



Universität
zu Köln

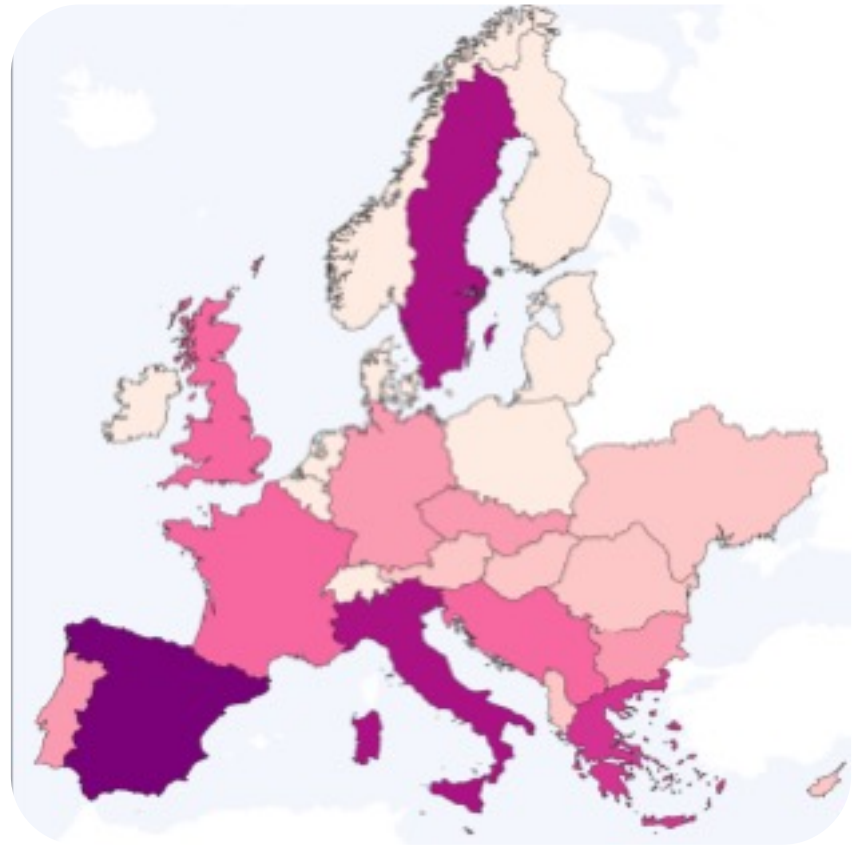


trr_341 plant
ecological
genetics

Polygenic adaptation and the evolution of gene expression in *Arabidopsis lyrata ssp. petraea*

J. de Meaux

Endangered species and biodiversity loss



Number of species at risk of extinction

1 - 10	10 - 33	33 - 56	56 - 101	101 - 156	156 - 332	332 - 578
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Among vascular species

- 7-9% are threatening to go extinct
- 80% are about to disappear for at least part of their range
- Mediterranean countries host most of the globally threatened species
- Genetic variation is dropping
- Alien species spread

Biodiversity is our living fabric and it is under threat

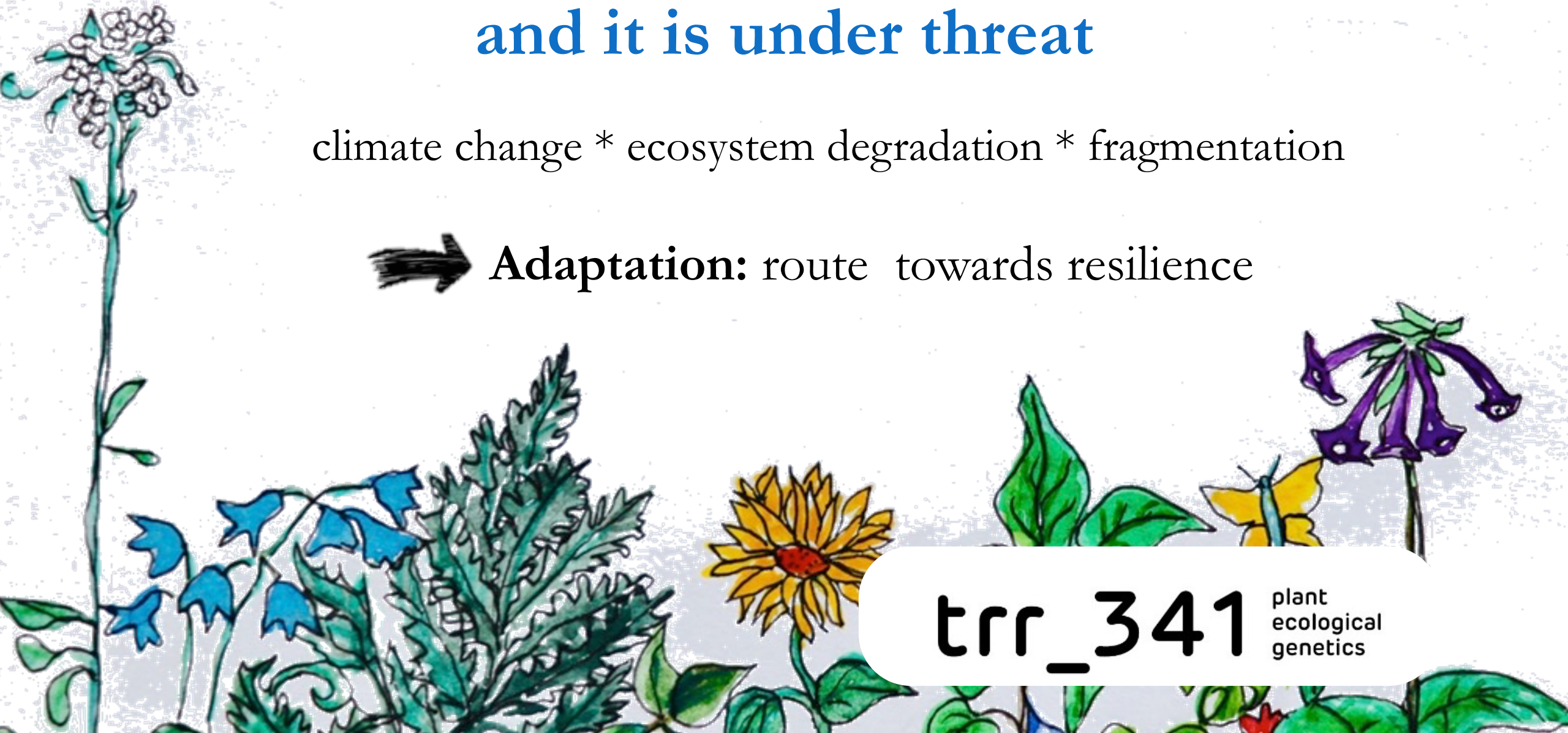
climate change * ecosystem degradation * fragmentation



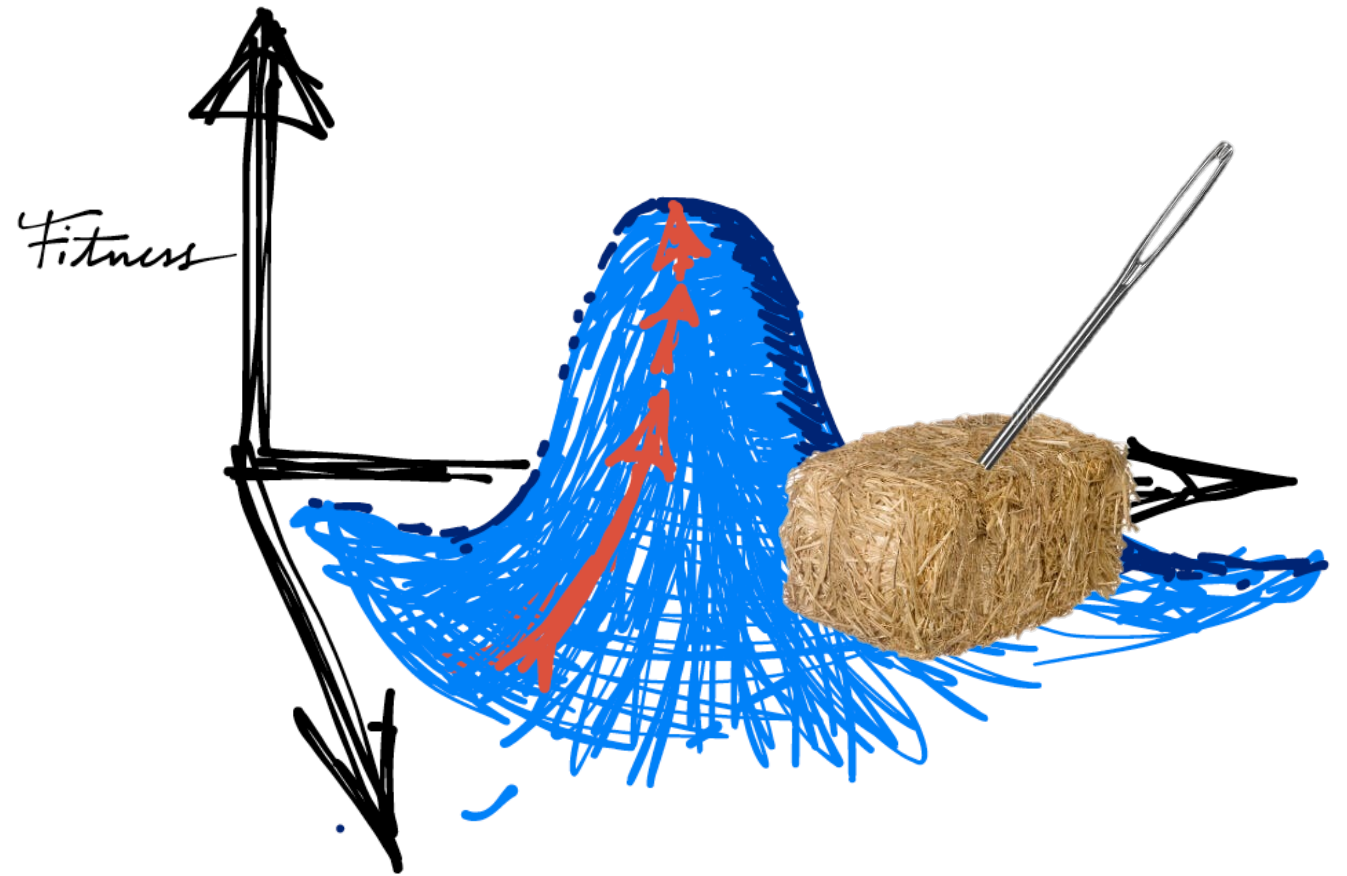
Adaptation: route towards resilience

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plant
ecological
genetics

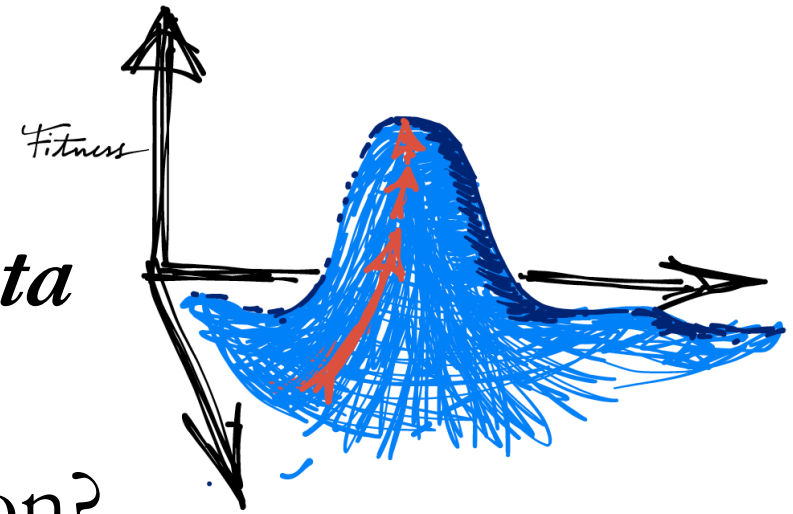


What are the adaptive resources of endangered species?



Outline

1. Our model system: *Arabidopsis lyrata* *ssp* *petraea*
2. How bad is the load after recolonization?
3. Does selection against deleterious variation shape genetic variation?
4. Can gene expression reveal the effect of polygenic selection?
5. Can we determine the adaptive relevance of plastic response to stress?



A. lyrata sp. petraea: highly fragmented populations of a rare species

A. lyrata ssp petraea is present in Central and Northern Europe
adapted to calcareous dolomitic outcroppings



Highly fragmented populations, endangered and protected
Diverged from *A. thaliana* 6 MY ago

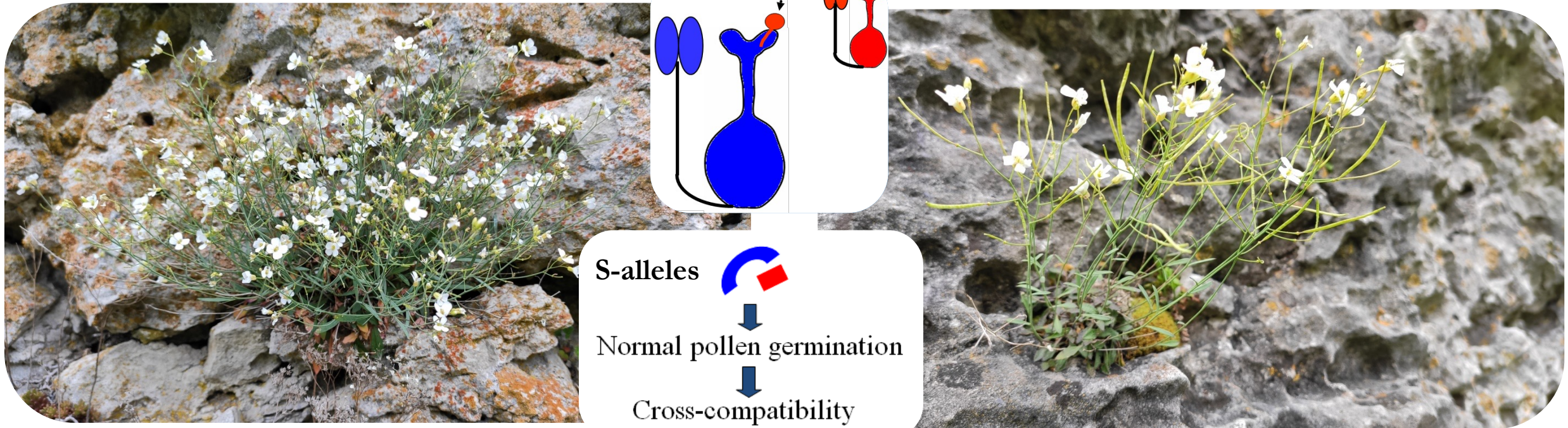


Arabidopsis lyrata subsp.
petraea, a perennial in
the field (Plech, Ger.)



