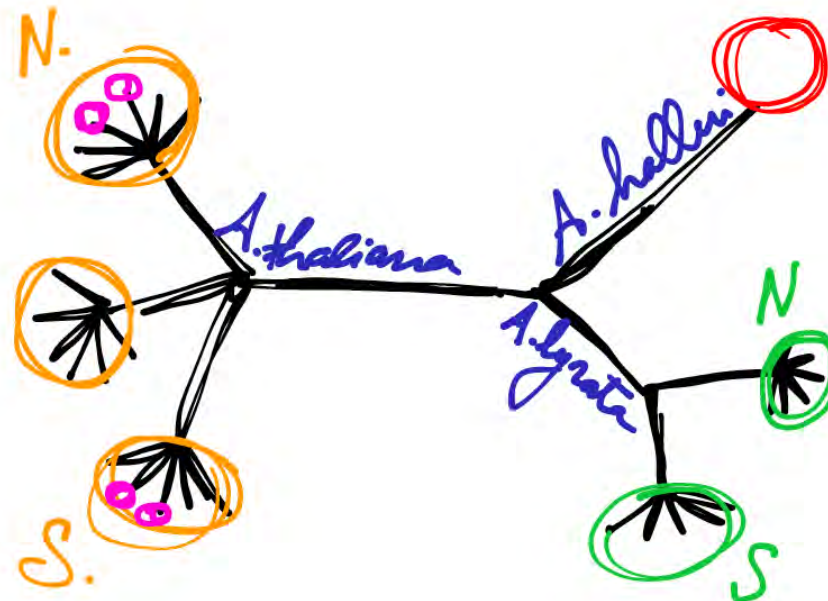
# What do small effect mutations do?

Fisher's infinitesimal model is valid



R.A. Fisher

Adaptation proceeds via many mutations of small effect



The *Arabidopsis* genus

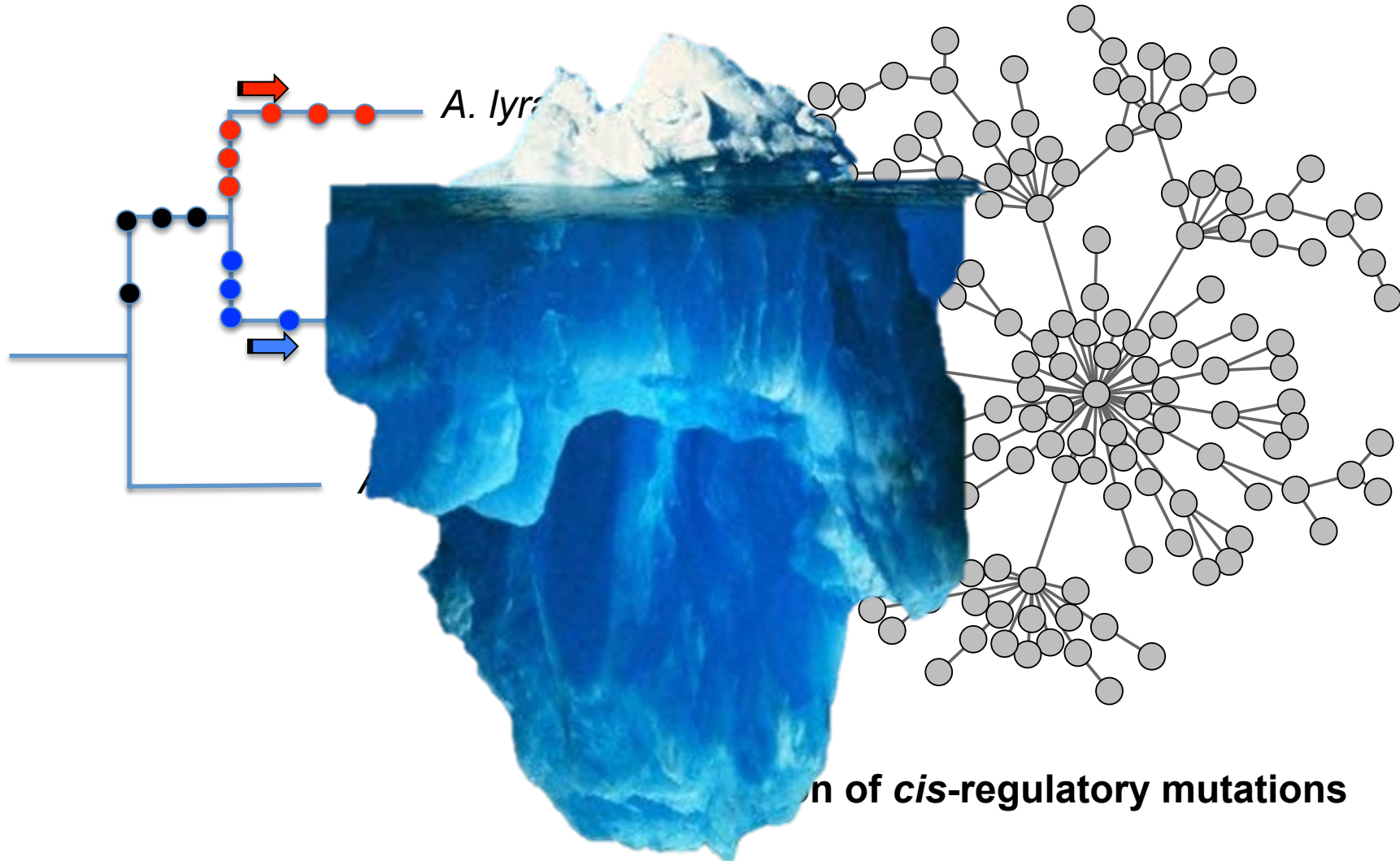
what do *infinitesimal* mutations do?  
Study the *collective* properties of mutations

# OUTLINE

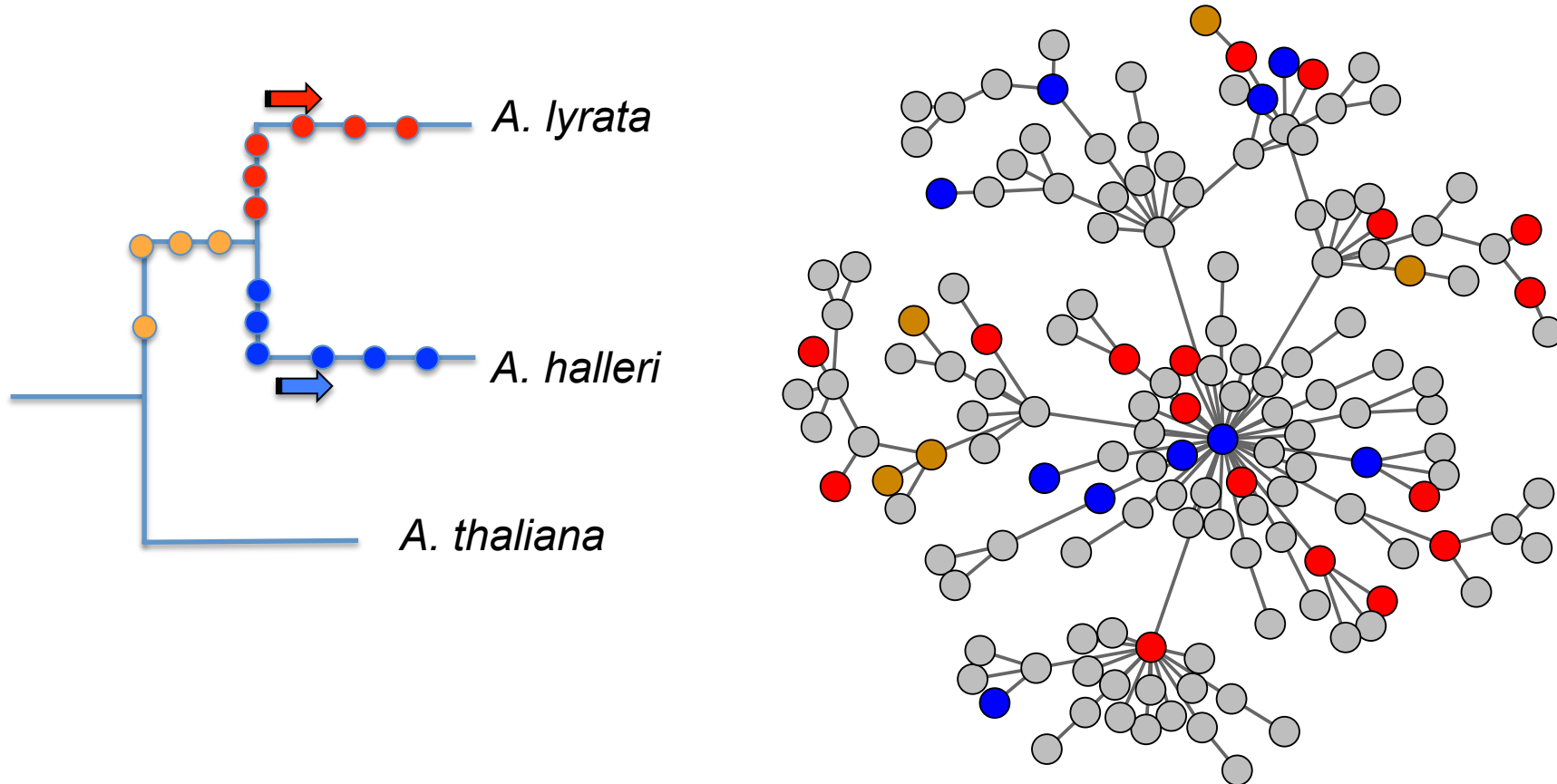
1. How to track polygenic selection?
2. The signature of Polygenic selection in the *Arabidopsis* genus
3. Biological interpretation
4. Perspectives