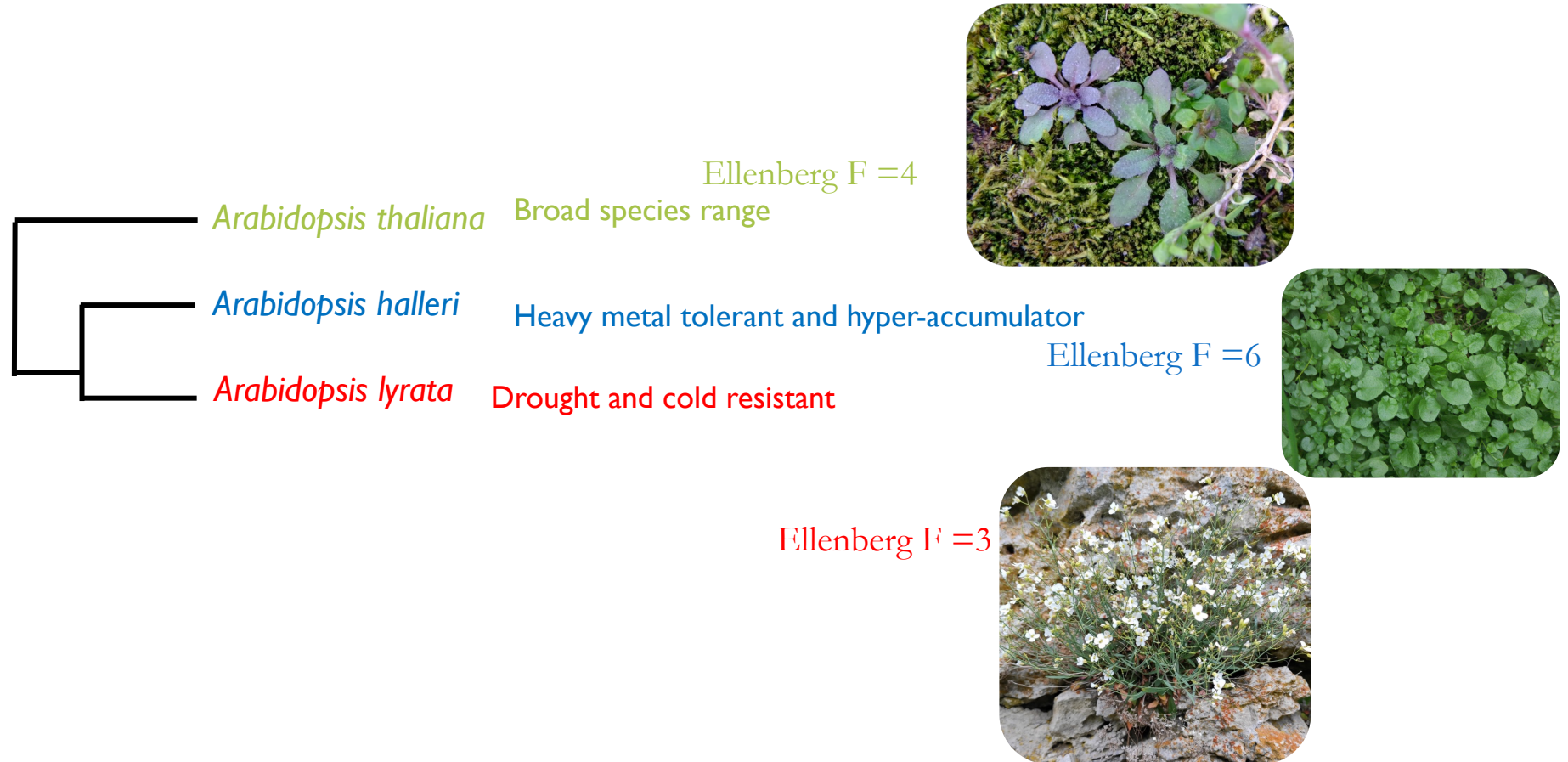
A. lyrata ssp *petraea*: a (human-like) system for understanding adaptation

A. lyrata ssp *petraea* an obligate **outcrosser**: a challenge for many years

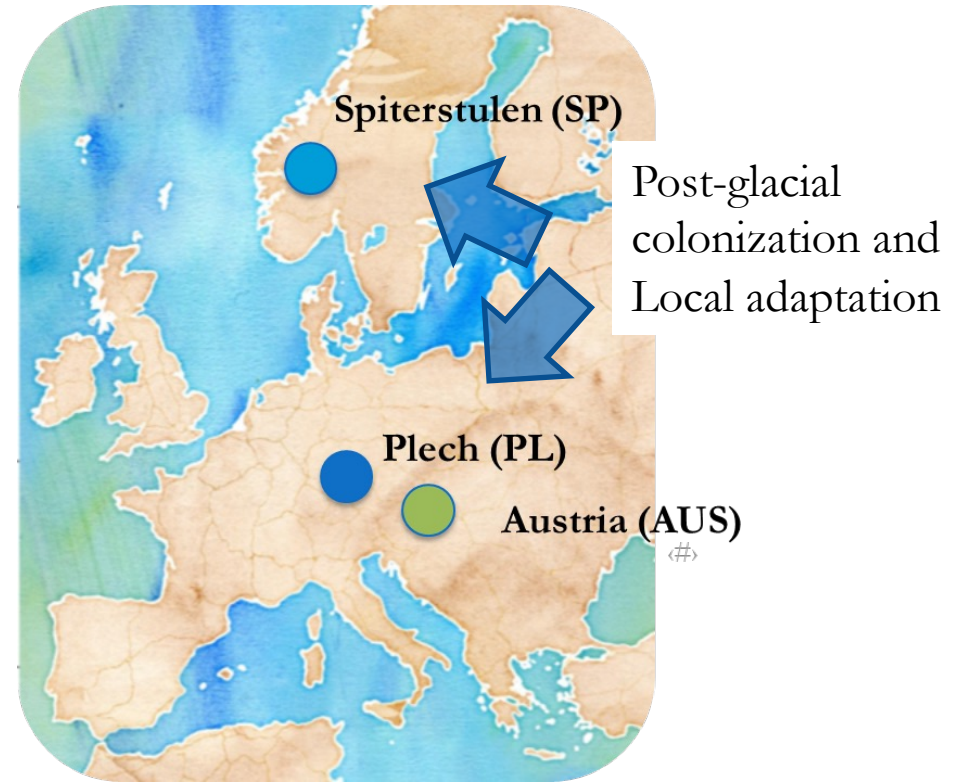
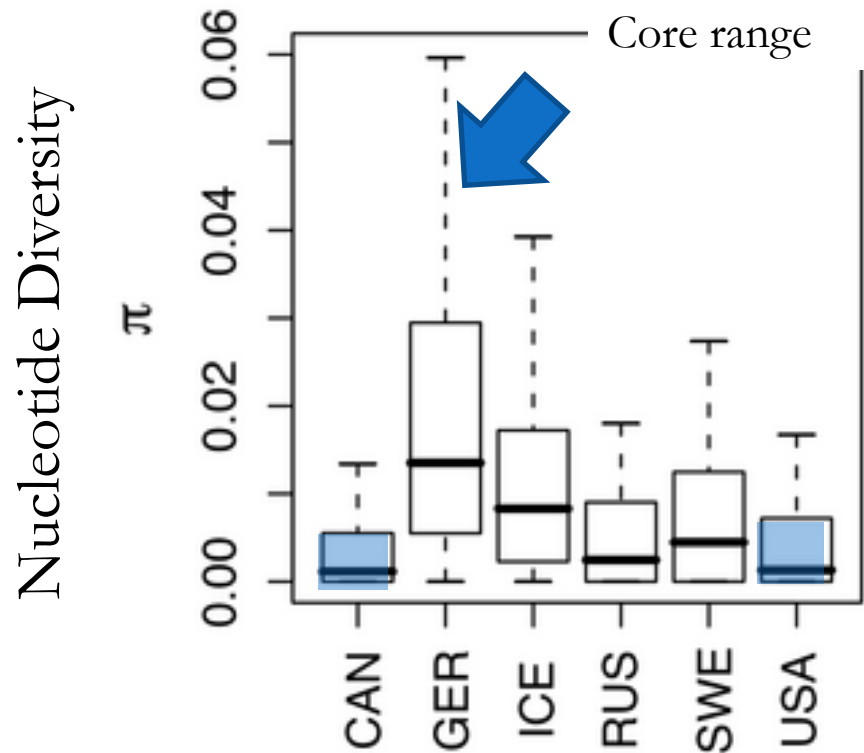


Arabidopsis lyrata: ecologically distinct from congeneric species

Arabidopsis species display distinct life history and ecological characteristics:



High intra-population diversity & local adaptation



Clauss & Mitchell-Olds 2006, Ross-Ibarra et al. 2008, Willi 2013

Takou et al. 2021, Härmälä & Savolainen 2018, Leinonen et al. 2009

Outline

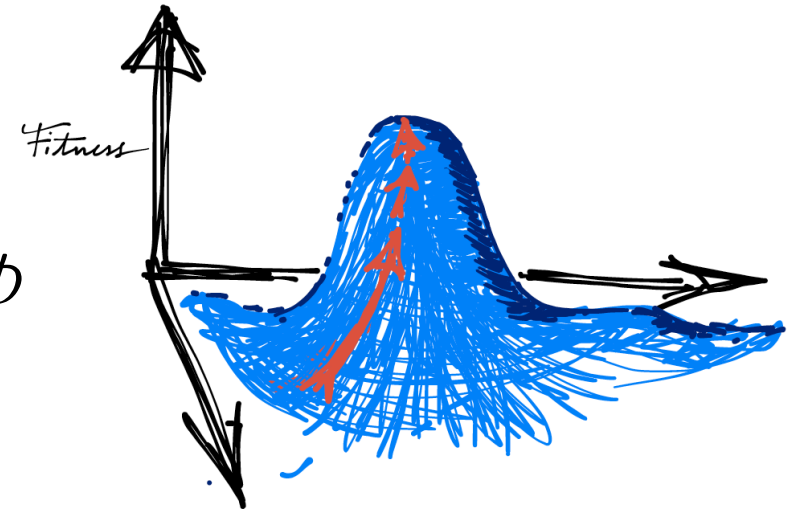
1. Our model system: *Arabidopsis lyrata* ssp *petraea*

2. **How bad is the load?**

3. Does selection against deleterious variation shape genetic variation?

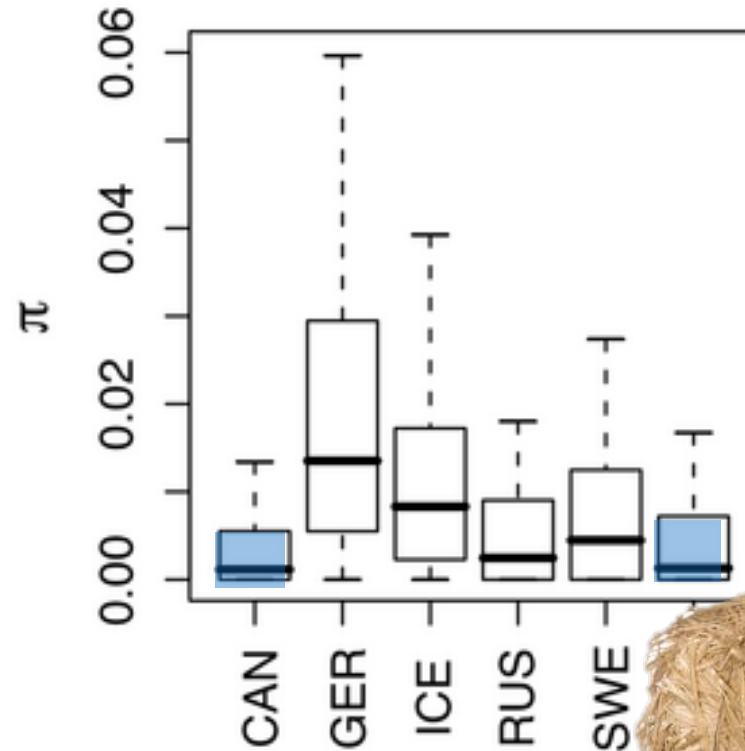
4. Can gene expression reveal the effect of polygenic selection?

5. Can we determine the adaptive relevance of plastic response to stress?



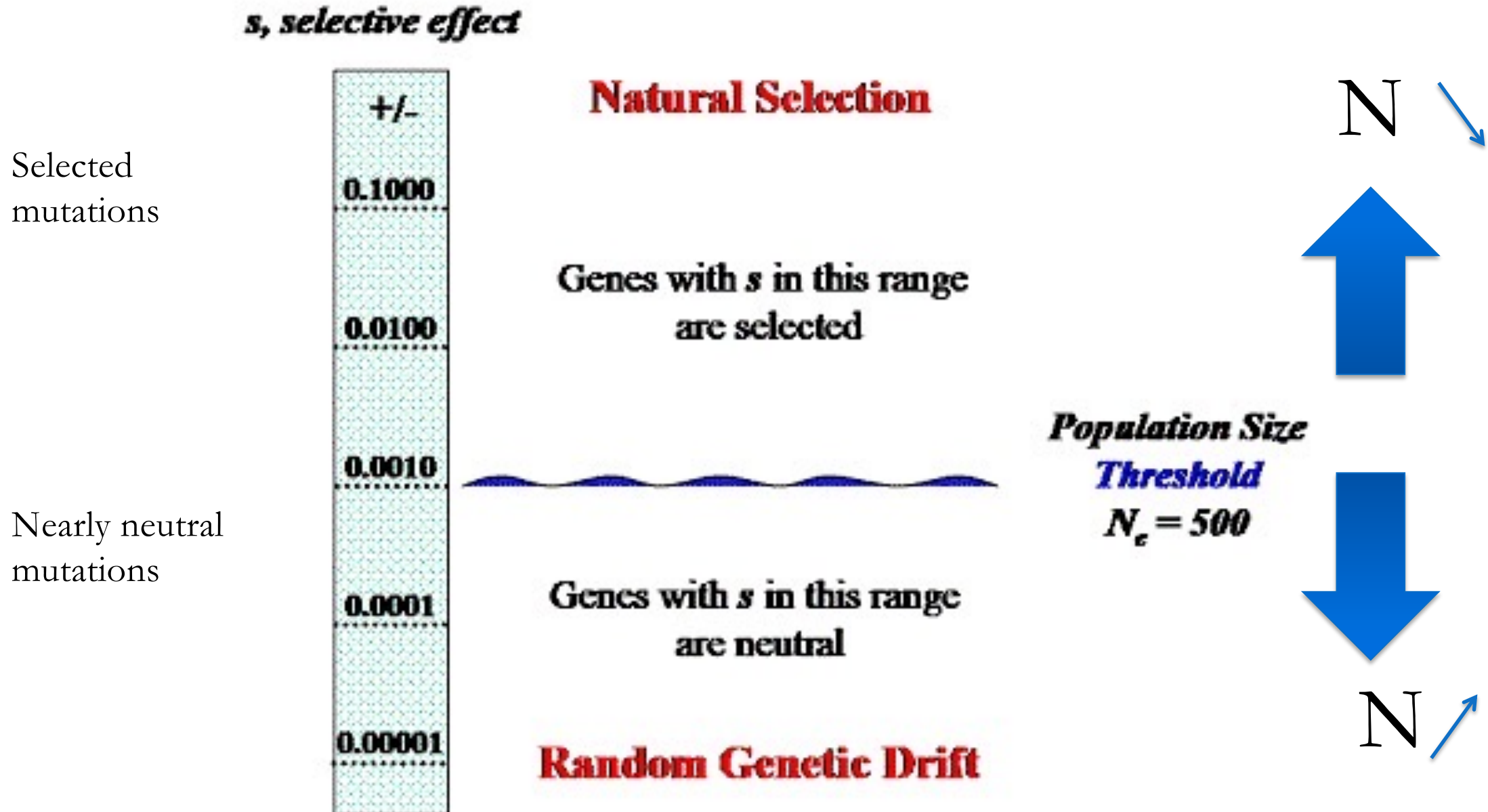
How bad is the load?

How much of the genetic variation are adaptive variants waiting to be selected?
How much is simply load that accumulated?



Ross-Ibarra et al. 2008

Efficiency of selection: what is this?

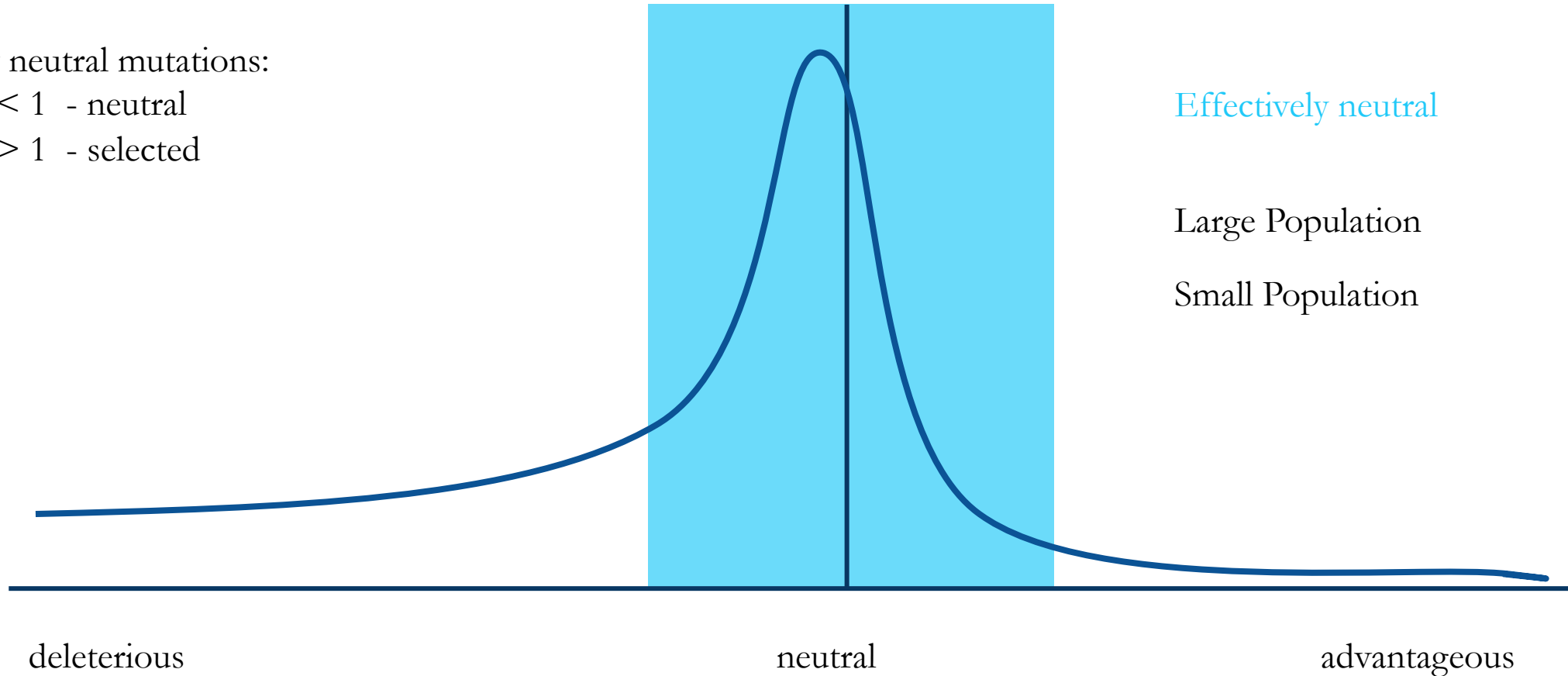


Many variants are deleterious variants not removed by selection

Nearly neutral mutations:

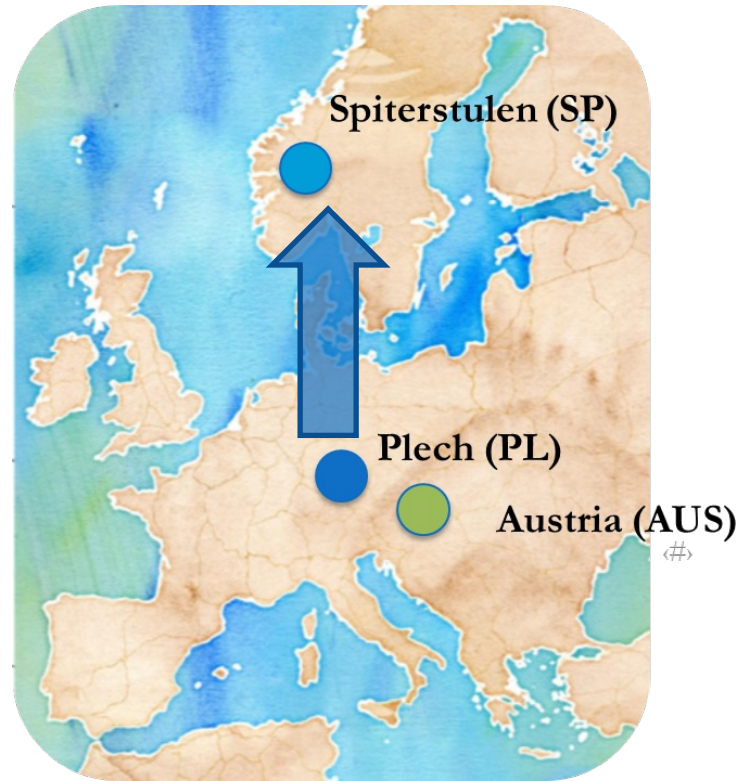
$Nes \ll 1$ - neutral

$Nes \gg 1$ - selected



Increased drift complicates selection!

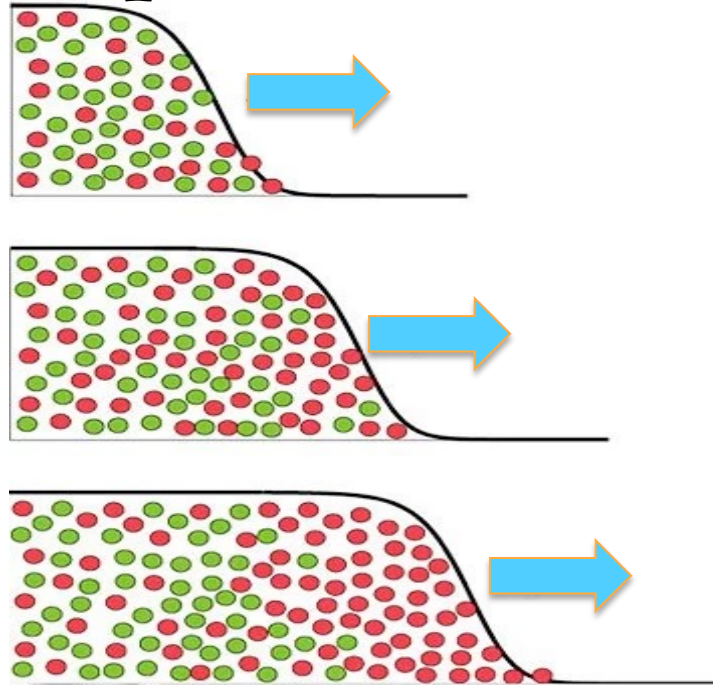
Postglacial colonization



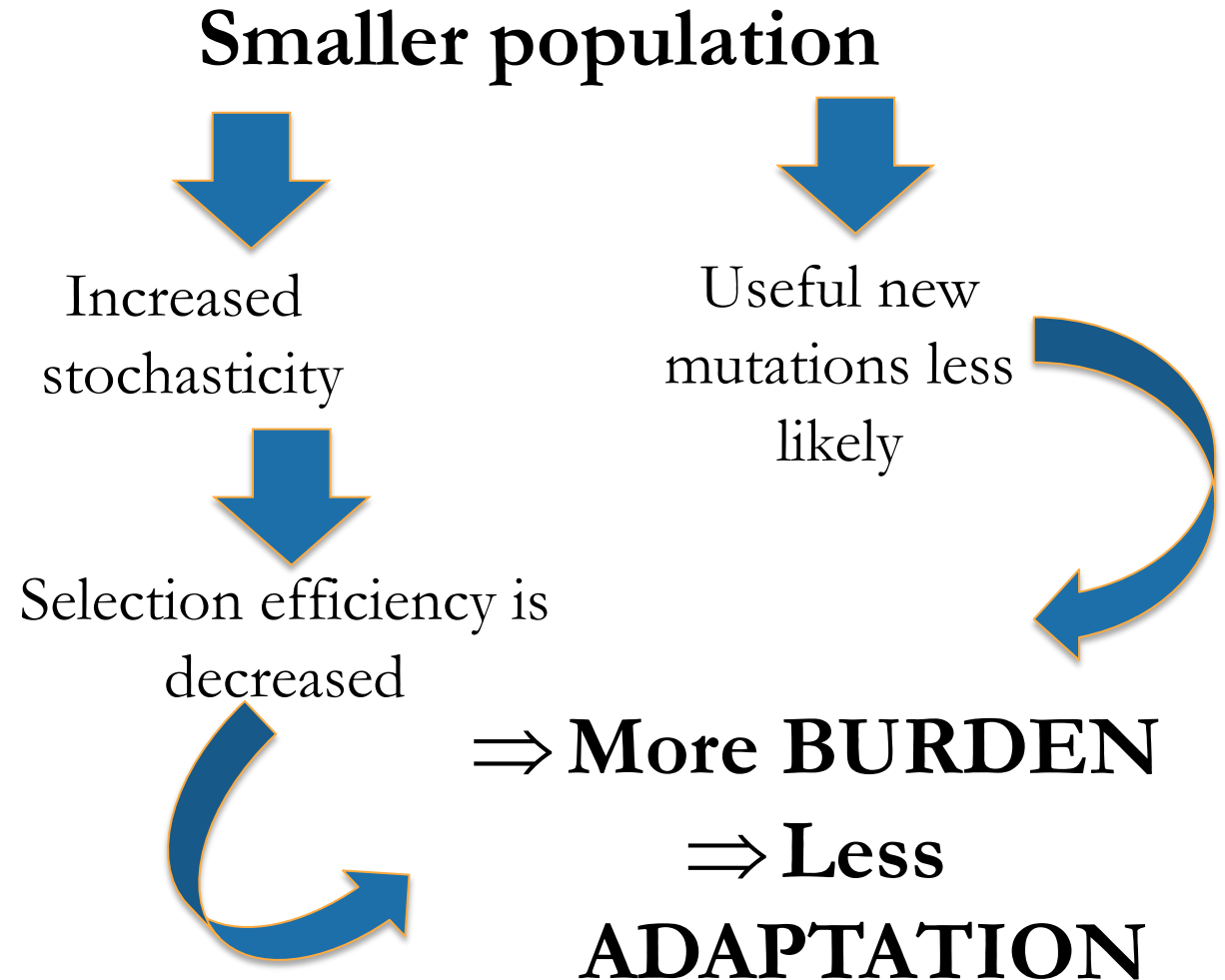
Takou et al. 2021, Hämälä &
Savolainen 2018, Leinonen et al.
2009

Expanding into new territories can alter the dynamics of genetic drift and selection.

Expansion => Burden



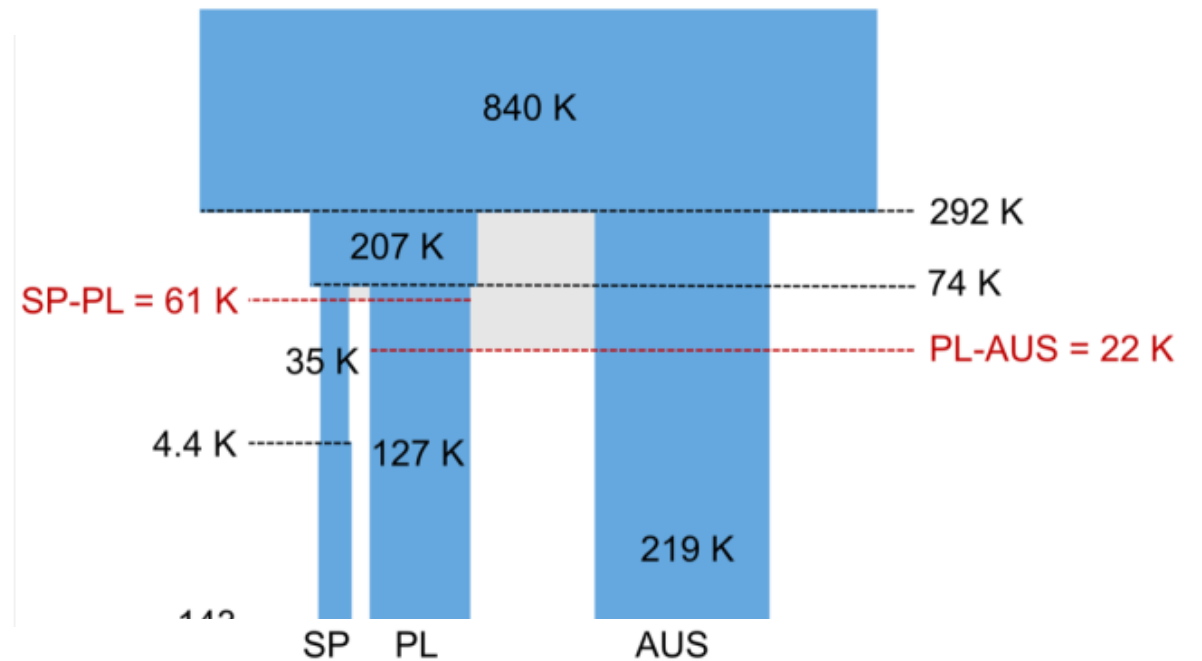
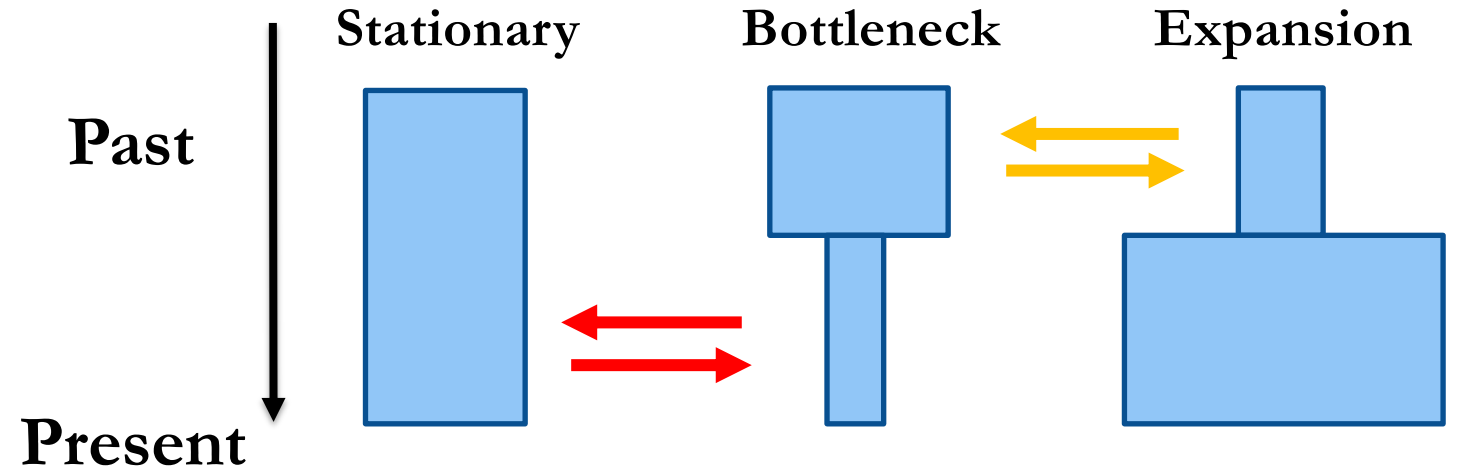
Hallatscheck et al. PNAS 2007



Lynch 2016, Nature Reviews

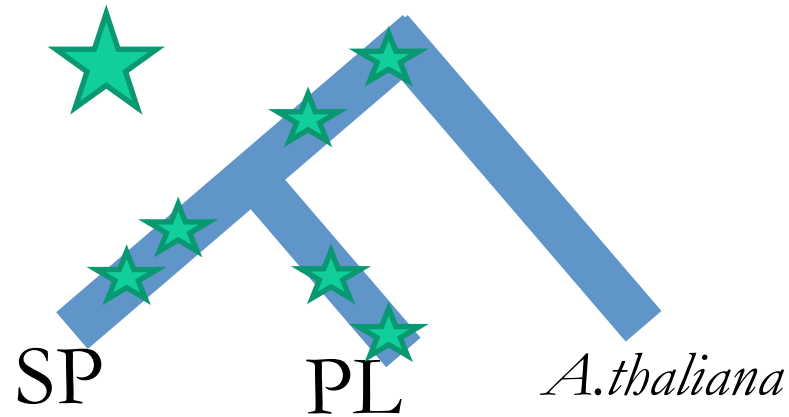
A. lyrata post-glacial expansion: bottleneck in the North

- ❖ Fastsimcoal2, N_e , m
- ❖ Sequence 47 individuals
- ❖ Fit 3 Site Frequency Spectra



Did the colonization bottleneck increase the burden?