# Mutations cluster in targets of polygenic selection

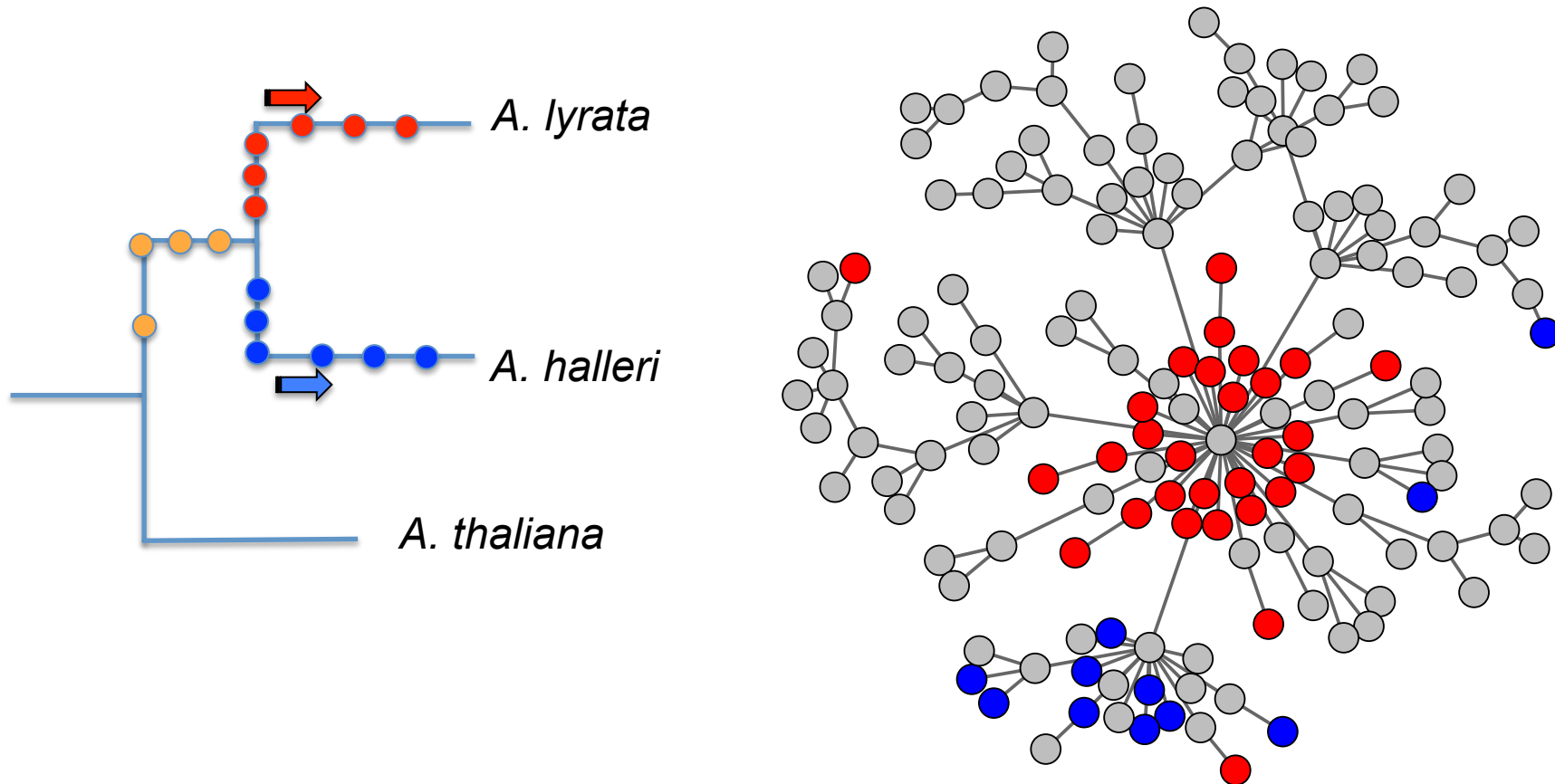


# No selection: random distribution of mutations



Distribution of *cis*-regulatory mutations

# Mutations cluster in targets of polygenic selection

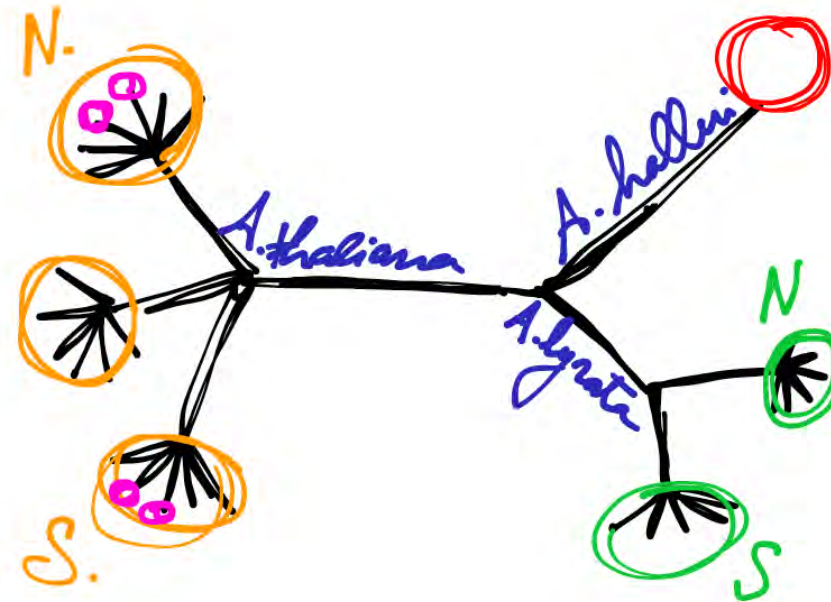


Distribution of *cis*-regulatory mutations

# Which small effect mutations?

## Small effect mutations:

- ✓ many
- ✓ functional
- ✓ small effect on fitness
- ✓ easy to type



The *Arabidopsis* genus

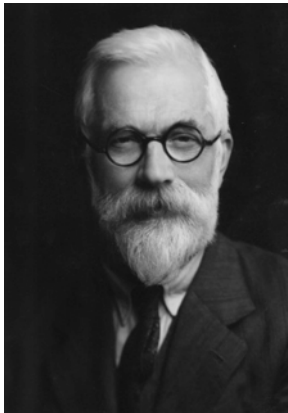
→ *Cis*-regulatory mutations?





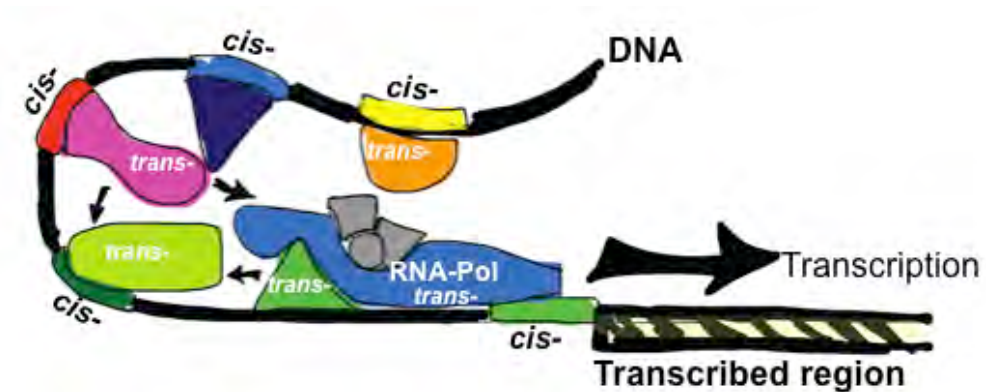
# The (biological) importance of small effect mutations

de Meaux et al. 2005, 2006,  
He et al. 2012a, 2012b,  
de Meaux et al. in prep.



R.A. Fisher

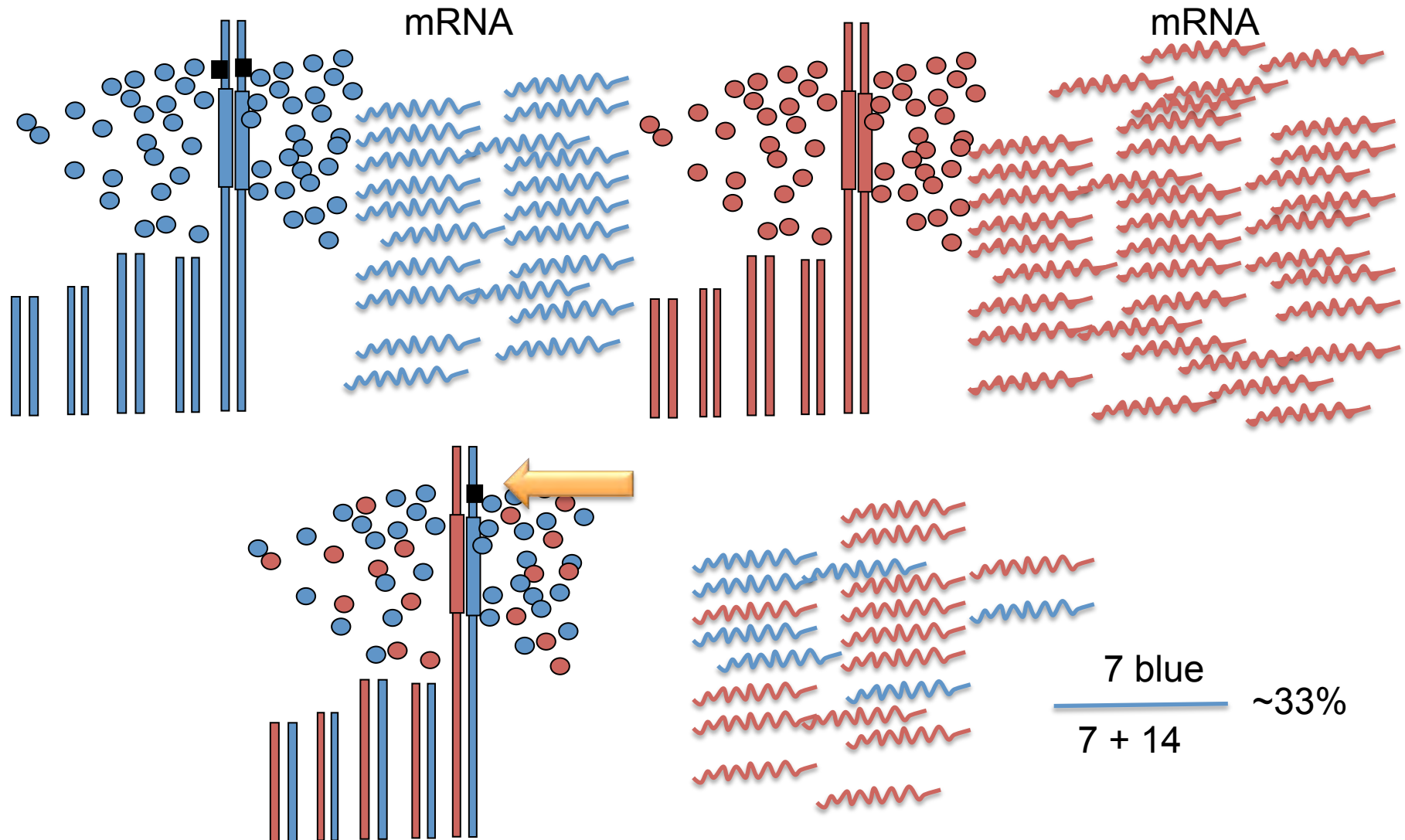
Adaptation is the sum of  
small effect mutations



## **cis-regulatory mutations**

- ❖ significant molecular phenotype
- ❖ active and environment responsive
- ❖ occur frequently
- ❖ most have small effects on fitness
- ❖ can be identified in crosses

# Cis-acting mutations are identified in crosses



# *Ecological diversity in the Arabidopsis genus*



- broad species range

- heavy-metal tolerant and hyperaccumulator

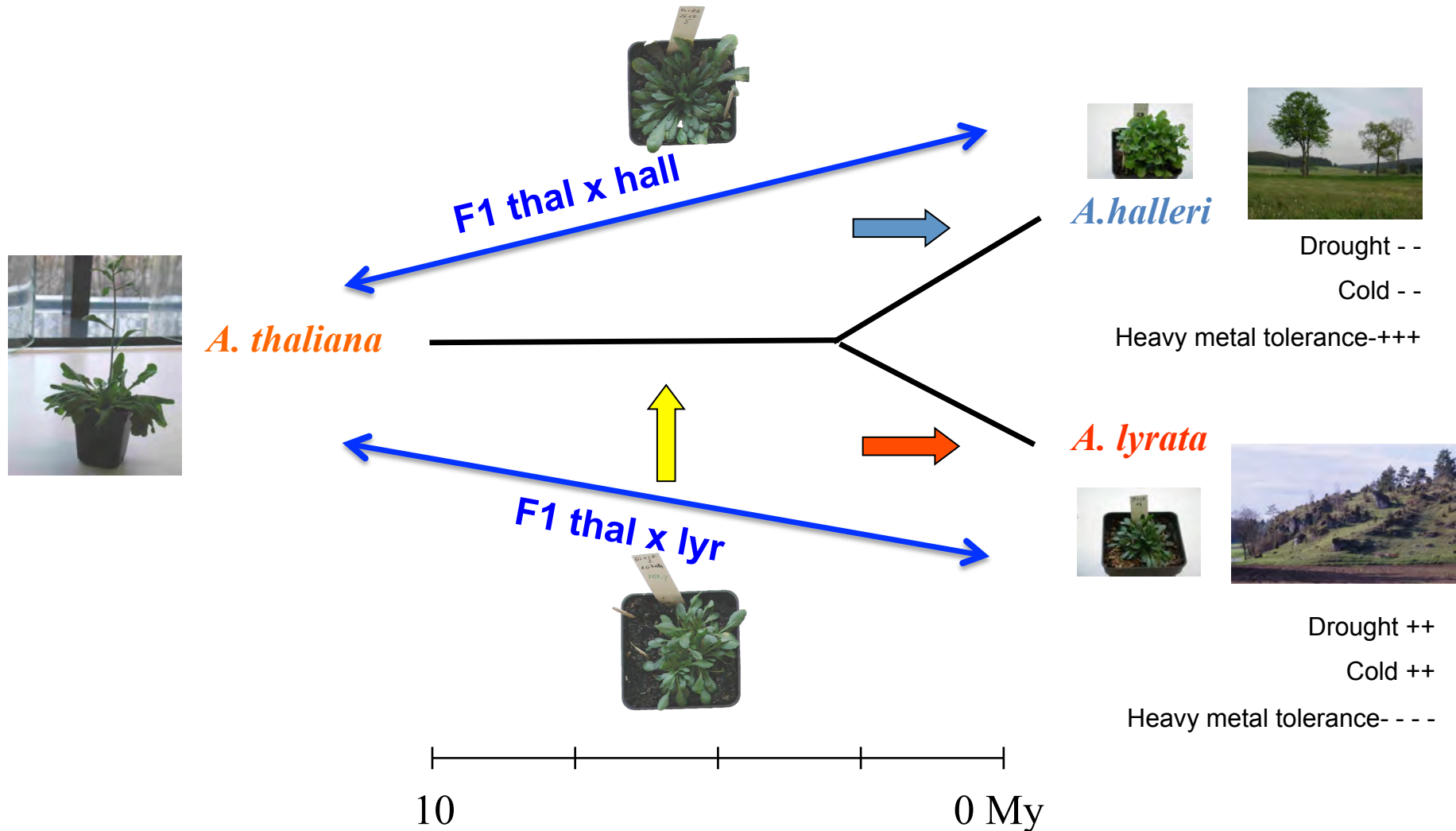
- cold and drought tolerance ?



## *Question*

Which molecular system were involved in adaptive divergence between species?