Per-individual burden

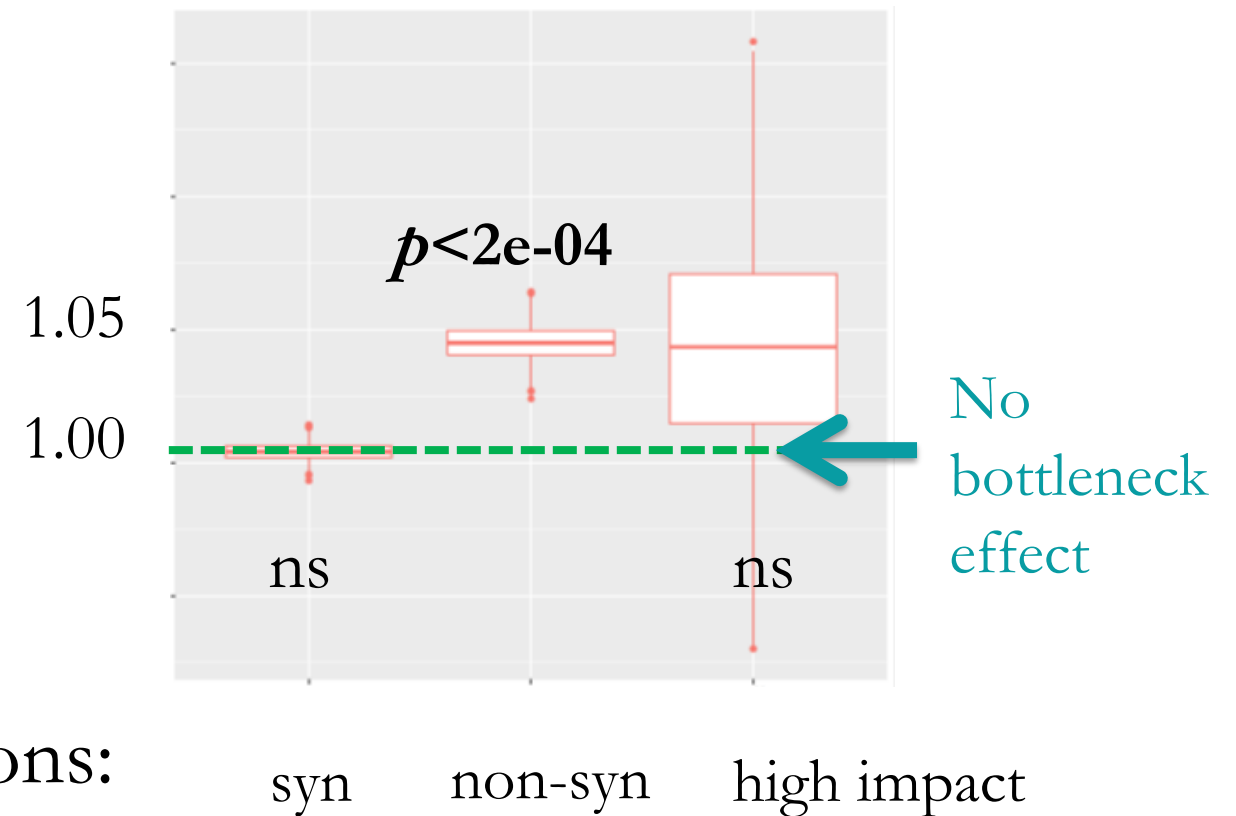


❖ #syn = $f(t)$

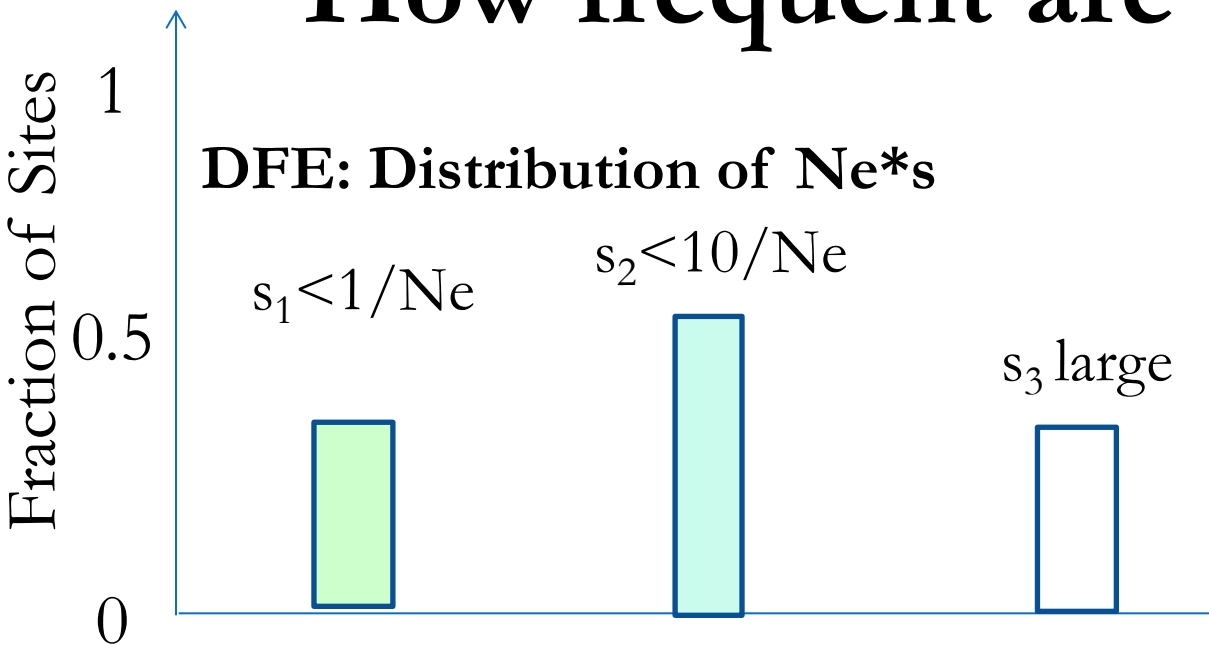
❖ Independent of demographic history.

4% more non-synonymous mutations:
how much did fitness decrease?

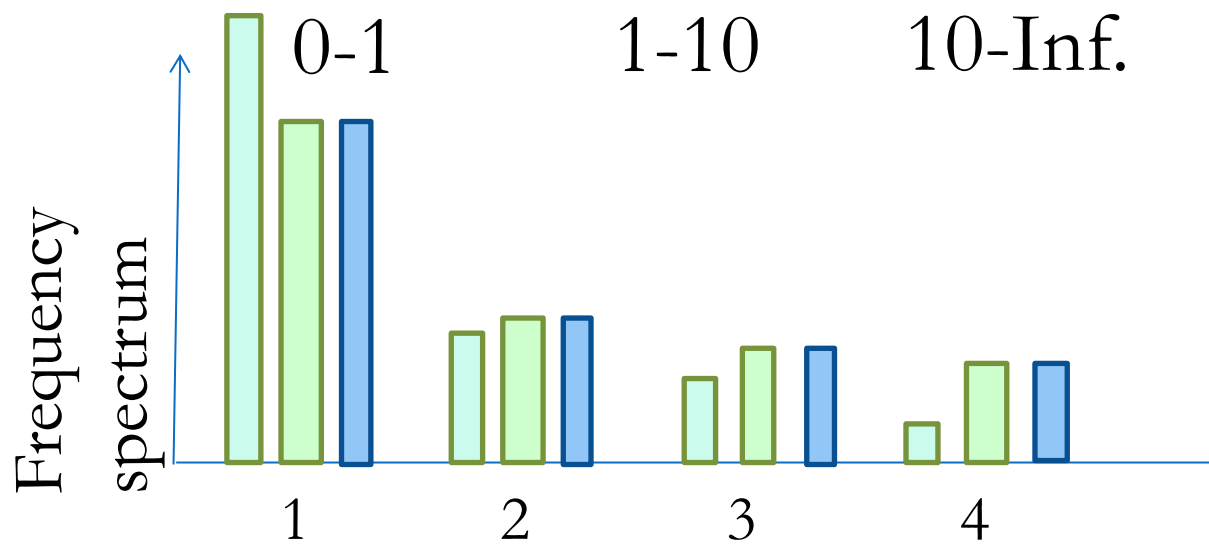
Excess per-individual burden SP



How frequent are deleterious mutations?

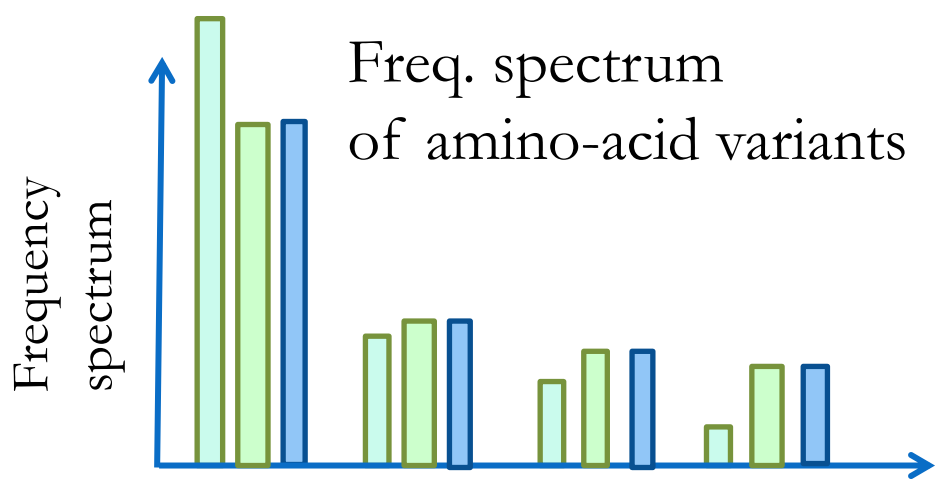
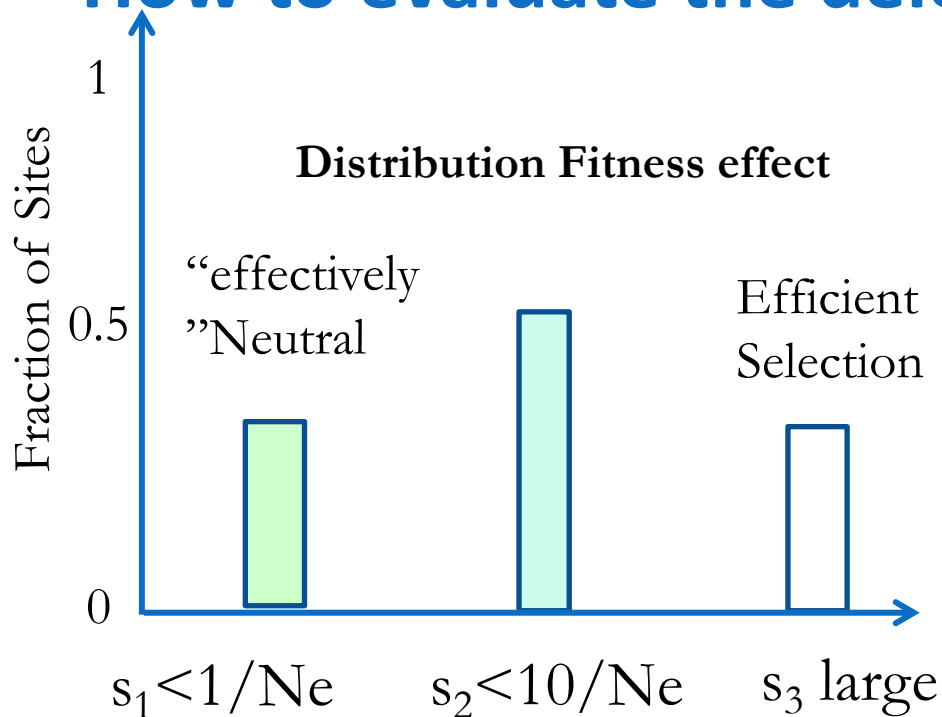


- ❖ s is determined by biology
- ❖ $\Sigma(s) = \text{Fitness Load}$
- ❖ N_e is determined by demography

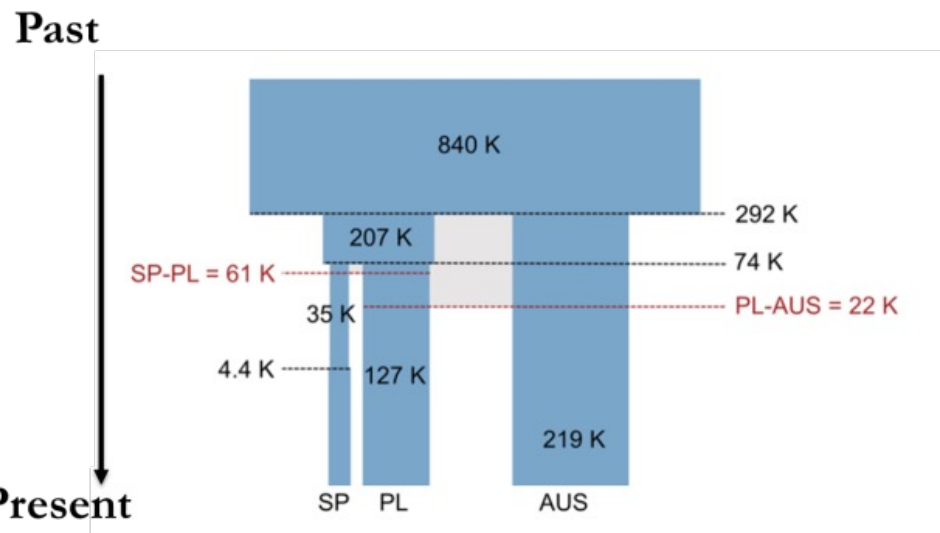


- ❖ N_e*s determines the frequency a new mutations is likely to reach
- ❖ < 1 :: “effectively” Neutral
- ❖ $>> 1$:: Efficient Selection

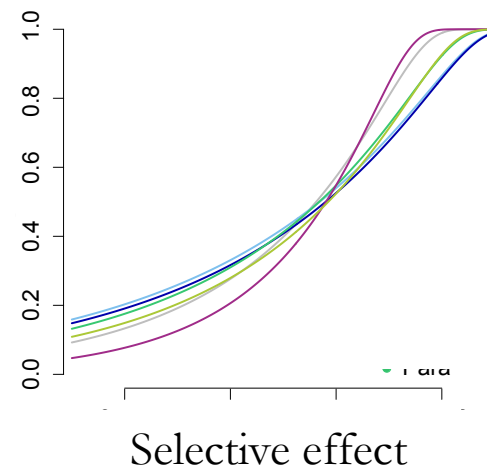
How to evaluate the deleteriousness of segregating variants?



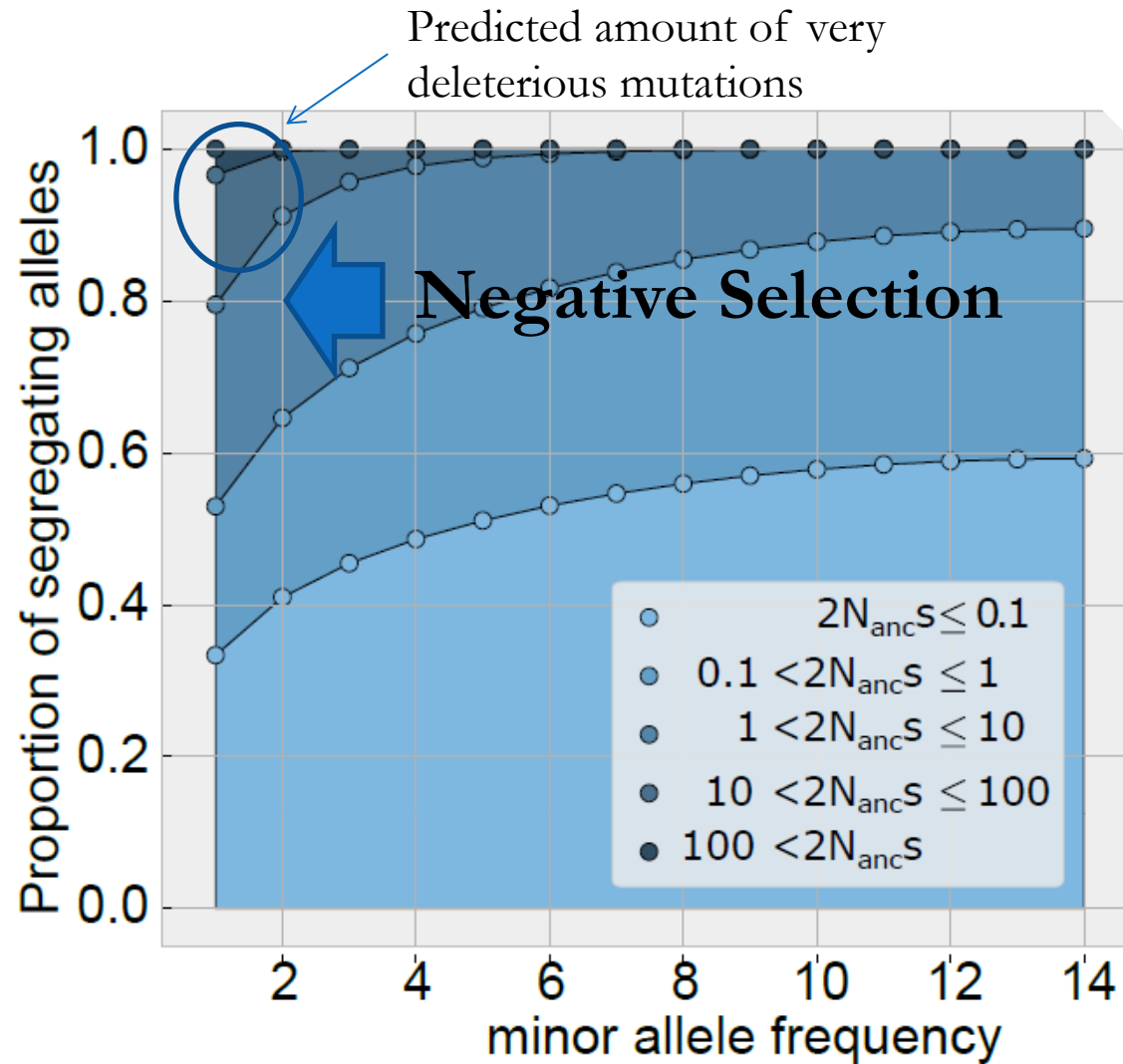
Population demography



Identify the distribution that best fits the data



Arabidopsis lyrata: an obligate outcrossing species subjected to negative selection



E. Koch
(Sunyaev lab)



K.A. Steige
Uni Hohenheim

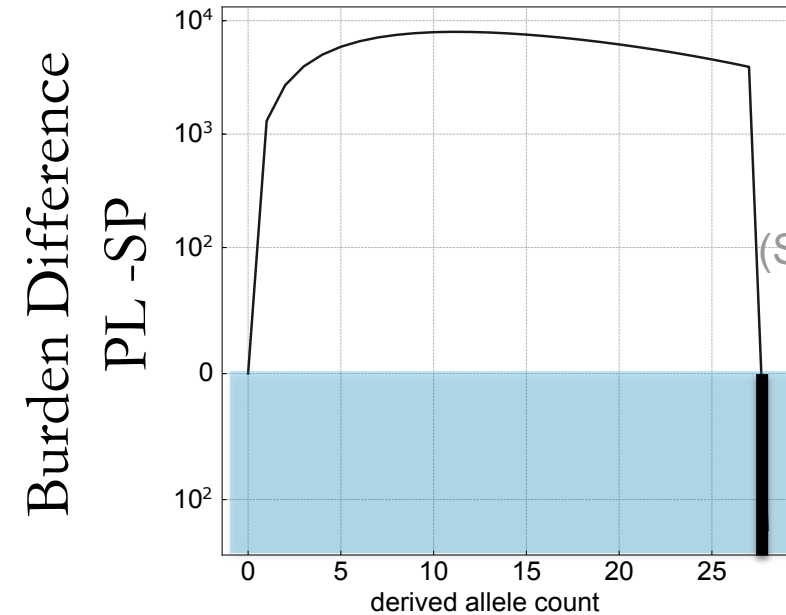
SP has a slightly higher burden with undetectable fitness effect



E. Koch
(Sunyaev lab)

BURDEN of deleterious mutations

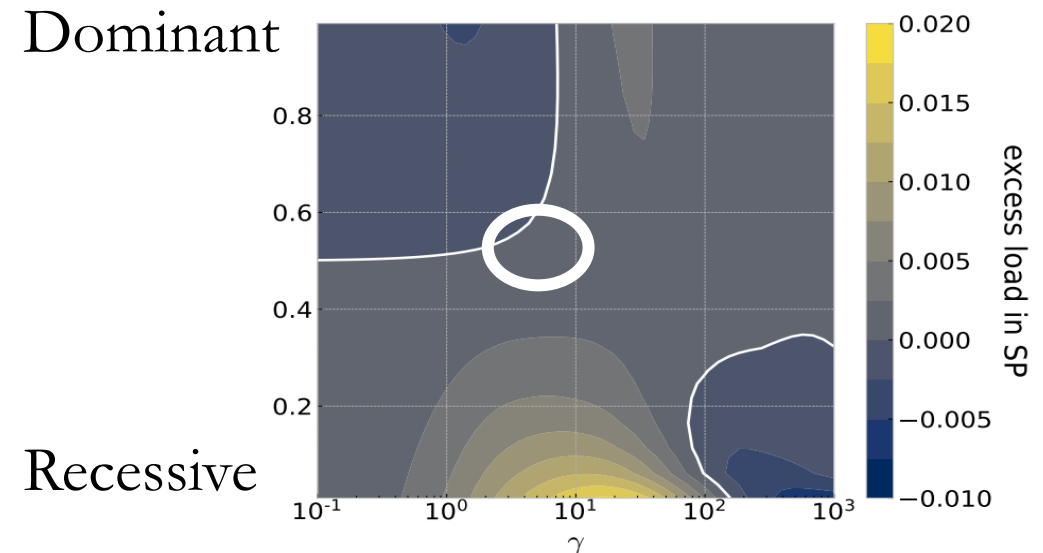
- PL more at low frequency
 - SP more at high frequency
 - Net difference is small
-
- the net difference is small, ~185 more in SP



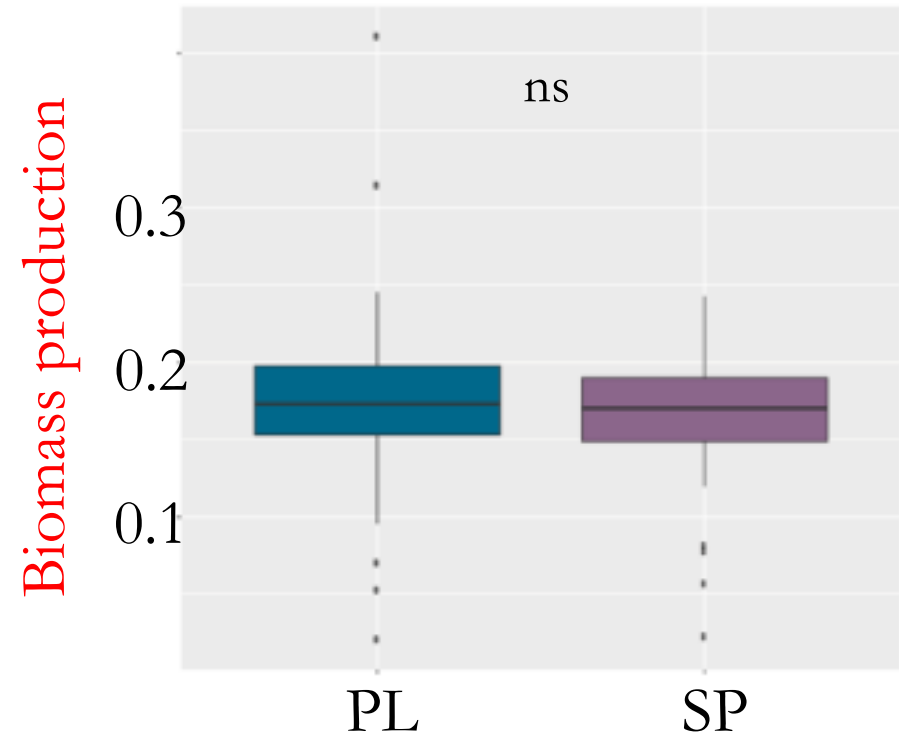
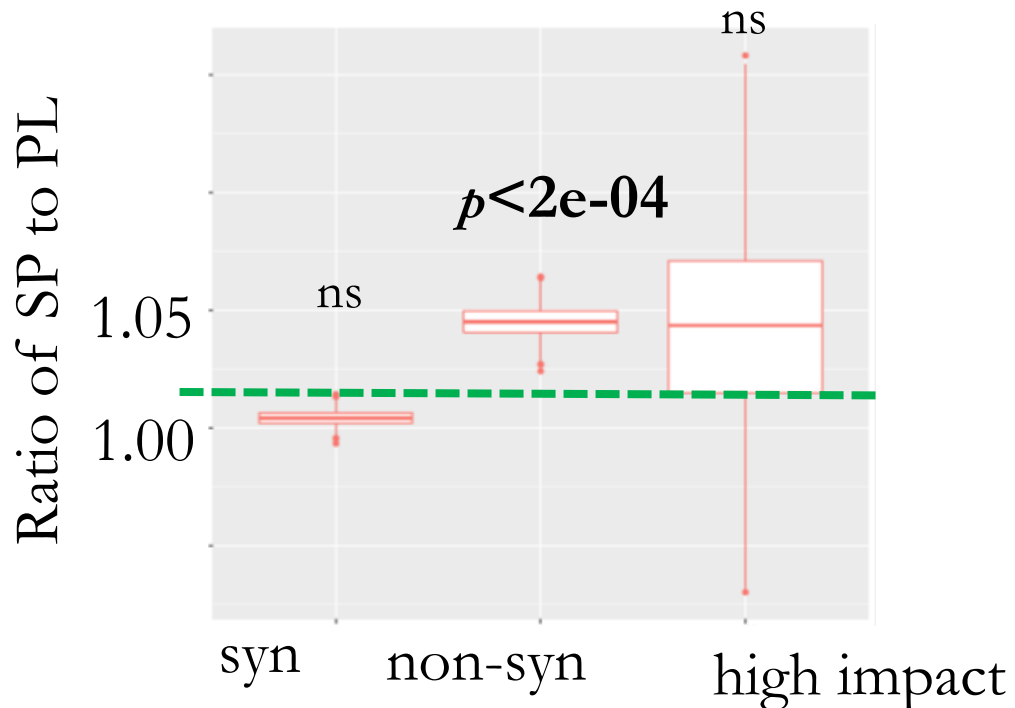
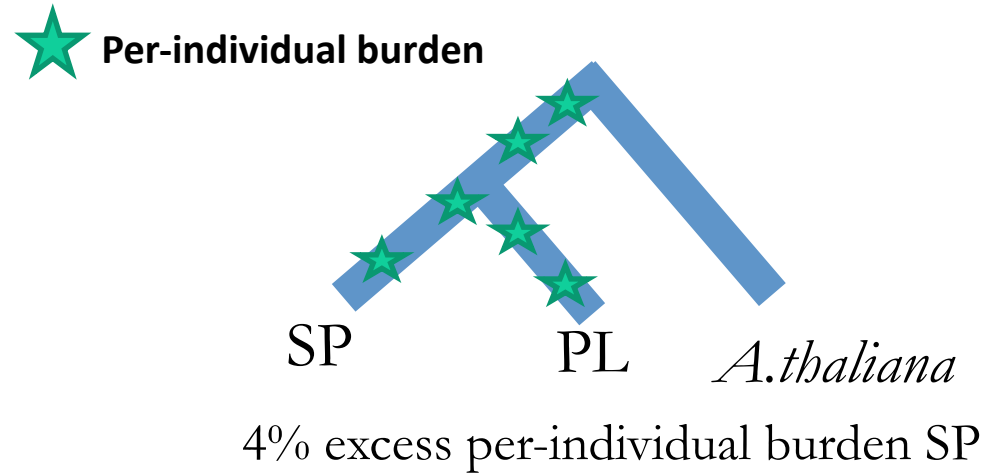
LOAD

Max:

- 2% for recessive mutations
 - 0.5% for codominant mutations
-
- Net load difference is expected to be UNDETECTABLE

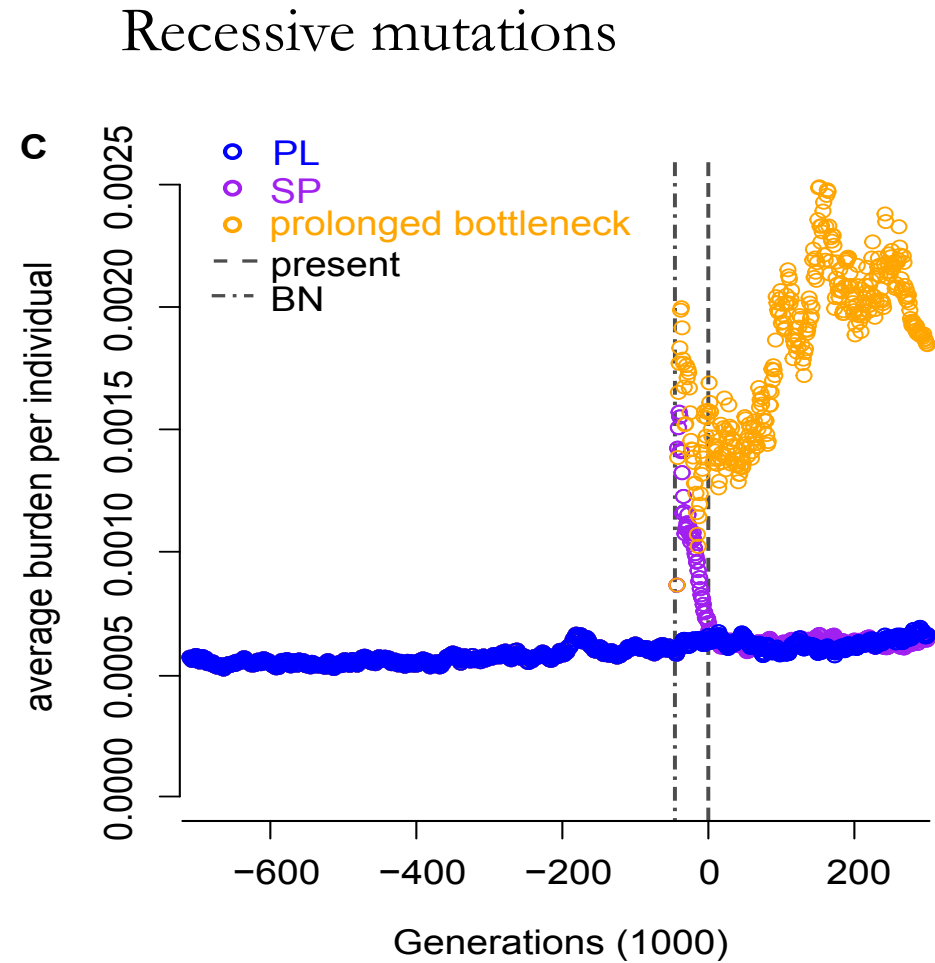
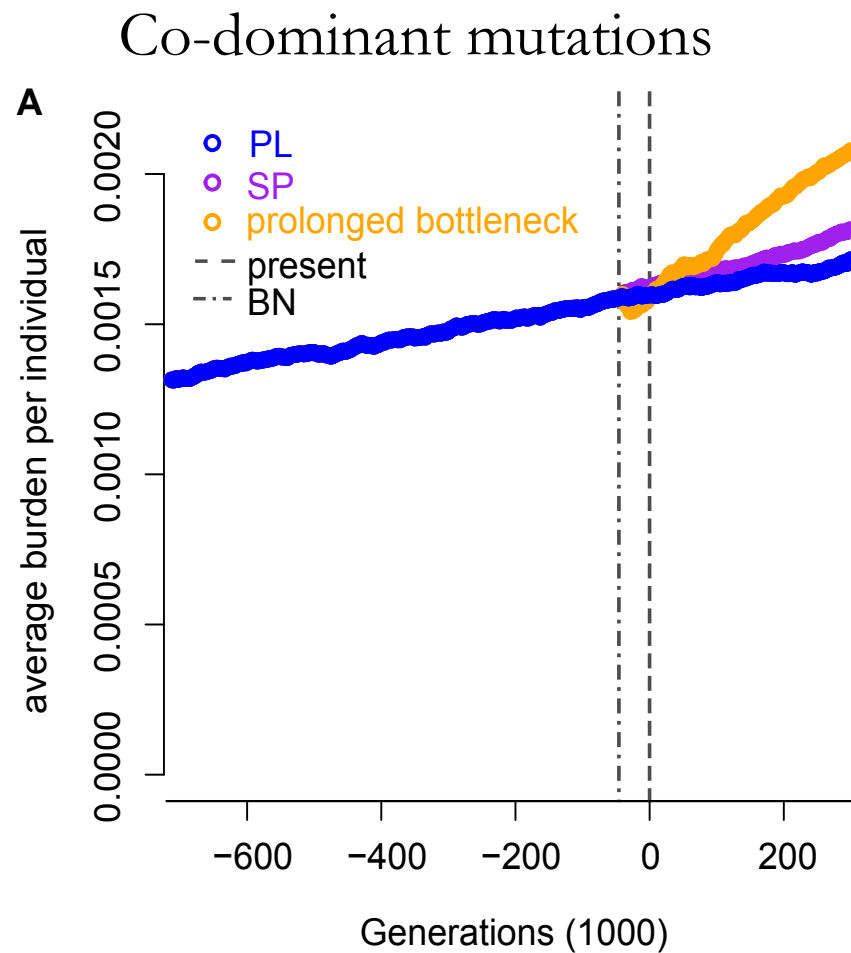


Accumulation of mutational burden with no impact on the fitness.