*cis-regulatory change in phylogenetic context*  
=> define derived changes



# *Distribution of cis-regulatory variation in two hybrid types under two stresses*

thaliana x lyrata

Col-0 x MN47



thaliana x halleri

Col-0 x hall2



## RNA-seq

- ✓ Control
- ✓ Exposed to 4°C, 1h
- ✓ Dehydration at room temperature, 1 h
  
- ✓ 3 replicates
- ✓ Paired-end read sequencing
- ✓ 13.9 to 160 M uniquely mapped reads

# OUTLINE

1. How to track polygenic selection?
2. The signature of Polygenic selection in the *Arabidopsis* genus
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Photo MPI Tübingen



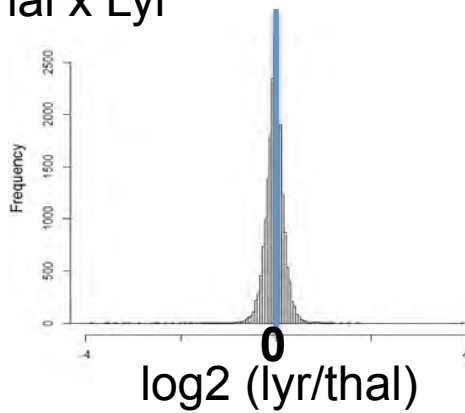
Photo Univ. Oulu



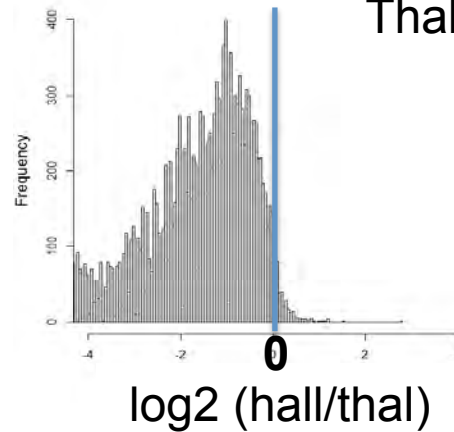
# Mapping bias need to be accurately corrected

## Direct mapping of hybrid DNA

Thal x Lyr

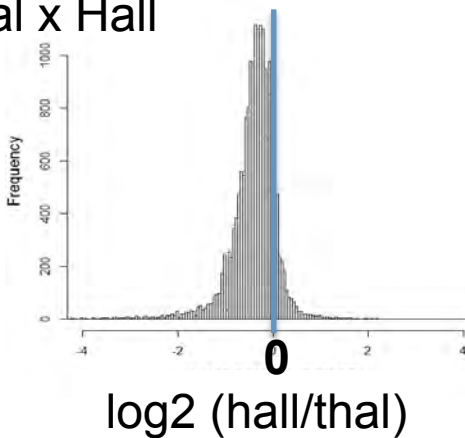


Thal x Hall



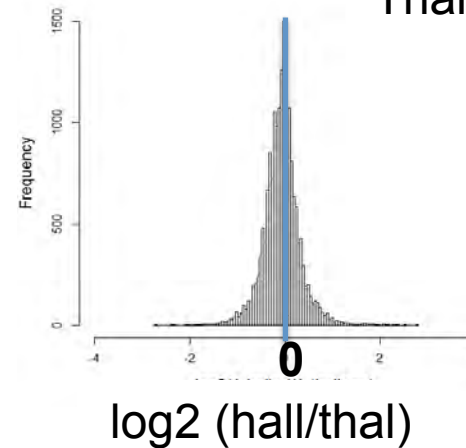
## Remapping of chopped reads

Thal x Hall



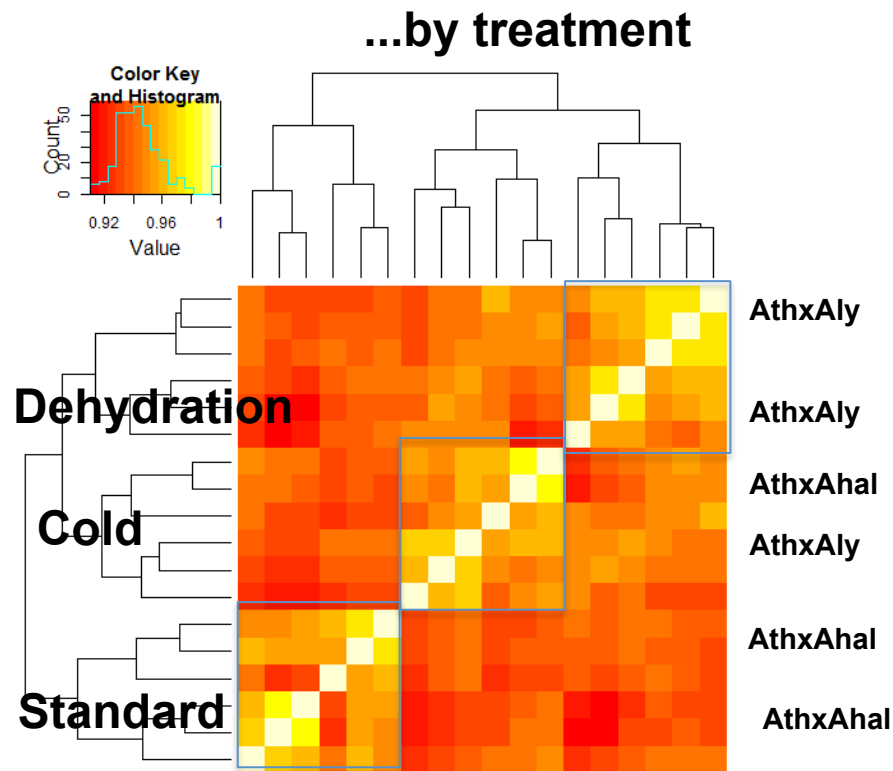
## Exclusion of highly divergent regions

Thal x Hall



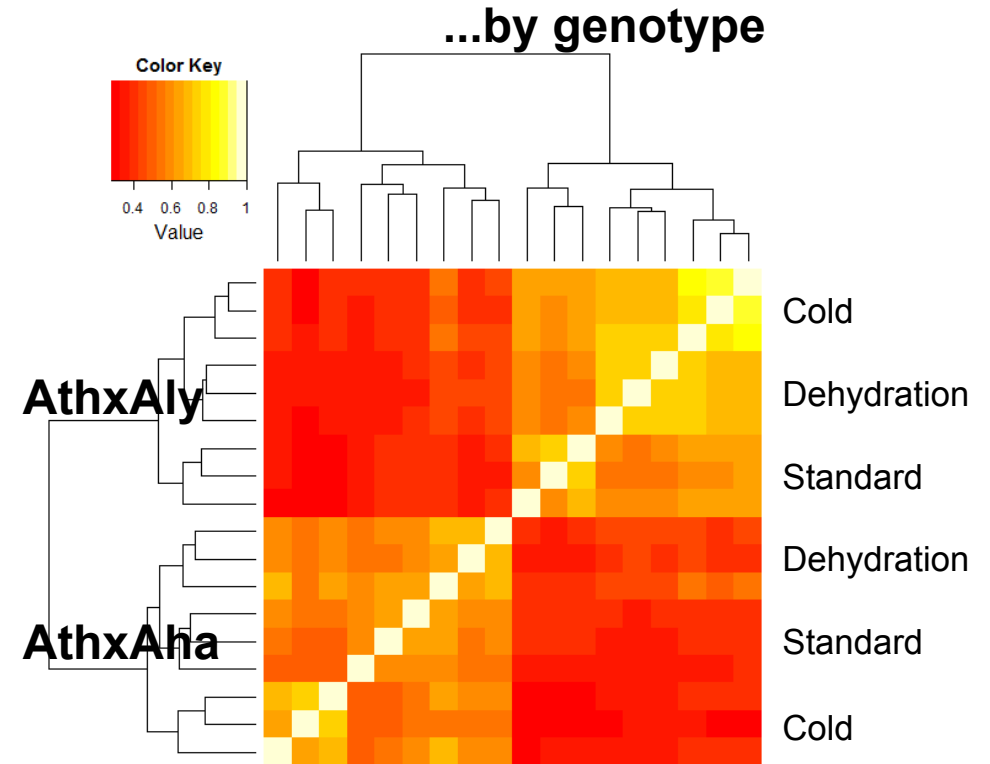


# Samples cluster by....



Correlation of expression levels (FPKM)

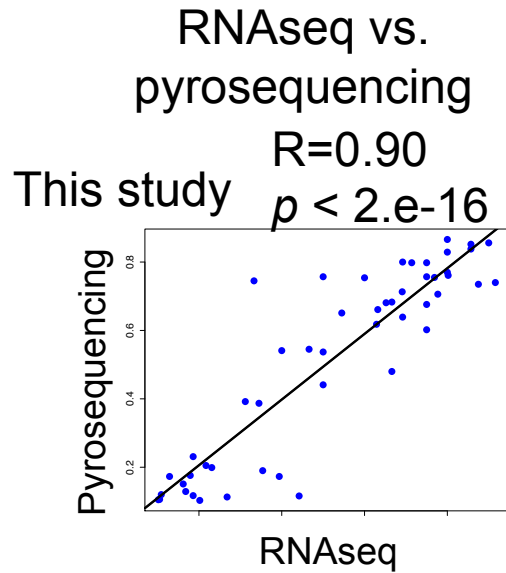
Plasticity is conserved between hybrids



Correlation of allelic ratio (log2)

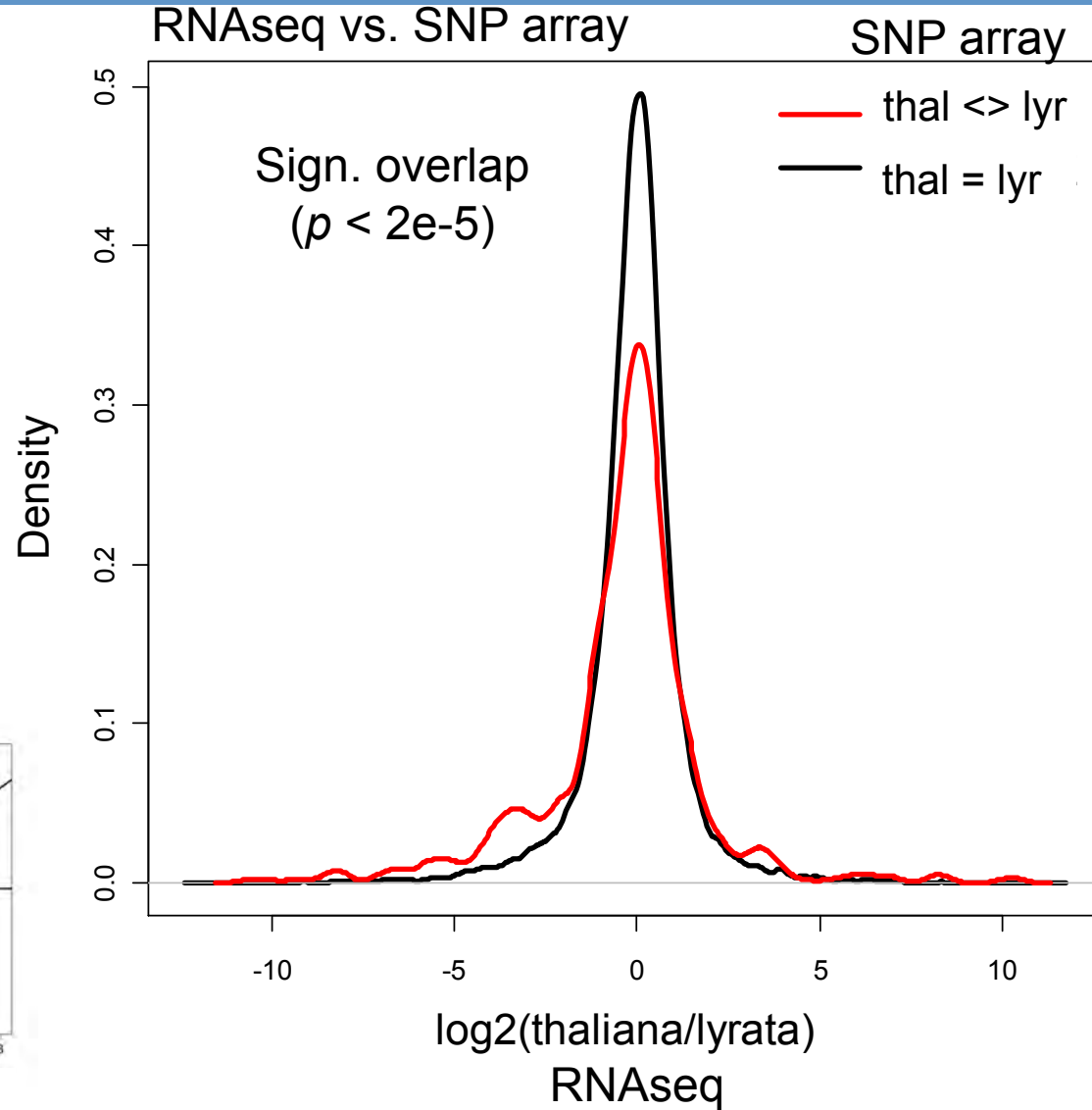
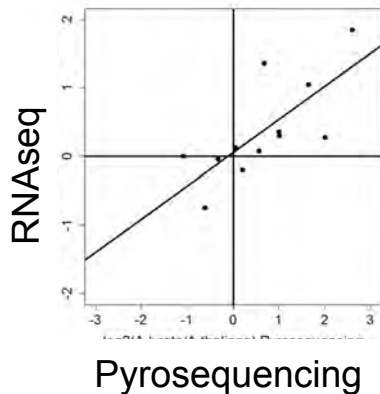
Cis-regulation differs between hybrids