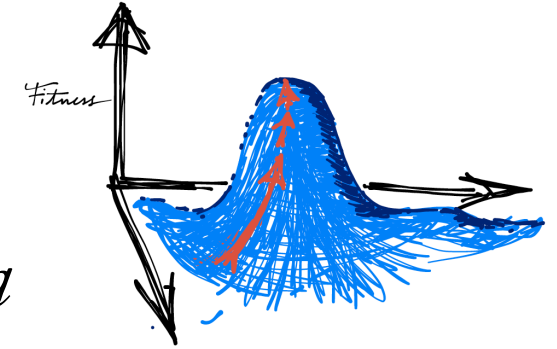


- ❖ Modeling of demographic history
- ❖ Common Garden Experiment

Too soon to see an increase in load

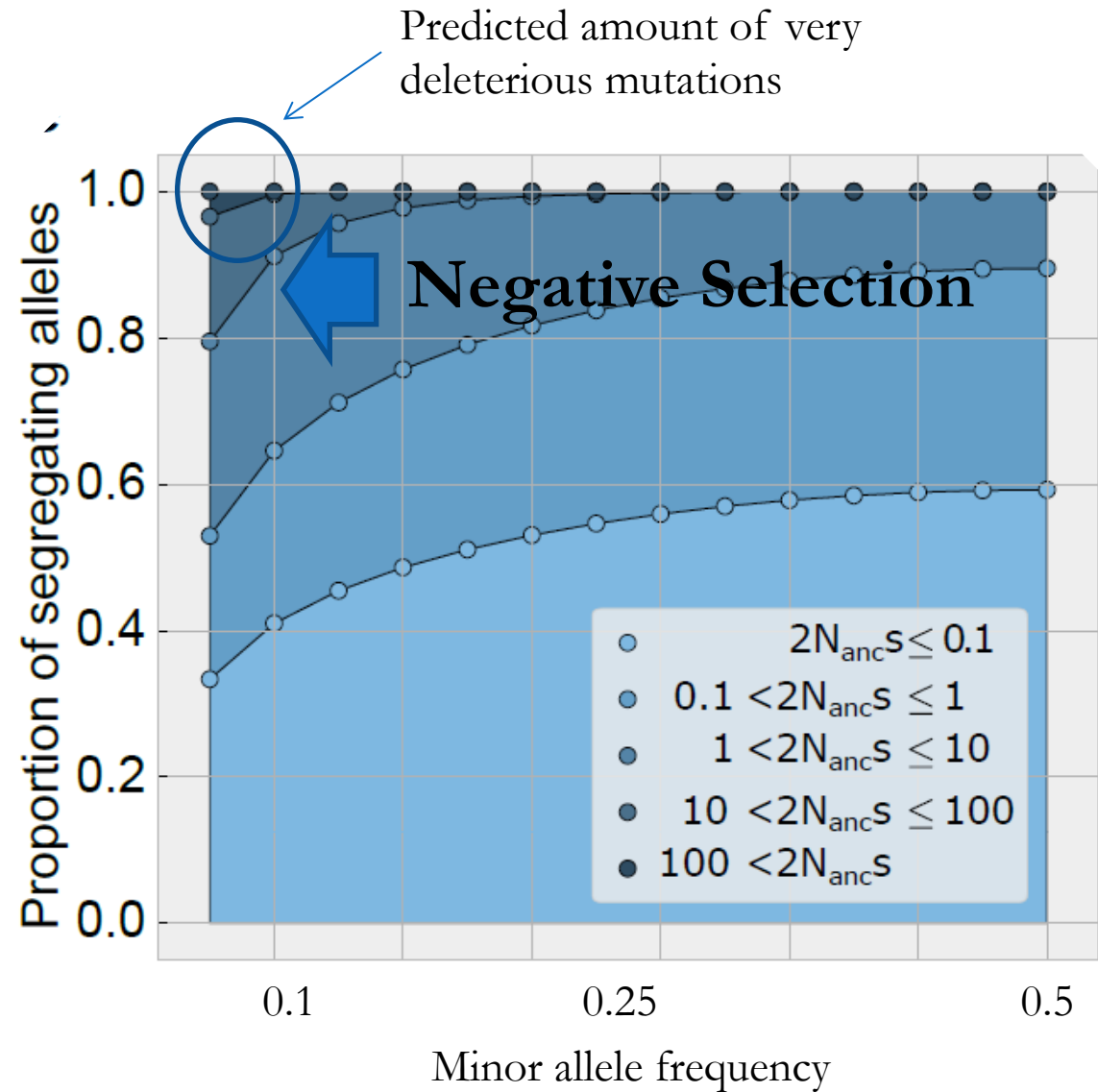


Outline

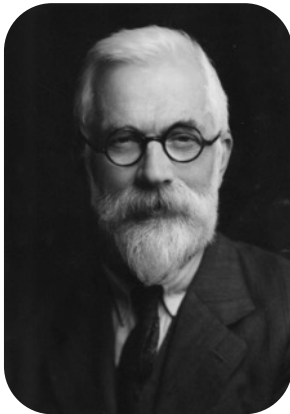


1. Our model system: *Arabidopsis lyrata ssp petraea*
2. The load is not significantly affected by recent bottlenecks
3. **Does selection against deleterious variation shape genetic variation?**
4. Can gene expression reveal the effect of polygenic selection?
5. Can we determine the adaptive relevance of plastic response to stress?

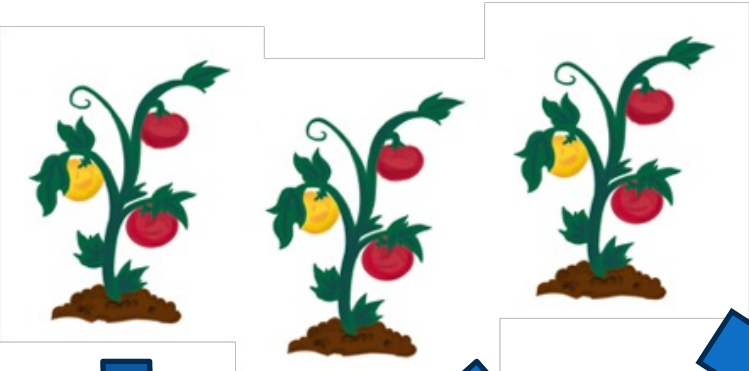
Does selection against deleterious variation shape gene expression variation?



Response to selection requires genetic variance



R.A. Fisher 1890 - 1962



The useful fraction of genetic variation
 V_A Additive genetic variance



V_{NA} Non-Additive genetic variance

The useless fraction of genetic variation

Non-heritable genetic variance?



H^2

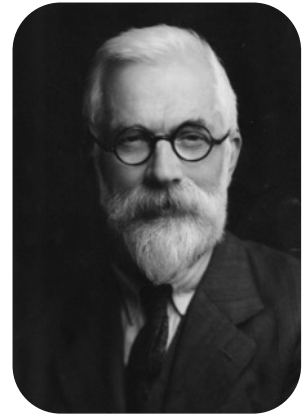
h^2

$$V_P = V_G + V_E$$

$$V_P = V_A + V_{NA} + V_E$$

Heritable Component
Additive Variance

Non-Heritable Component
Non -Additive Variance
Dominance Variance

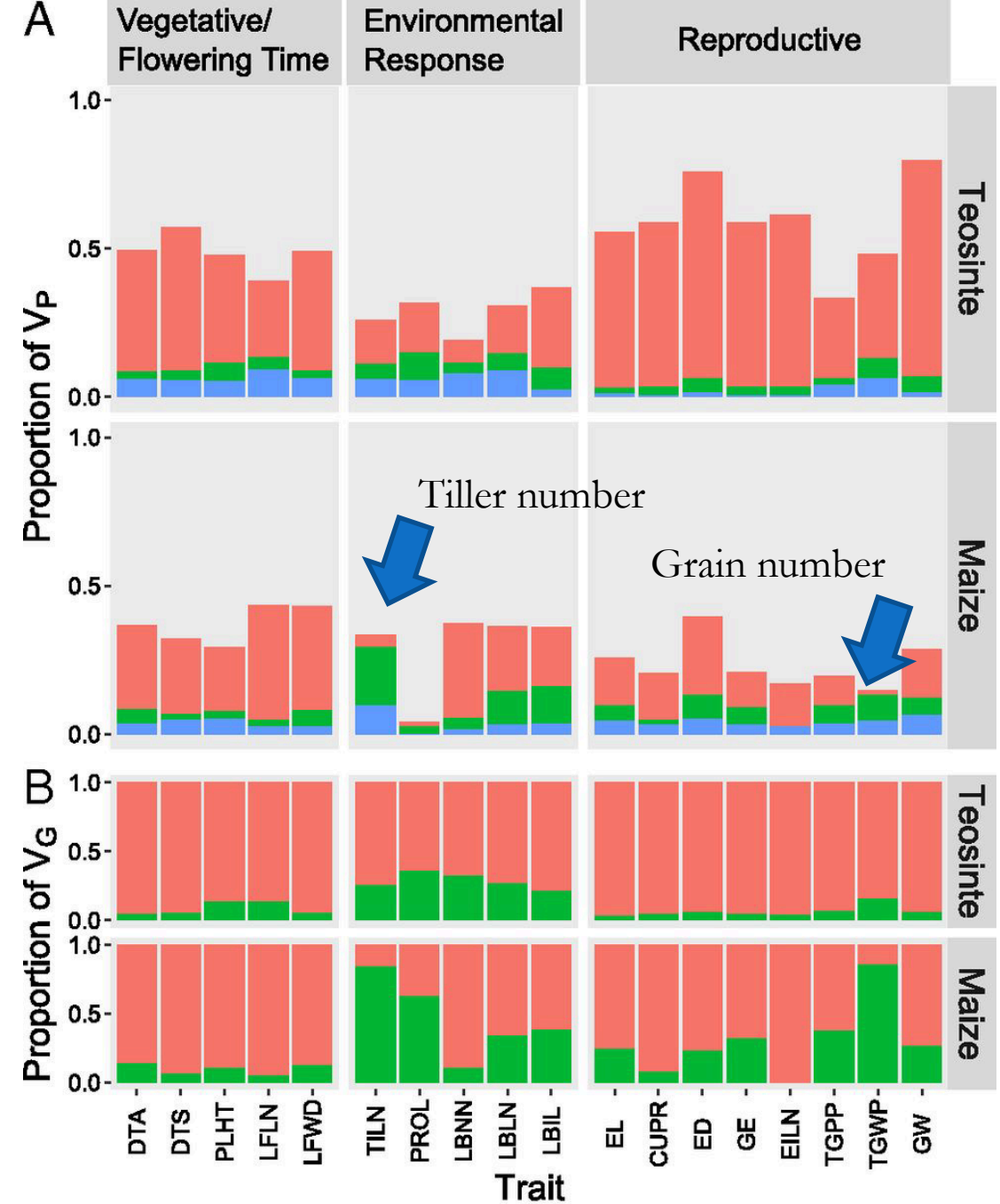
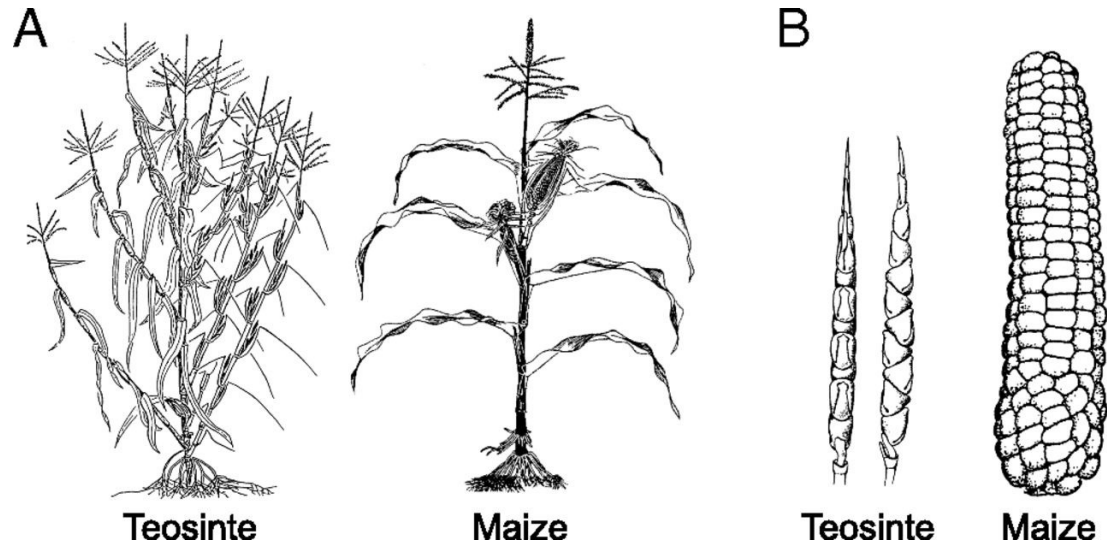


R.A. Fisher 1890 - 1962

V_A , the useful fraction of genetic variation

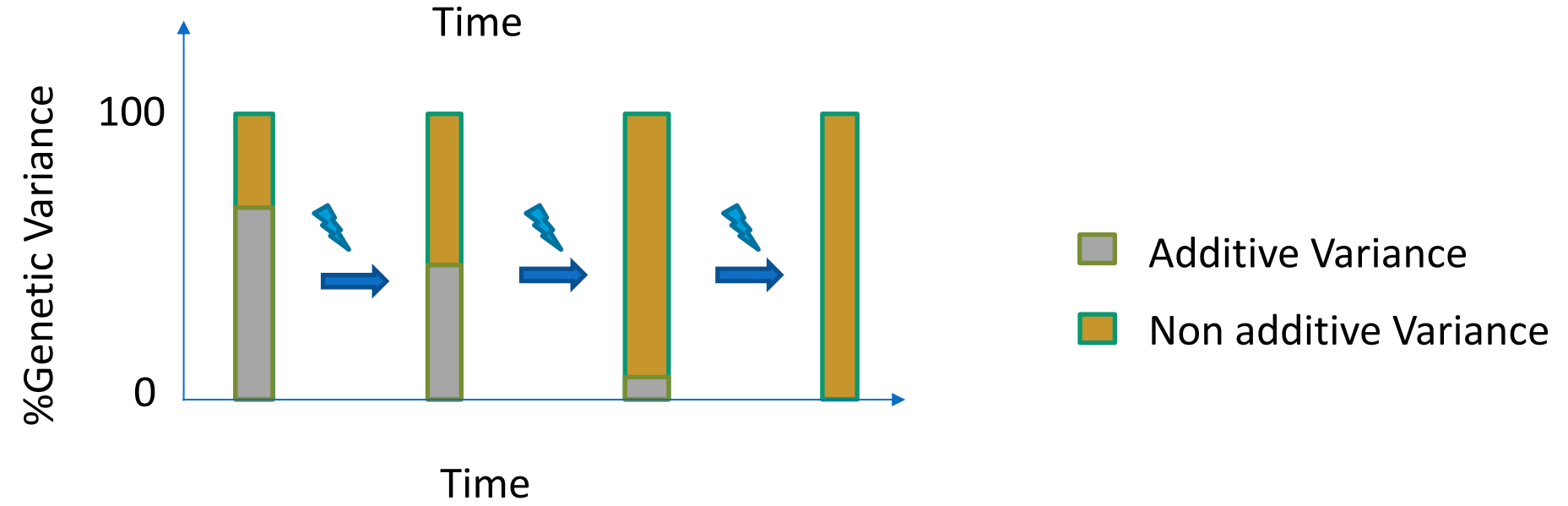
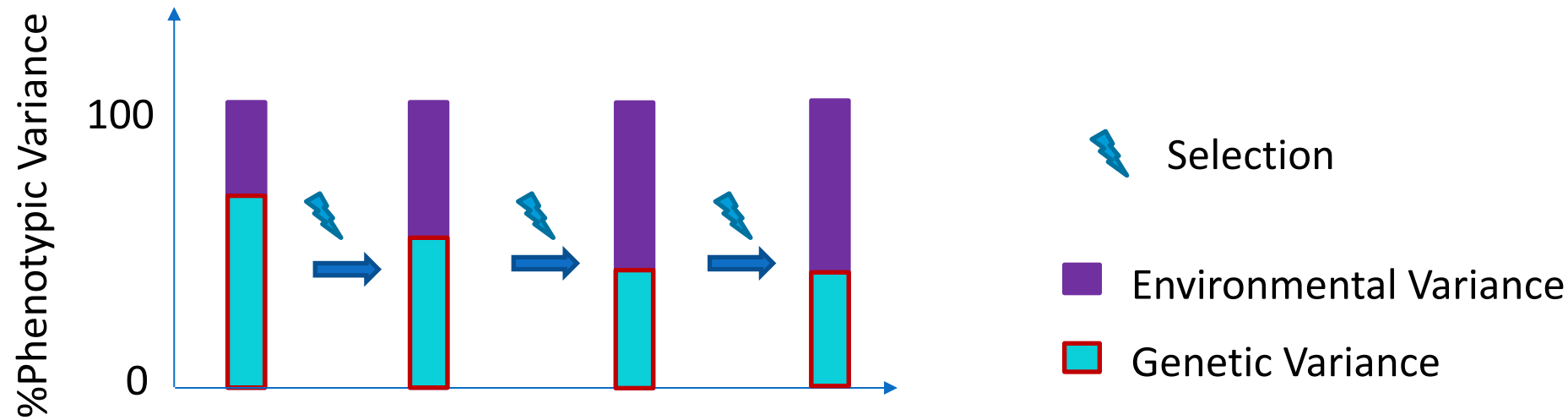
V_{NA} , the useless fraction of genetic variation

Some strongly selected traits display high levels of non-additive variance



Variance V_A V_D $V_{G \times E}$

If selection depletes additive variance, is there non-additive variance left?



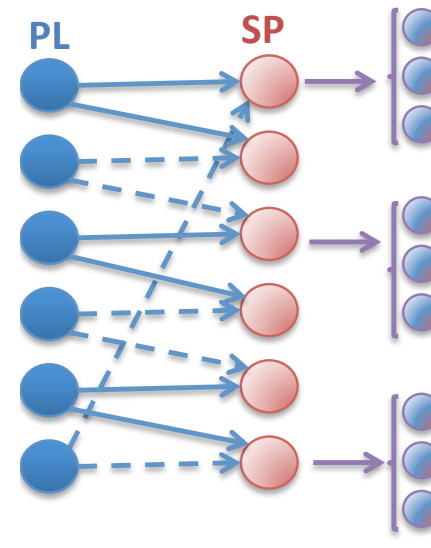
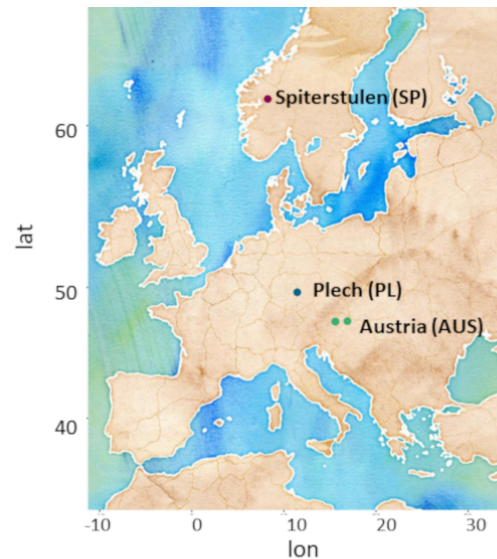
What determines levels of non-additive variance?