# Validation of RNAseq data



He et al. 2012

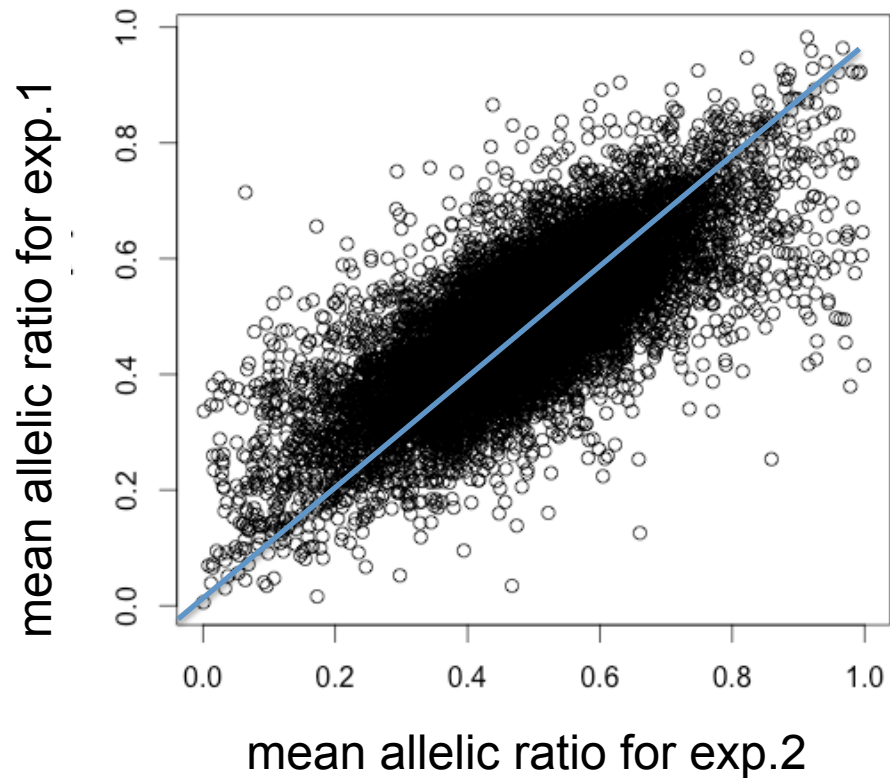
R=0.80  
 $p = 0.000$



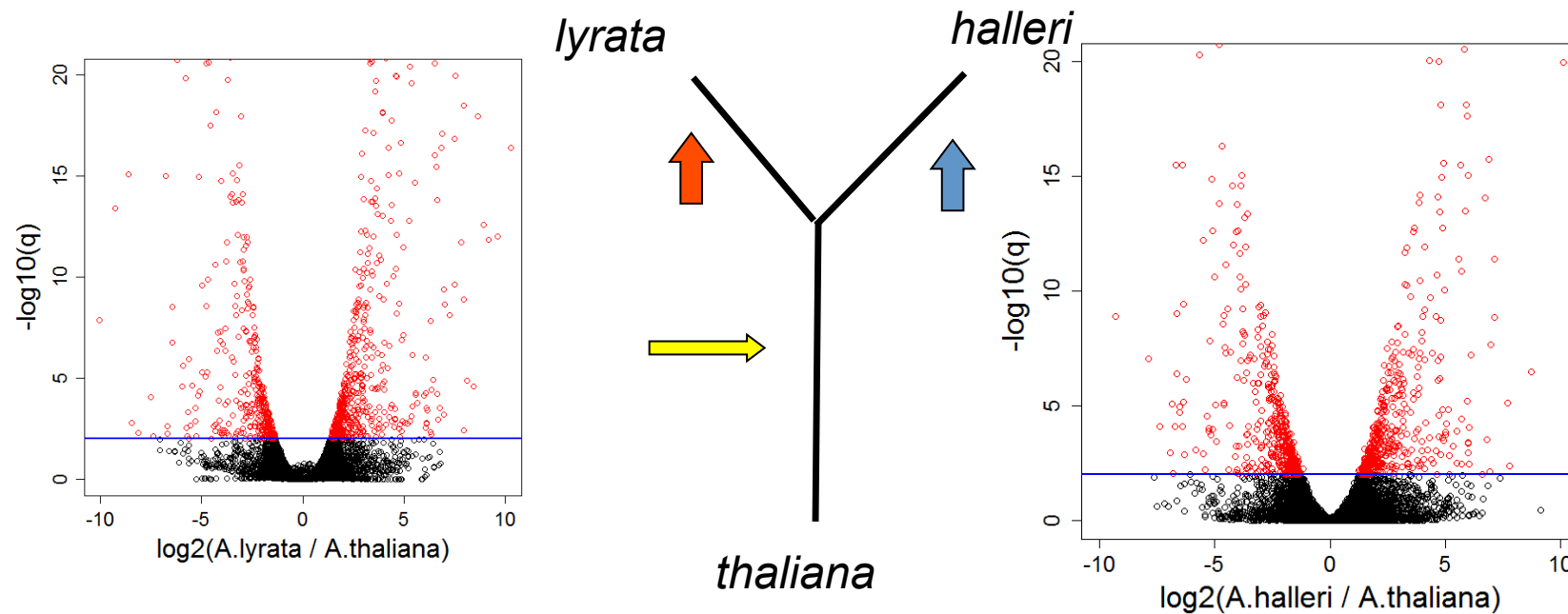
# *Replicability of Allelic ratios*

Standard conditions  
Ath-Aly hybrids  
2 trials  
Each 3-4 replicates

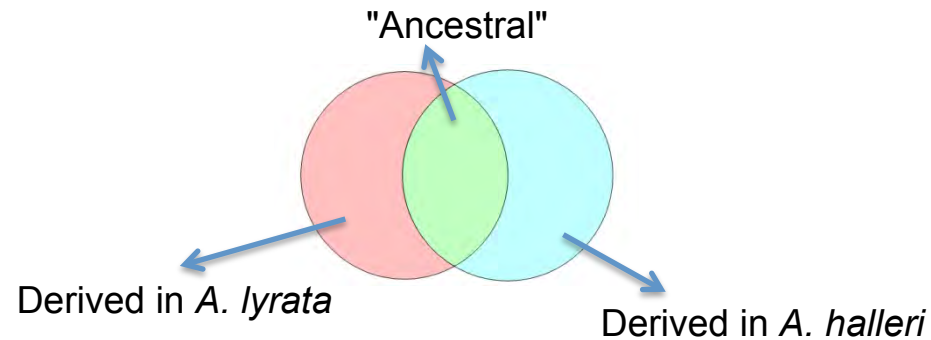
**thal/(thal+lyr)**  
 $R^2 = 0.81, p < 2.2e-16$



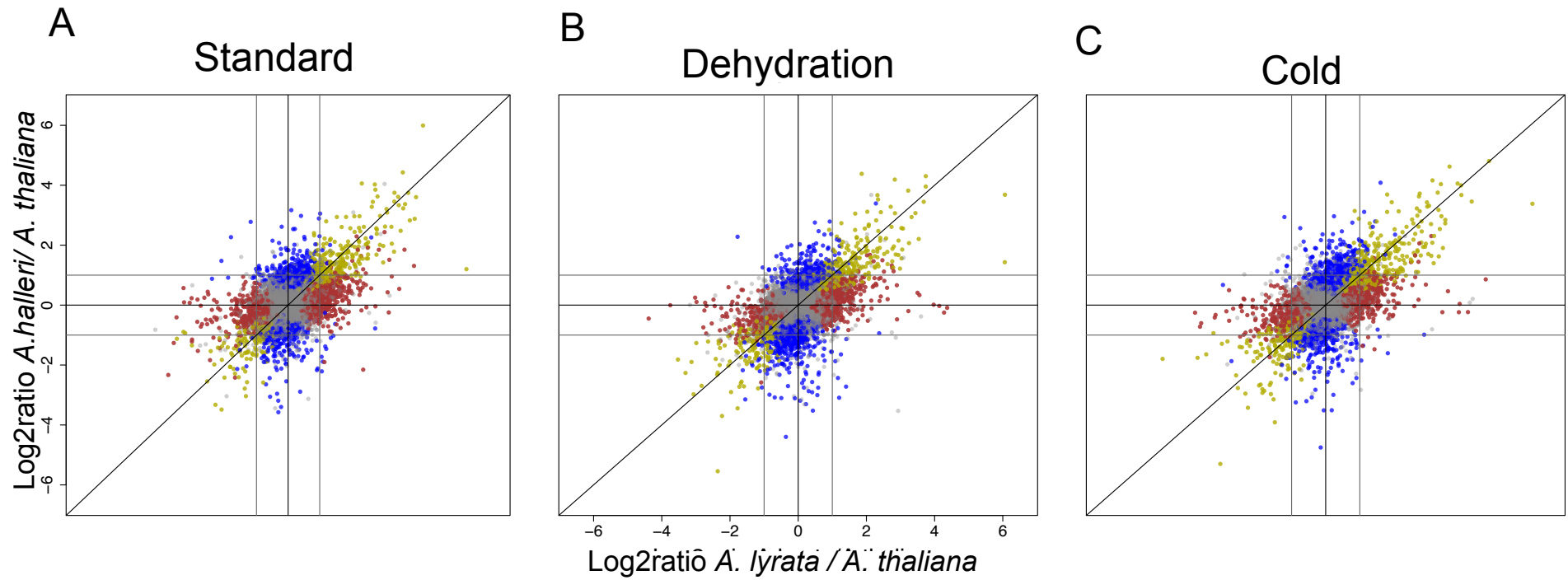
# Phylogenetic analysis of cis-regulatory changes



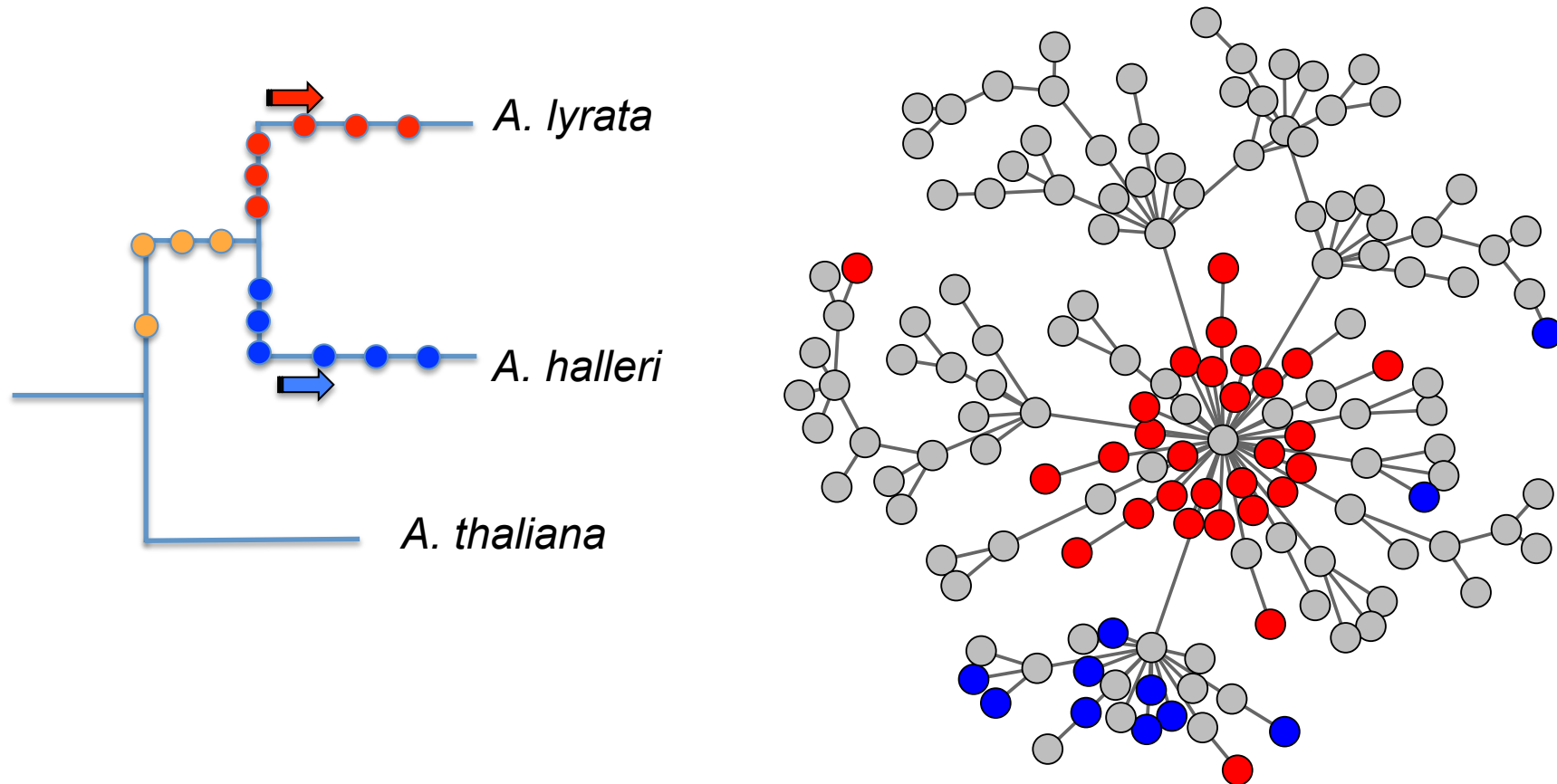
**Up-regulating *cis*-acting mutations in *A. halleri* and/or *A. lyrata***



# Phylogenetic analysis of cis-regulatory changes

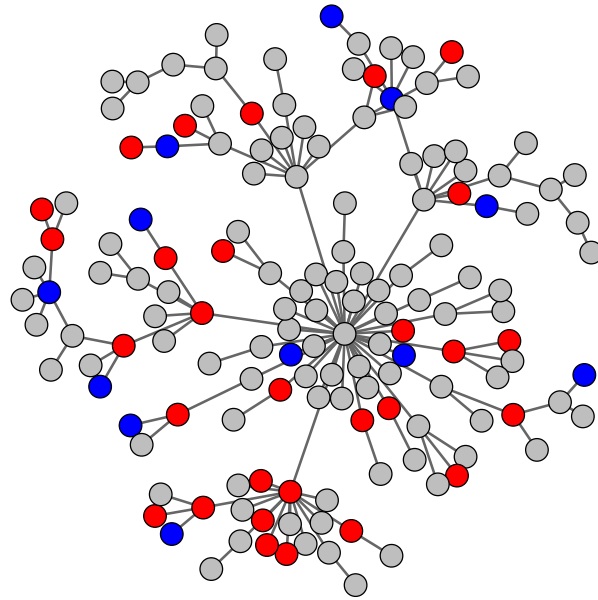


# Can we detect the action of polygenic selection on specific targets?



**Are *cis*-regulatory mutations enriched in specific molecular systems?**

# How to perform functional enrichments among ASE genes?



Universe

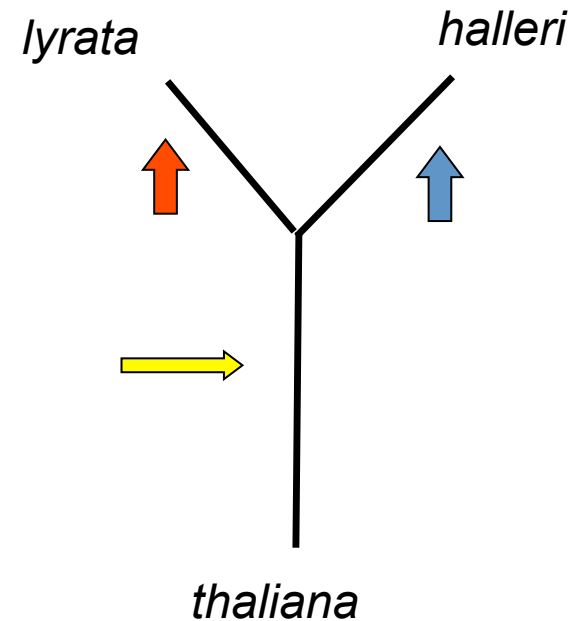
All expressed genes

All genes

ASE genes

ASE derived genes

ASE up-reg. genes



Expression category

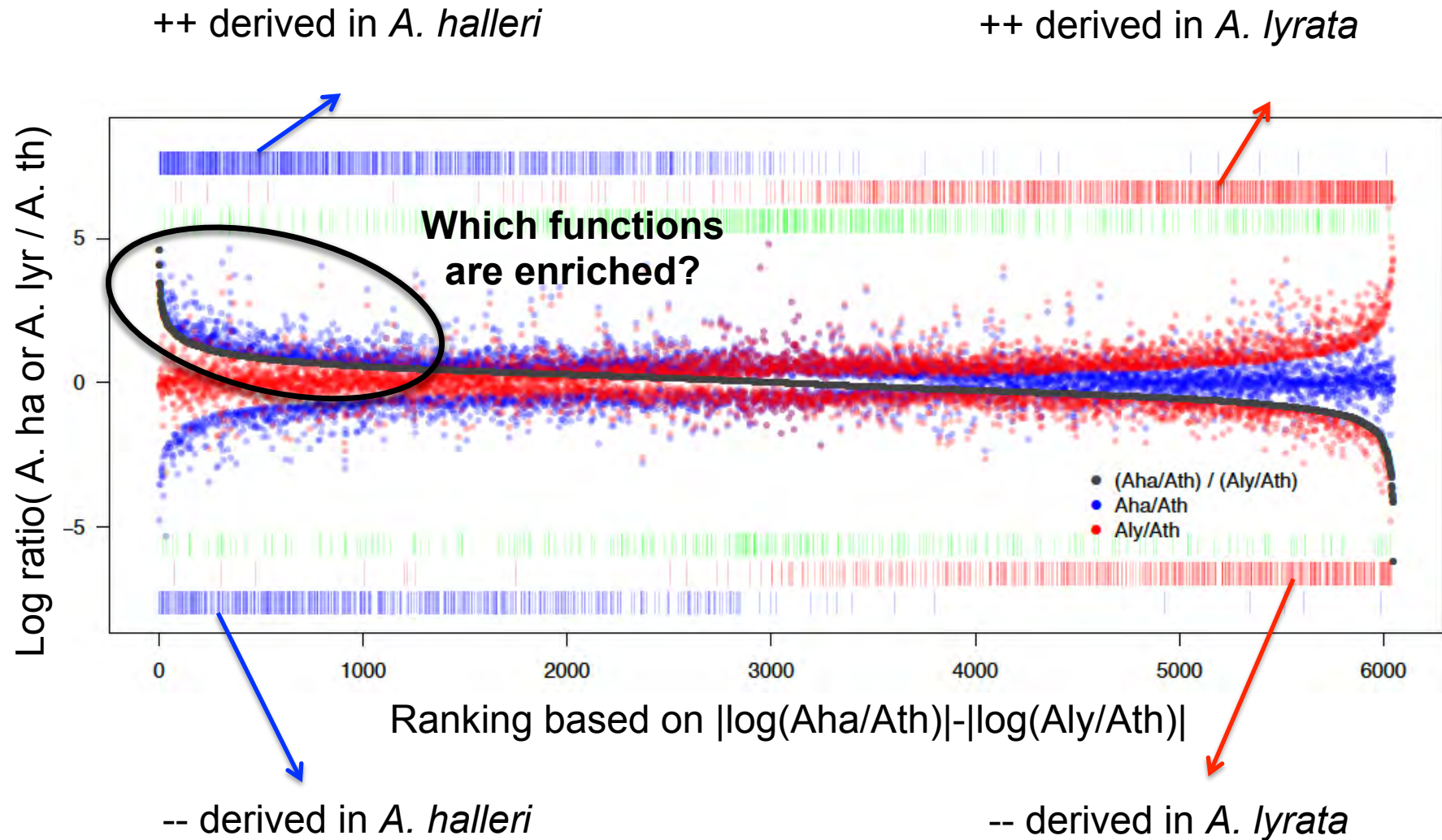
Stress expressed ASE

Stress responsive ASE?

Constitutive ASE?

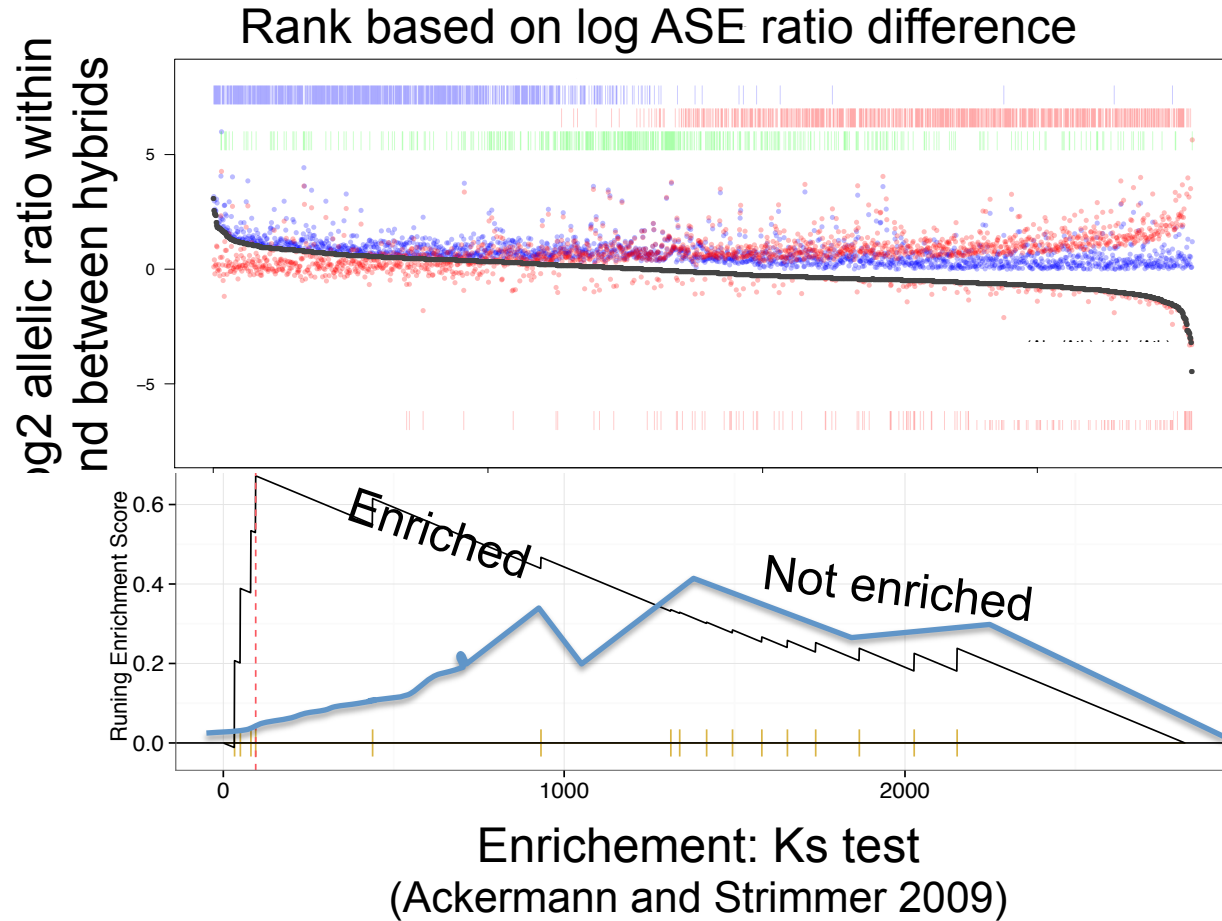
Which p-value cut-off?