History of Selection?
Biology?



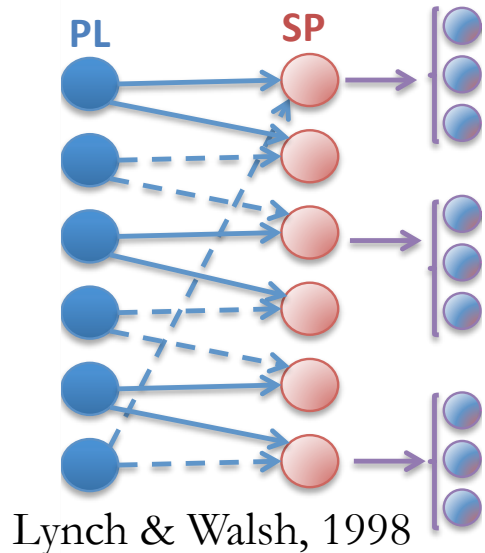
Margarita Takou

Genetic composition of *Arabidopsis lyrata* gene expression



- 130 Full and $\frac{1}{2}$ sibs
- Facilitates detection of non-additive variance
- RNAseq
- Estimates of V_A and V_{NA} for $\sim 17,000$ transcripts

Experimental estimation of additive and dominance variance components of genetic variation



- Full sibs

$$t_{FS} = \frac{1}{2} \frac{V_A}{V_p} + \frac{1}{4} \frac{V_{NA}}{V_p} + \frac{V_M}{V_p}$$

- $1/2$ sibs

$$t_{1/2S} = \frac{1}{4} \frac{V_A}{V_p}$$

Plant advantage

- Control for maternal environment (reciprocal crosses)
- Control for environment (common garden)
- V_A and V_{NA} can be estimated

Precise estimation of heritability components is possible in plants

- 131 inter-population families
- RNAseq
- Estimates of V_A and V_{NA} for $\sim 17,000$ transcripts

Holger Schielzeth,
FSU, Jena

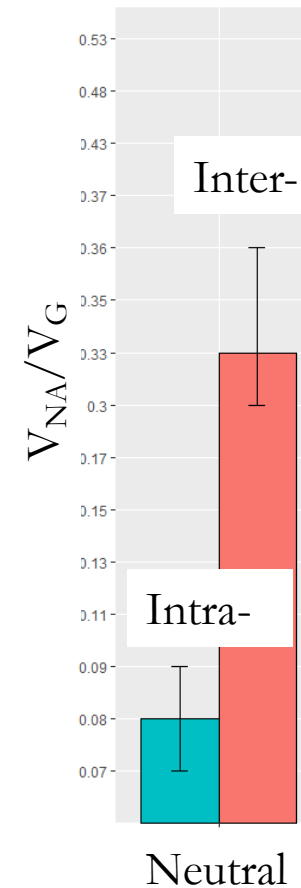
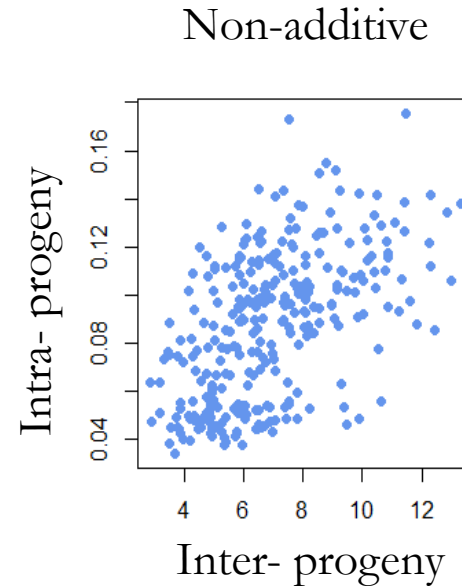
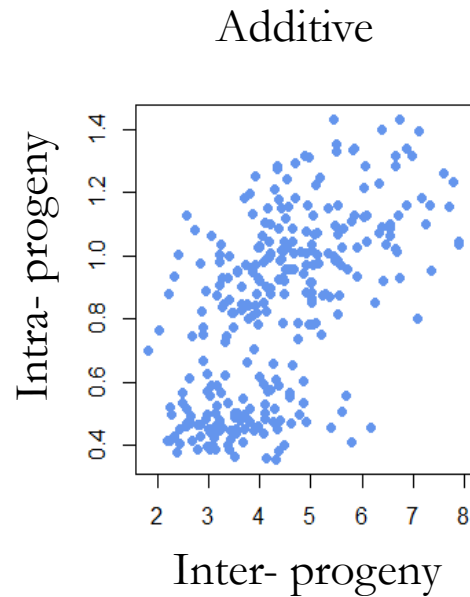
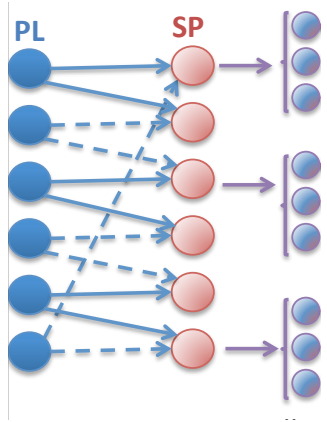


Inter-population progenies facilitate quantification of non-additive genetic variance



Josselin Clo,
CNRS Lille

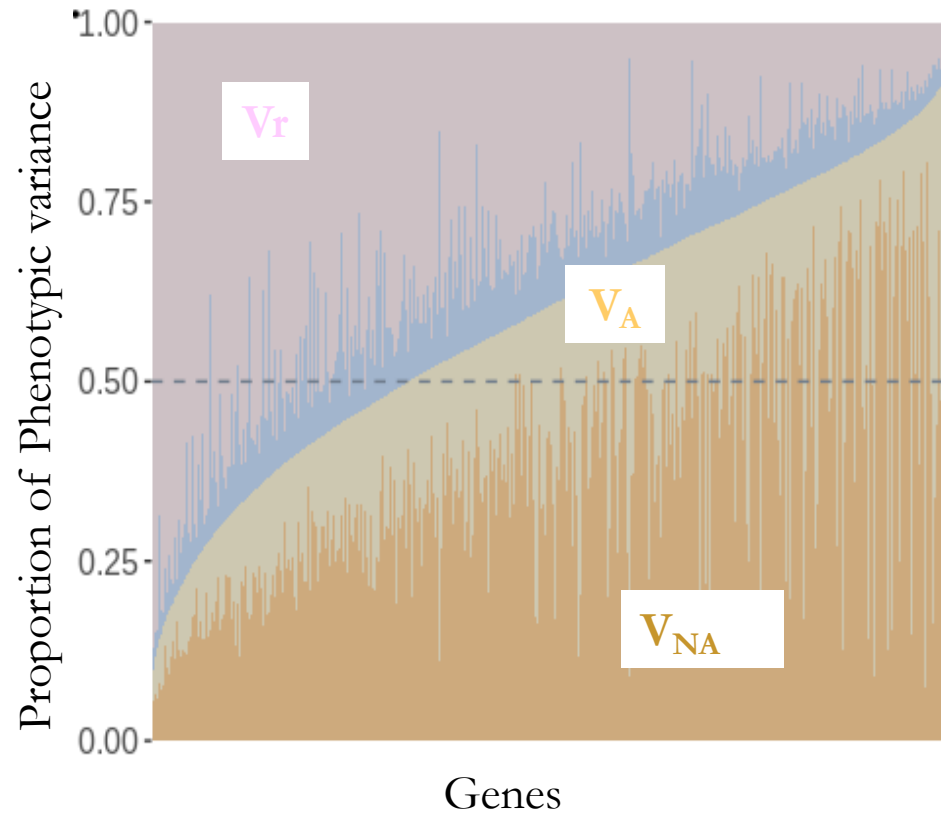
Clo & Opedal, 2021



Interpopulation progeny

- more heterozygotes
- more variants
- Correlates with intra-population variance
- Higher genetic variance
- Higher non-additive variance

Genetic composition of *Arabidopsis lyrata* gene expression



- **67% of genes with $V_A + V_{NA} > 50\% V_P$**
- **Mean $V_A = 0.21 V_P$, Mean $V_{NA} = 0.37 V_P$**
- **V_{NA} predominates for 25% of transcripts**
- **Does the strength of negative selection associate with V_{NA} ?**

Some traits display high levels of non-additive variance, but which ones are linked to selection?

338 traits: Higher fraction of V_{NA} in life history traits (assumed to be more closely related to fitness than morphological traits)

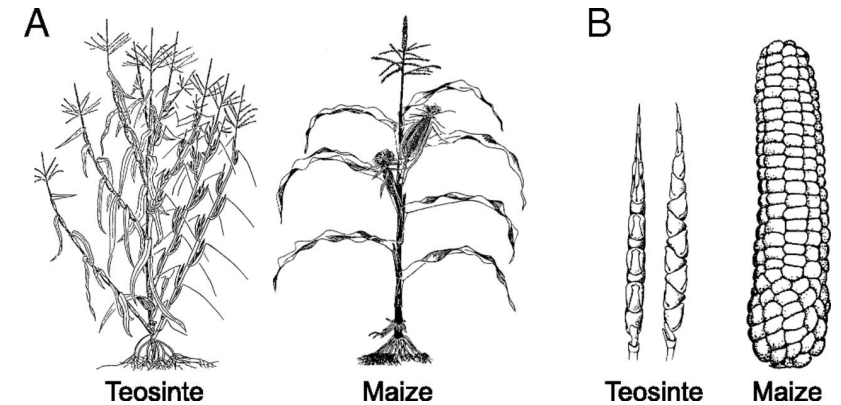
Crnokrak and Roff 1995

8 wing traits in *Drosophila*: no significant covariance of V_{NA} with male competitive ability

Sztepanacz and Blows 2015

18 traits in maize and teosinte : Tiller number and grain number show higher fraction of V_{NA} in maize, not teosinte.

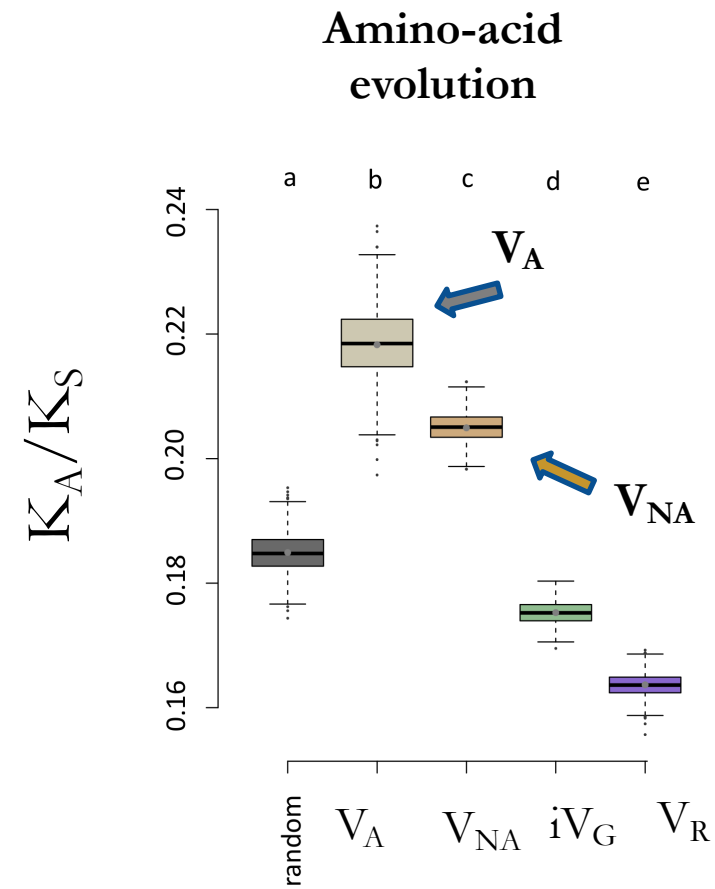
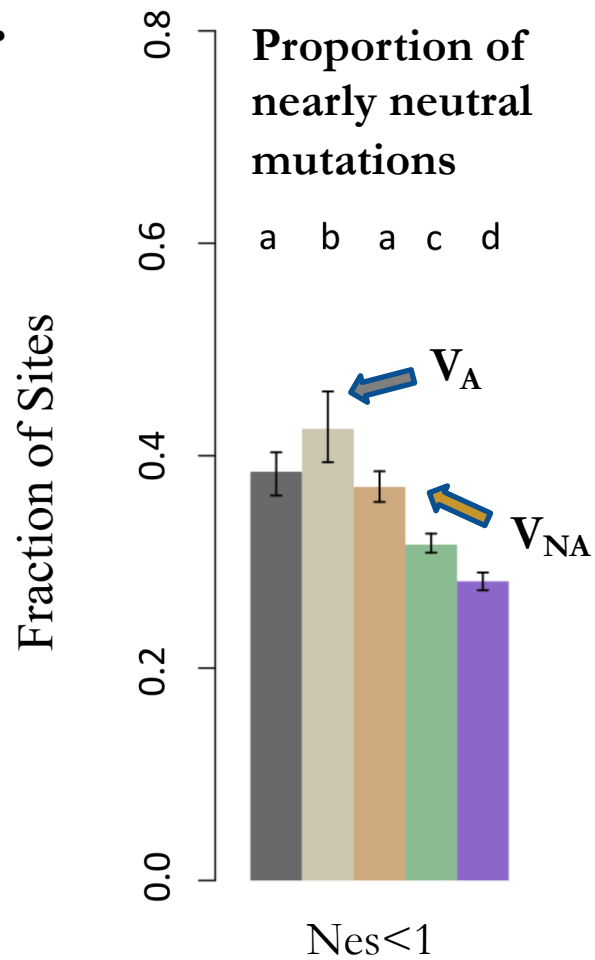
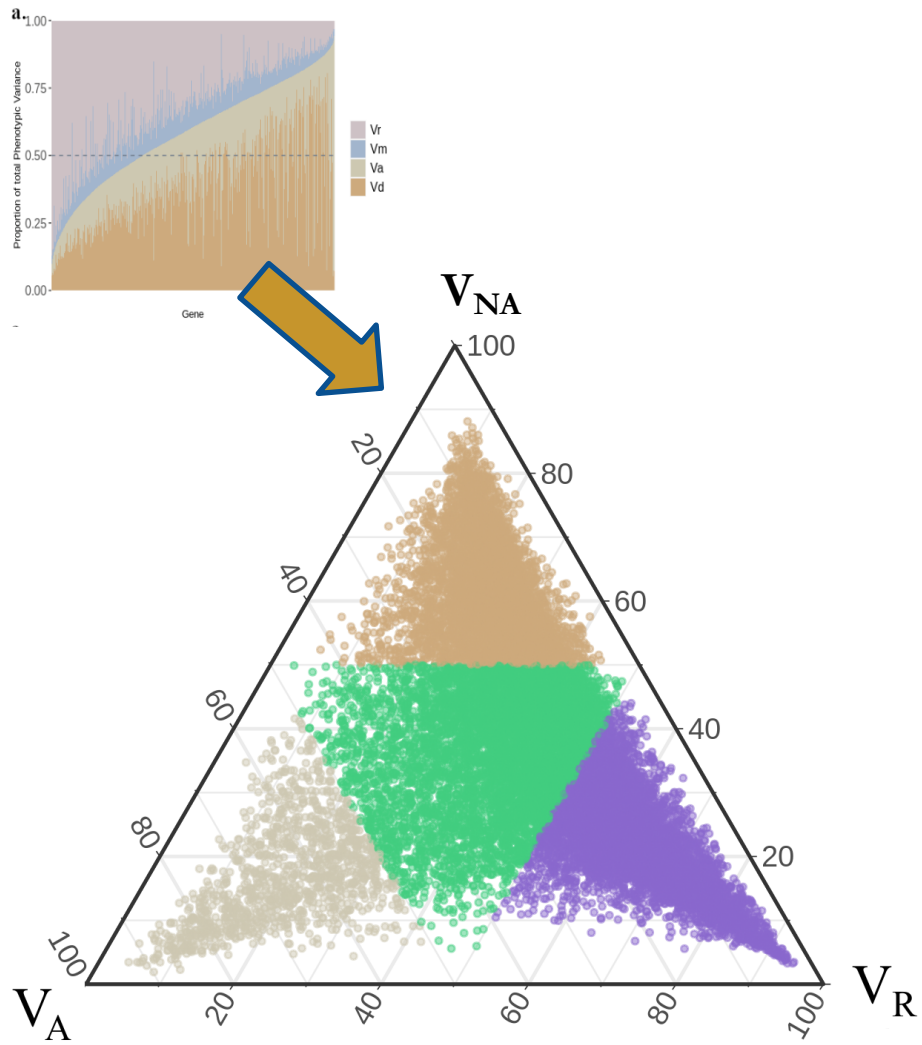
Yang et al. 2019