# Ranking differences in ASE

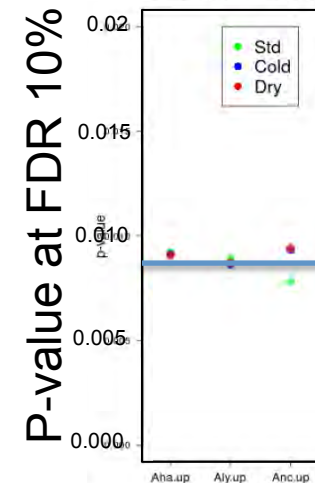




# Focus on up-regulatory derived cis-acting changes



Threshold p-value:  
Enrichment in randomized  
rankings



0.009  
cut-off

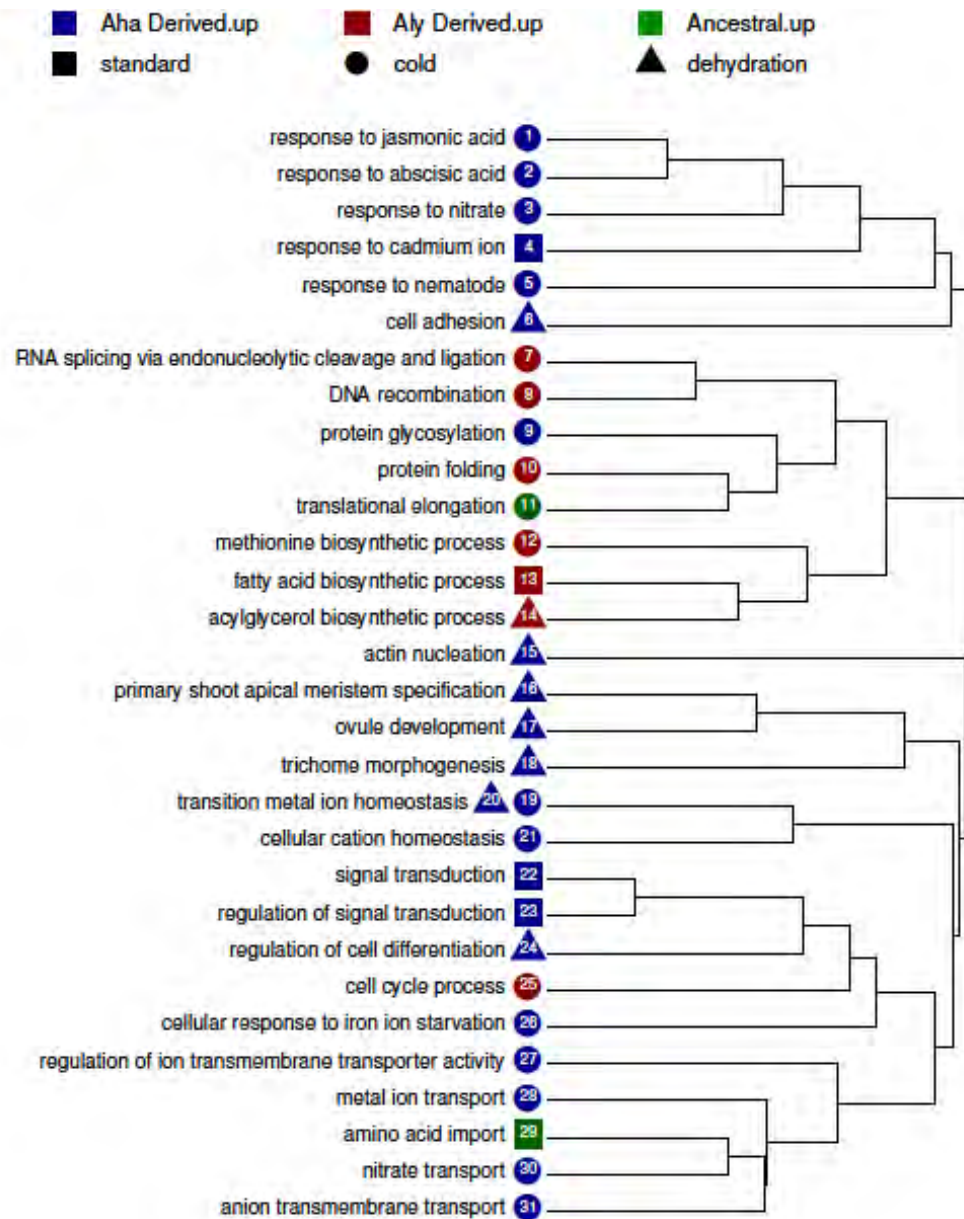
	GO.ID	Term	P.value	Treatment	GO.domain
A. halleri derived ASE genes	GO:0009966	regulation of signal transduction	0.0046	standard	BP
	GO:0007165	signal transduction	0.0084	standard	BP
	GO:0046686	response to cadmium ion	0.0087	standard	BP
	GO:0010167	response to nitrate	0.0015	cold	BP
	GO:0009624	response to nematode	0.0019	cold	BP
	GO:0006486	protein glycosylation	0.0025	cold	BP
	GO:0010106	cellular response to iron ion starvation	0.0037	cold	BP
	GO:0015706	nitrate transport	0.004	cold	BP
	GO:0055076	transition metal ion homeostasis	0.0045	cold	BP
	GO:0032412	regulation of ion transmembrane transporter	0.0053	cold	BP
	GO:0098656	anion transmembrane transport	0.0053	cold	BP
	GO:0030003	cellular cation homeostasis	0.0056	cold	BP
	GO:0009753	response to jasmonic acid	0.0059	cold	BP
	GO:0030001	metal ion transport	0.0077	cold	BP
	GO:0009737	response to abscisic acid	0.0089	cold	BP
	GO:0045010	actin nucleation	0.0012	dehydration	BP
	GO:0010090	trichome morphogenesis	0.0027	dehydration	BP
	GO:0010072	primary shoot apical meristem specificat...	0.0038	dehydration	BP
	GO:0048481	ovule development	0.0048	dehydration	BP
	GO:0007155	cell adhesion	0.0053	dehydration	BP
GO:0045595	regulation of cell differentiation	0.0057	dehydration	BP	
GO:0055076	transition metal ion homeostasis	0.006	dehydration	BP	
GO:0005385	zinc ion transmembrane transporter activ...	0.0046	standard	MF	
GO:0015103	inorganic anion transmembrane transporte...	0.0073	dehydration	MF	

Max FDR 10%

	GO.ID	Term	P.value	Treatment	GO.domain
A. lyrata derived	GO:0006633	fatty acid biosynthetic process	0.0055	standard	BP
	GO:0009086	methionine biosynthetic process	0.0065	cold	BP
	GO:0000394	RNA splicing, via endonucleolytic cleava...	0.0065	cold	BP
	GO:0022402	cell cycle process	0.0078	cold	BP
	GO:0006310	DNA recombination	0.008	cold	BP
	GO:0006457	protein folding	0.0088	cold	BP
	GO:0046463	acylglycerol biosynthetic process	0.0063	dehydration	BP
	GO:0016616	oxidoreductase activity, acting on the C...	0.0012	dehydration	MF

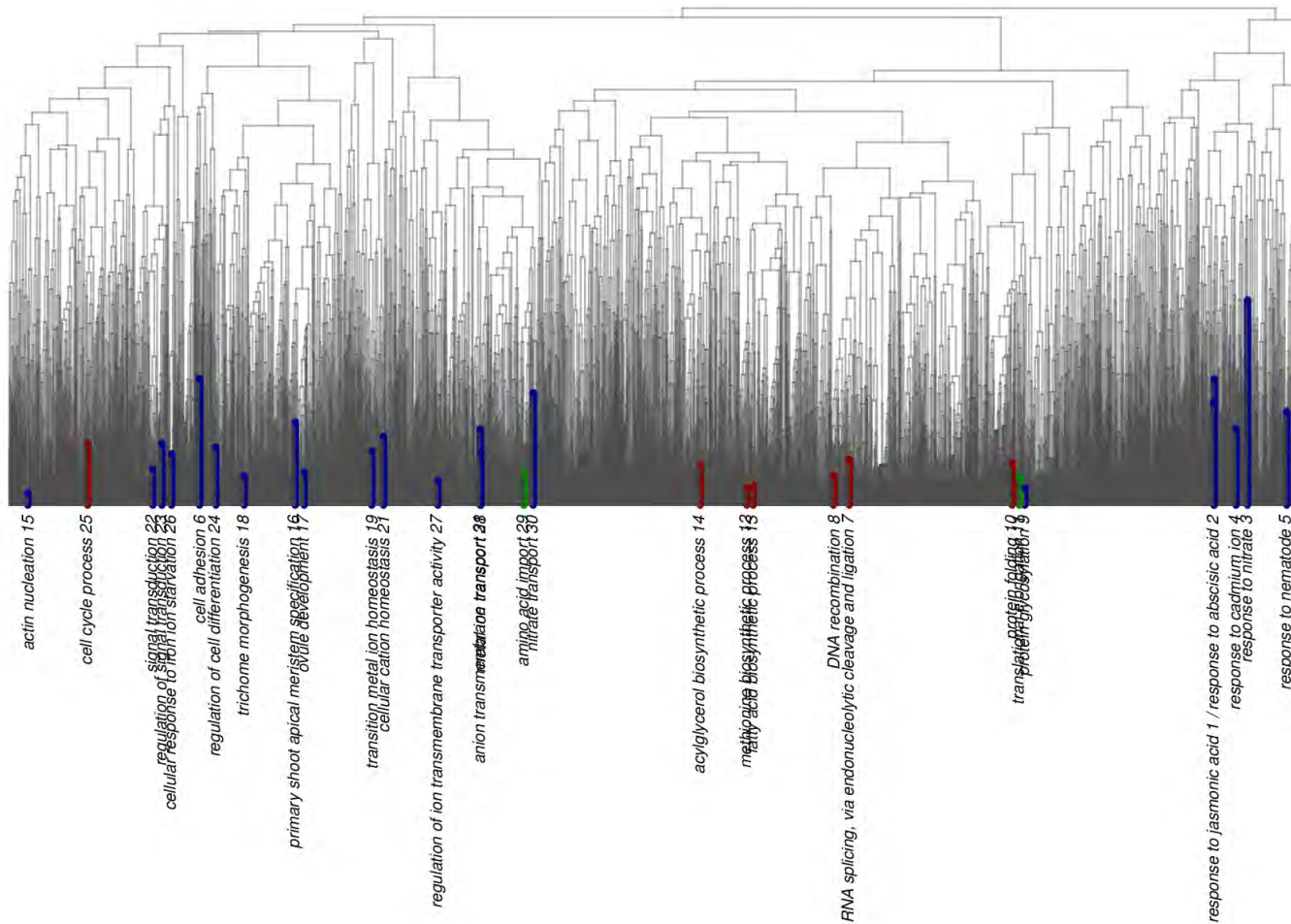
32 enriched GO categories  
p<0.009

*An excess of cis-regulatory mutations in distinct molecular systems*



# An excess of cis-regulatory mutations in heavy metal homeostasis

- ✓ Not drift
- ✓ Not relaxed constraints
- ✓ Selection



# OUTLINE

1. How to track polygenic selection?
2. The signature of Polygenic selection in the *Arabidopsis* genus
3. **Biological interpretation**
4. Perspectives



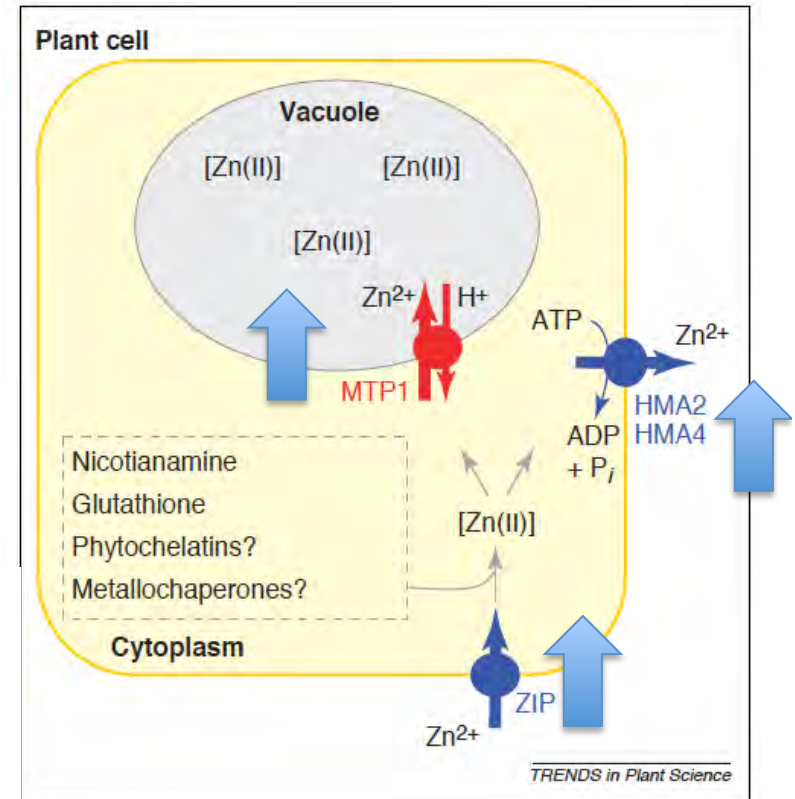
# An excess of cis-regulatory mutations in heavy metal homeostasis



## Metal homeostasis/transport is enriched in all 3 treatments

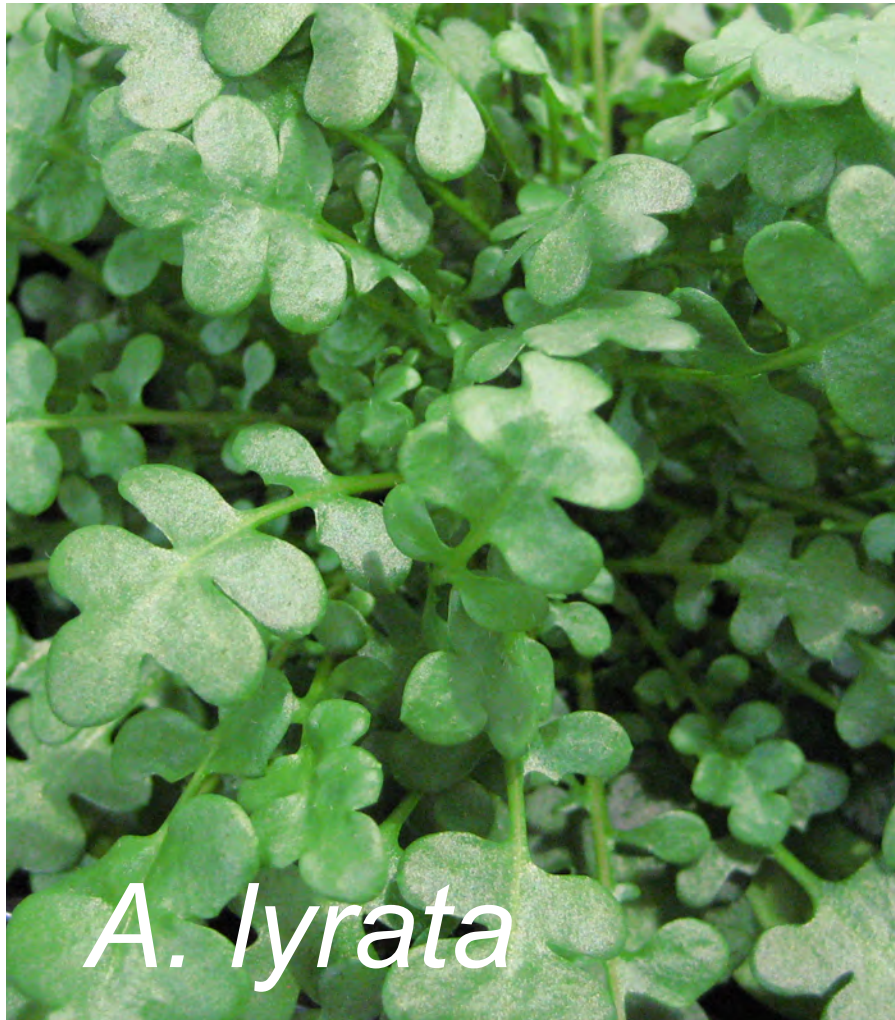
- Transition metal ion homeostasis (cold, drought)
- Cellular cation homeostasis (cold)
- Metal ion transport (cold)
- Zinc ion transmembrane transporter activity (standard)
- Response to cadmium ion (standard)

=> includes many known metal homeostasis genes



Krämer *et al.* 2005  
Krämer, 2010

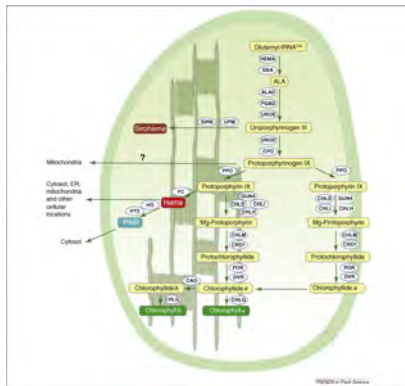
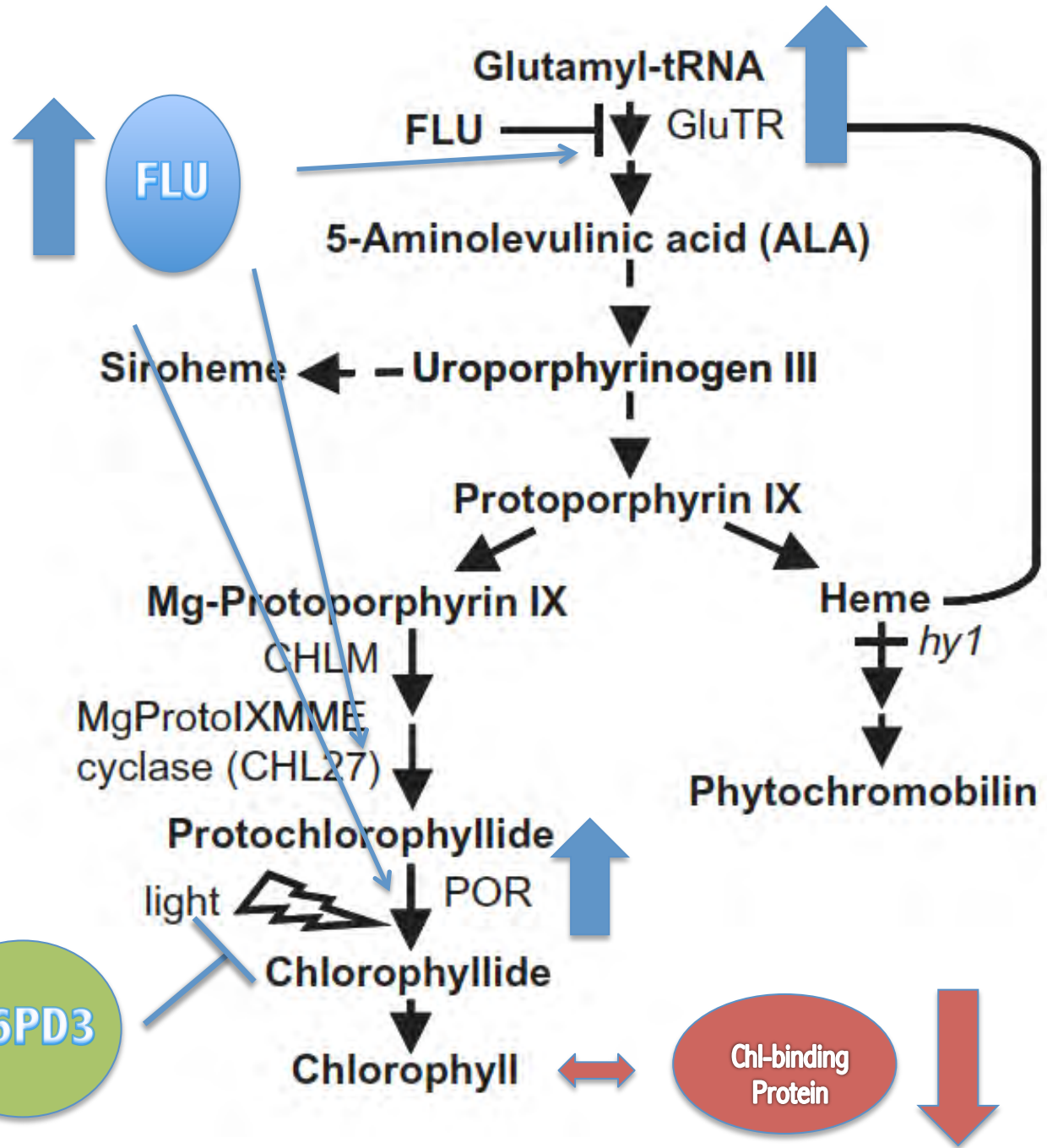
*Fatty acid metabolism enriched in A.lyrata  
In standard conditions matches the phenotype*



Acyl carrier proteins  
fatA acyl-ACP thioesterase  
KCS genes (Wax synthesis)

Torres-Franklin et al. 2007

Convergent changes in the chlorophyll biosynthesis pathway in standard conditions

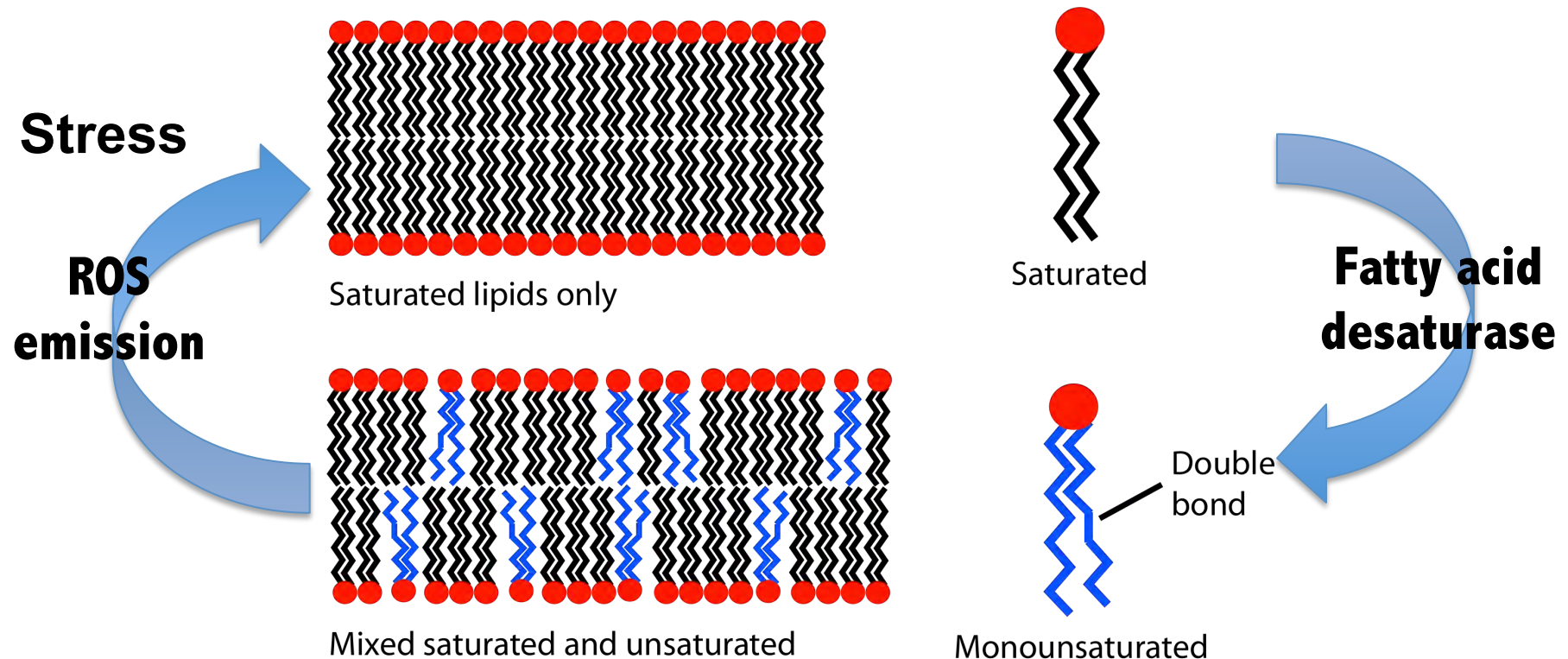


Kauss et al. 2012



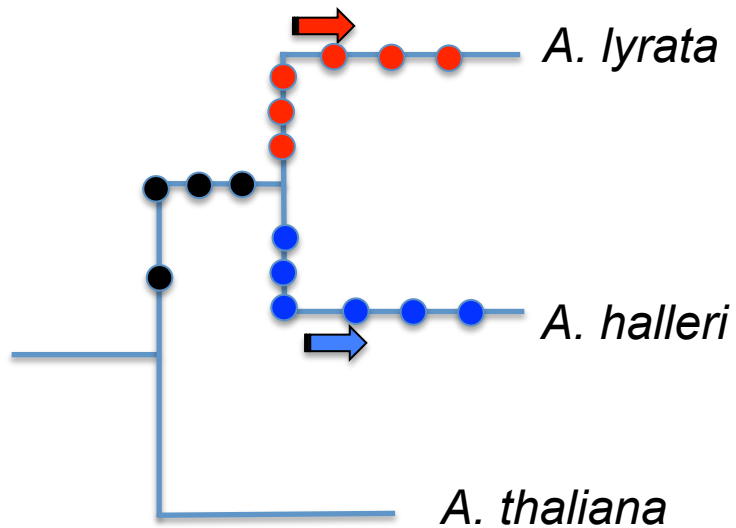
# Acyl-glycerol biosynthetic process

## Selection for optimizing membrane stability in the face of drought stress?

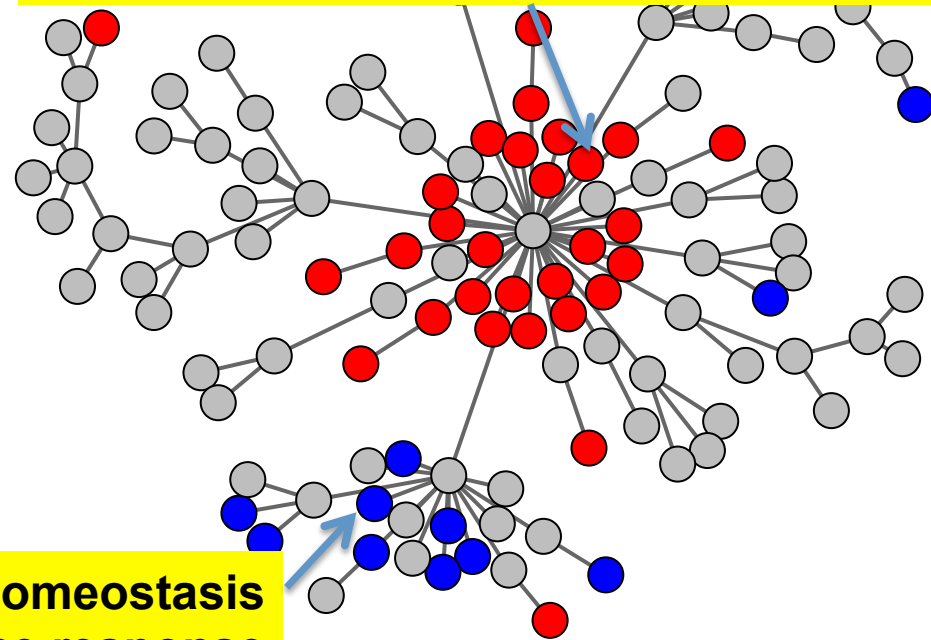


- Phosphatidic acid phosphatases
- Membrane-bound O-acyl transferases

# Mutations cluster in targets of polygenic selection



**Standard: fatty acid pathway (wax, chlorophyll)**  
**Dehydration: acyl-glycerol pathway (membrane integrity)**  
**Cold: protein folding (chaperones)**



**Metal homeostasis**  
**Stress hormone response**

Distribution of *cis*-regulatory mutations

# Collective properties of derived cis-regulatory changes

- Enrichment occurs in different functions: no obvious effect of variation in evolutionary constraints

✓ **PROOF of PRINCIPLE**

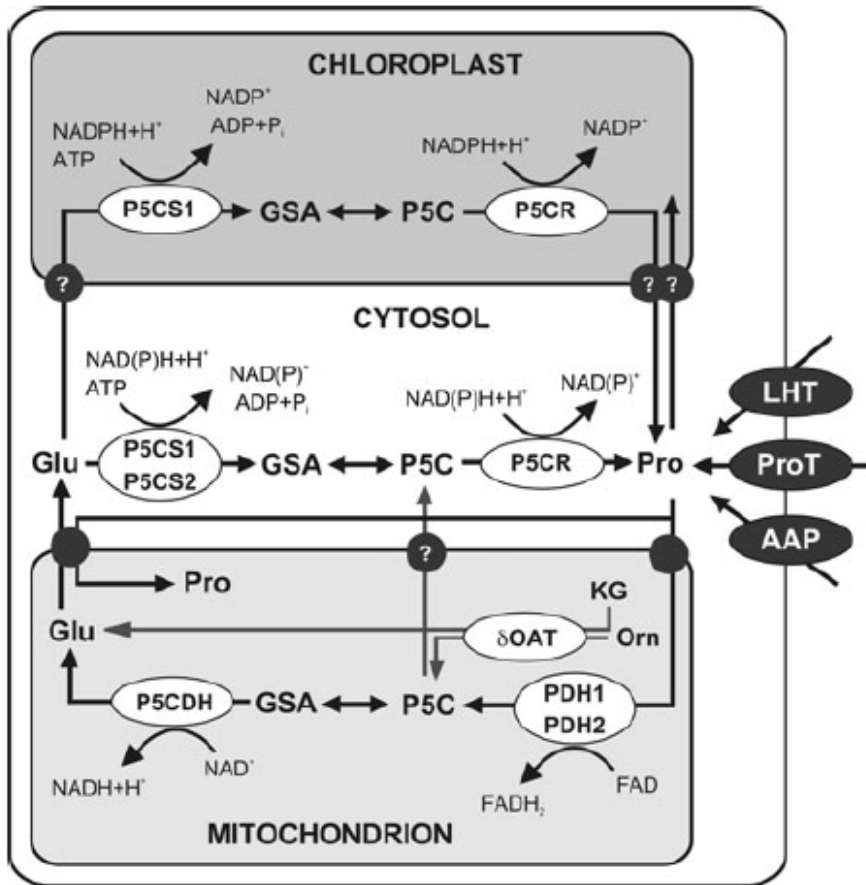
- *Cis-reg.* mutations derived confirm that heavy metal tolerance in *A. halleri* is the result of a polygenic response to selection
- *Cis-reg.* mutations derived in *A. lyrata* suggest the rewiring of fatty acid biosynthesis and stress response
- Biological interpretation of GO enrichment limited
  - Ex: Proline metabolism and accumulation

✓ **will now be tested**

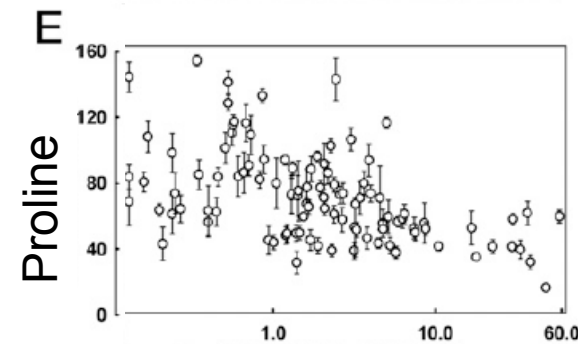
# Proline accumulation associated to variation in drought tolerance in *A. thaliana*

Kesari, Juenger et al. 2012

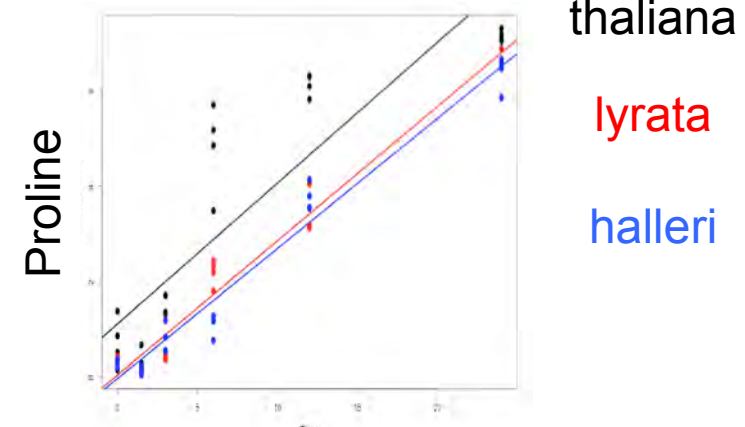
*A. thaliana*



Lehmann et al. 2010

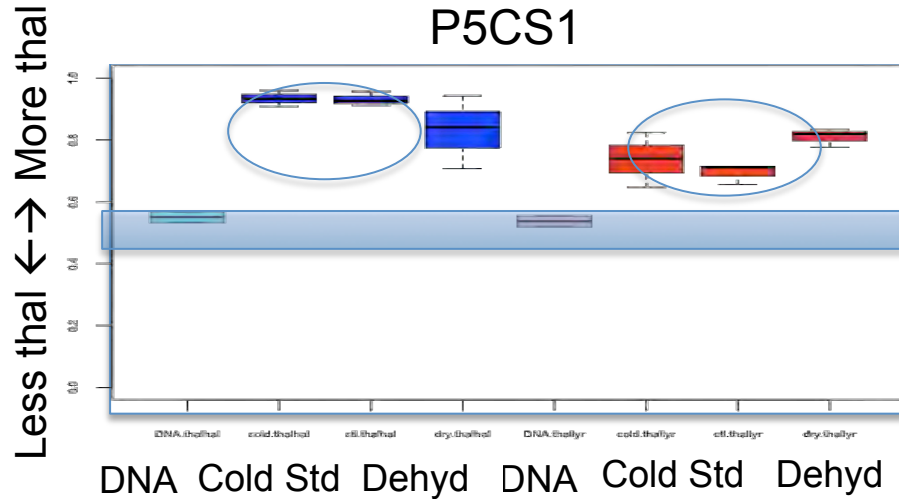


Splicing of P5CS1

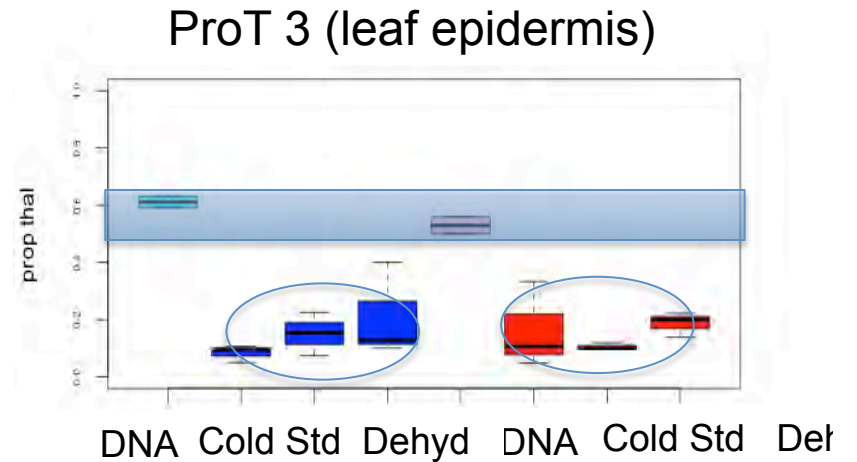


Dehydration (h)

# Proline metabolism in standard conditions selected in the outgroup lineage?



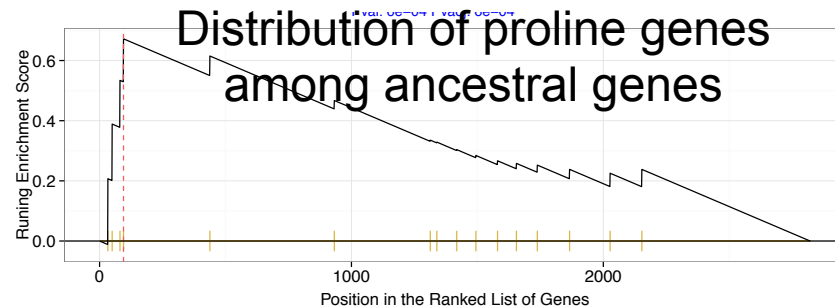
P5CS1 allele of *A. thaliana*  
=> more expressed



proline transmembrane transporter:  
ProT3 allele of *A. thaliana*  
=> less expressed

## Enrichment of changes in Proline genes:

Ancestral (standard)  $p = 6e-4$   
Aly-derived down (cold)  $p = 0.005$



# Collective properties of derived cis-regulatory changes

- Enrichment occurs in different functions: no obvious effect of variation in evolutionary constraints

✓ **PROOF of PRINCIPLE**

- *Cis-reg.* mutations derived confirm that heavy metal tolerance in *A. halleri* is the result of a polygenic response to selection
- *Cis-reg.* mutations derived in *A. lyrata* suggest the rewiring of fatty acid biosynthesis and stress response
- Biological interpretation of GO enrichment limited
  - Ex: Proline metabolism and accumulation
  - Recent evolution of Proline metabolism in *A. thaliana*?

✓ **will now be tested**

✓ **Biological relevance of annotations critical for data interpretation**

# *Conclusion: mapping natural selection targets using cis-regulatory change*

- ✓ Enrichment in heavy-metal functions in *A. halleri*
- ✓ Several functional categories enriched in *A. lyrata*
- ✓ *Novel functional hypotheses*

## **A new approach to detect the molecular basis of adaptation**

- Genome scans: mutations subjected to hard sweeps
- QTLs – GWAS: large effect mutations
- Relative-rate test of Cis-regulatory evolution:  
small effect / soft sweep mutations

# *Conclusion: mapping natural selection targets using cis-regulatory change*

## **Advantages:**

- can be implemented in non-model species
- can be explored in ecologically relevant conditions
- shift towards collective properties of mutations

## **Limitations:**

- Dependent on the quality of functional annotations
- Models are needed to predict effects of combination of +/- cis-acting changes



# OUTLINE

1. How to track polygenic selection?
2. The signature of Polygenic selection in the *Arabidopsis* genus
3. Biological interpretation
4. Perspectives



# Perspective:

Survival in the absence of water

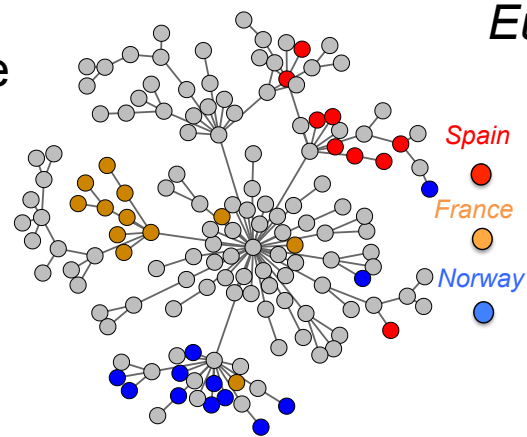
✓ Reaction to **drought** in the *Arabidopsis* genus

- Phenotype
- Transcriptome
- Metabolites



✓ Description of cis-acting divergence between *Arabidopsis thaliana* populations

Project „Adaptoscope“

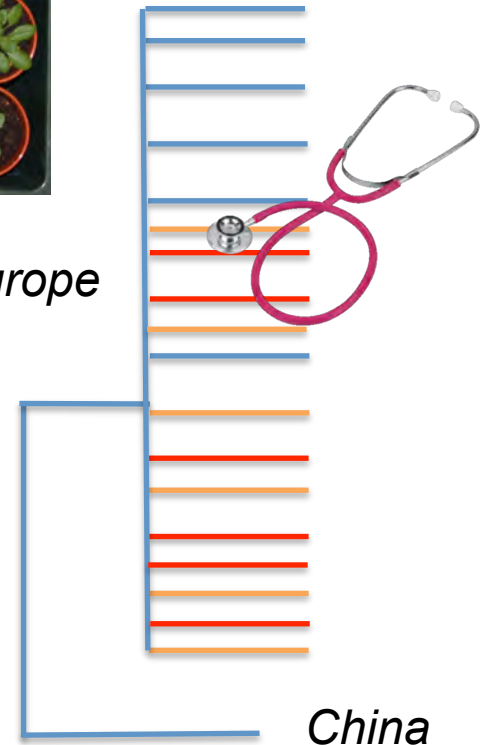


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