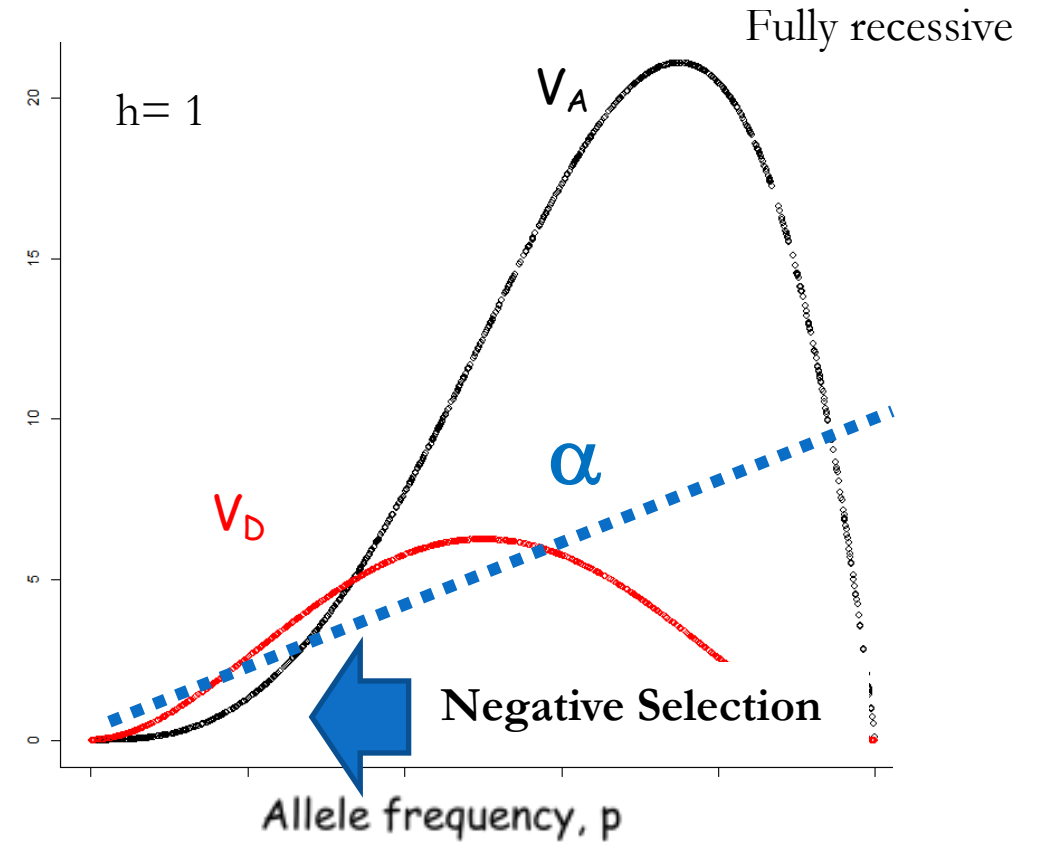
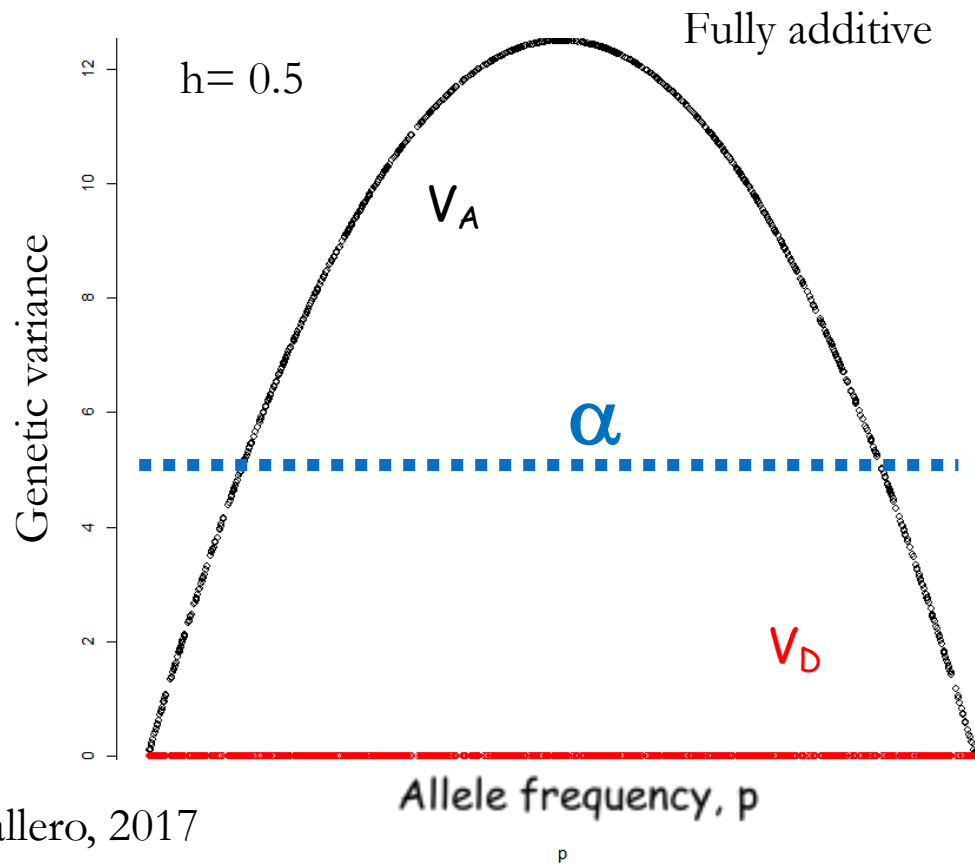
10 000 expressed genes in sticklebacks, no evidence for V_{NA} linked to Q_{st}

Leder et al. 2015

Fraction of V_{NA} depends on the strength of natural selection



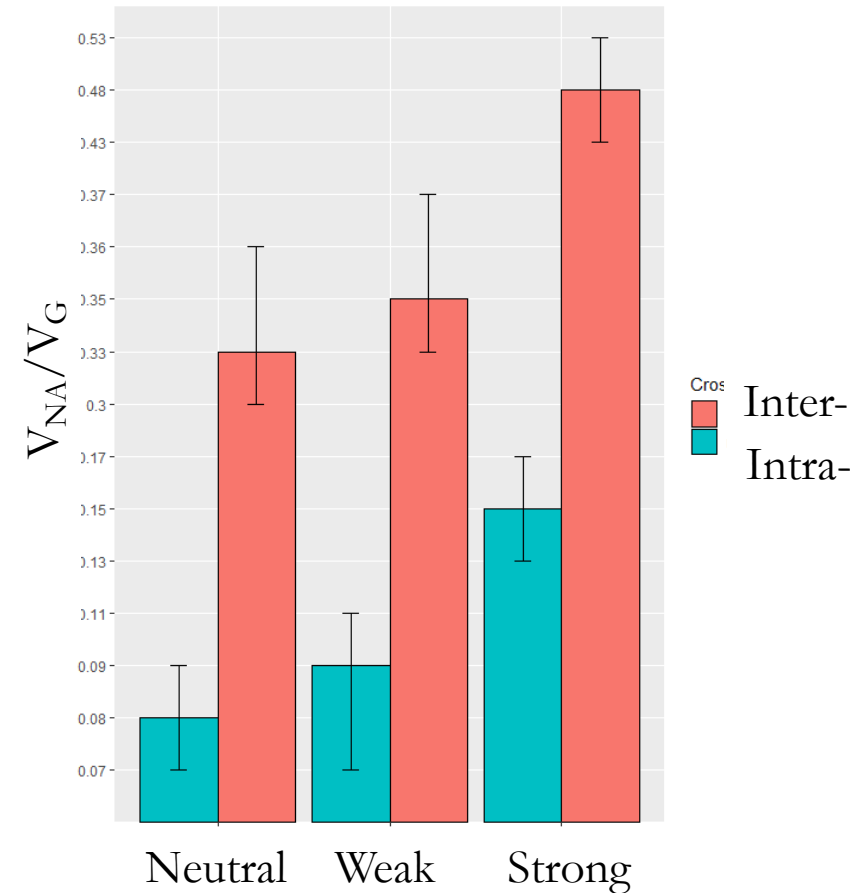
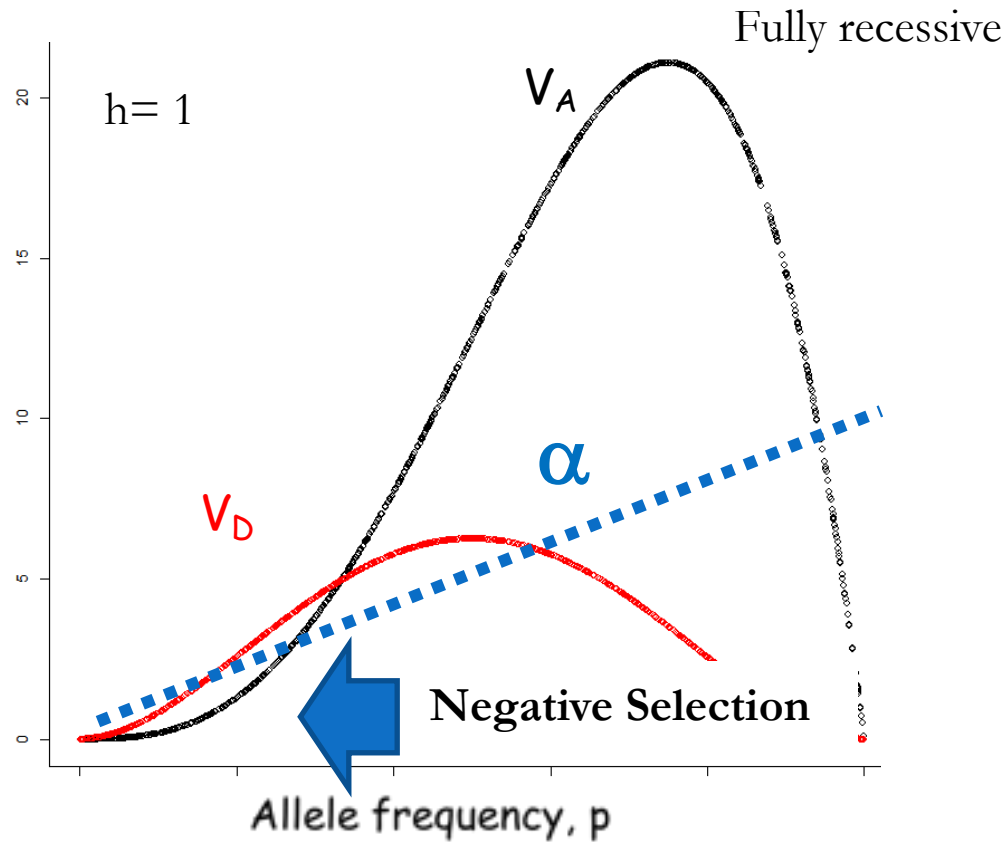
Why should non additive variance reflect selection?



Caballero, 2017

Allelic relationships modify the frequency range of maximum additive variance and allele substitution effects

Why should non additive variance reflect selection?

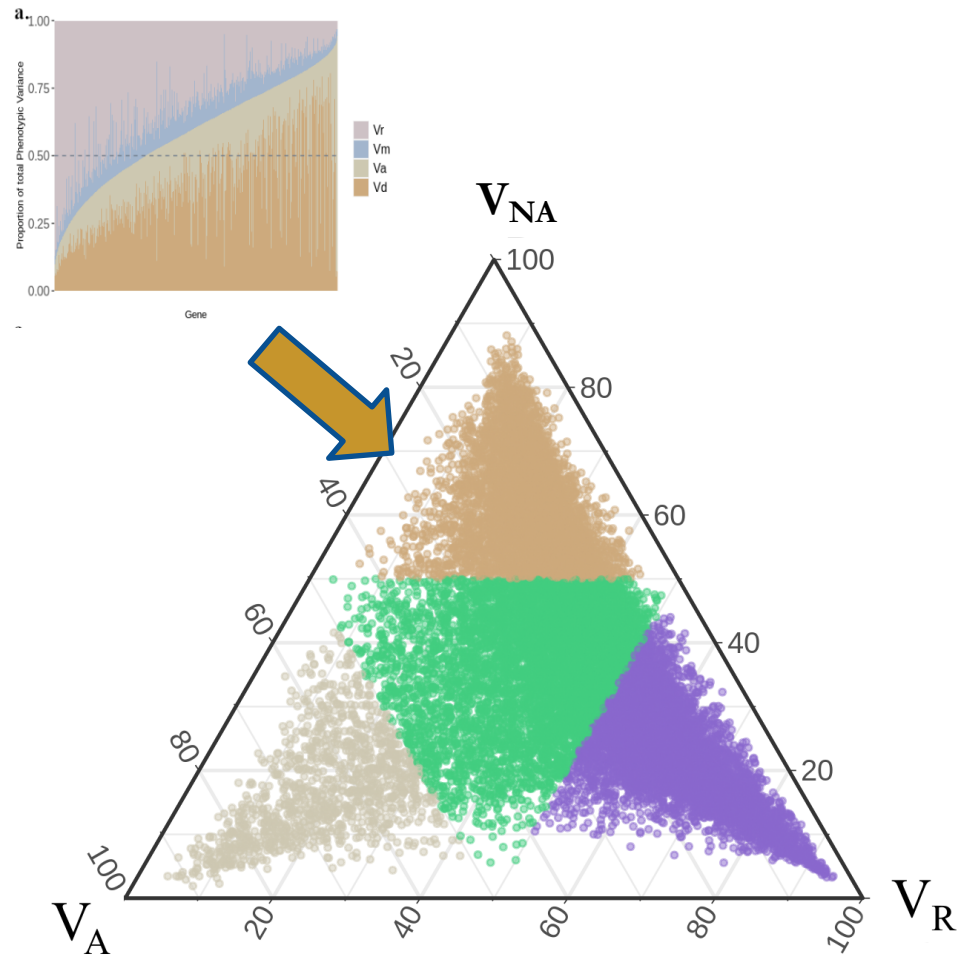


Selection decreases V_A more than V_{NA}
 Effect is stronger in inter-pop progenies

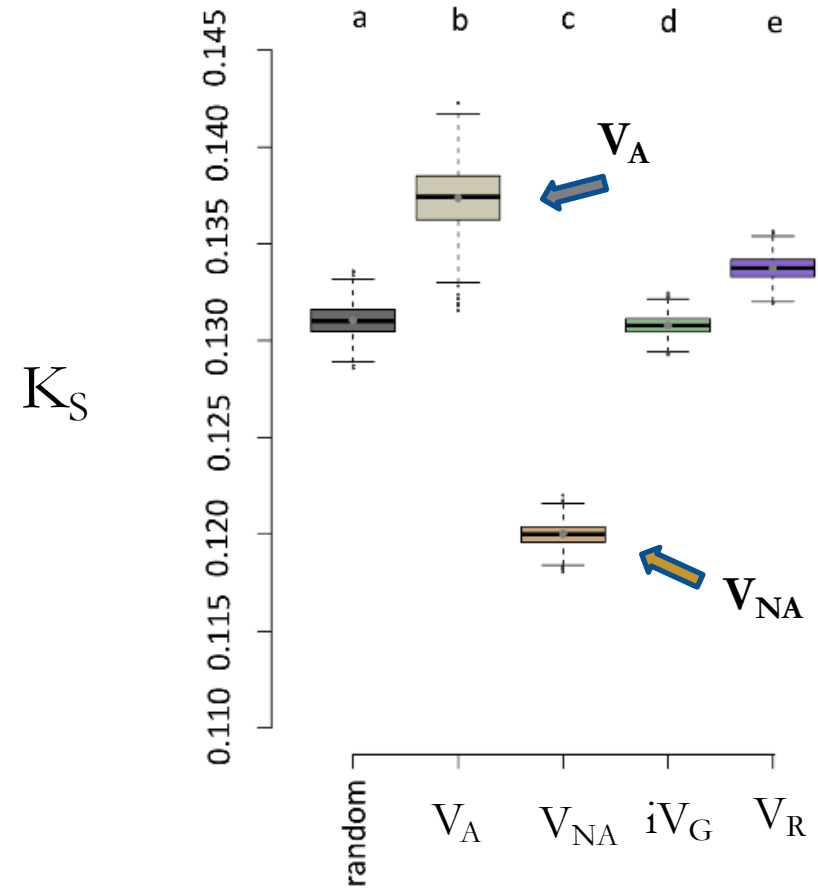
What determines levels of non-additive variance?

- ✓ **History of Selection
Biology?**

Mutational sources of V_{NA}



Lowest K_s for V_{NA} genes



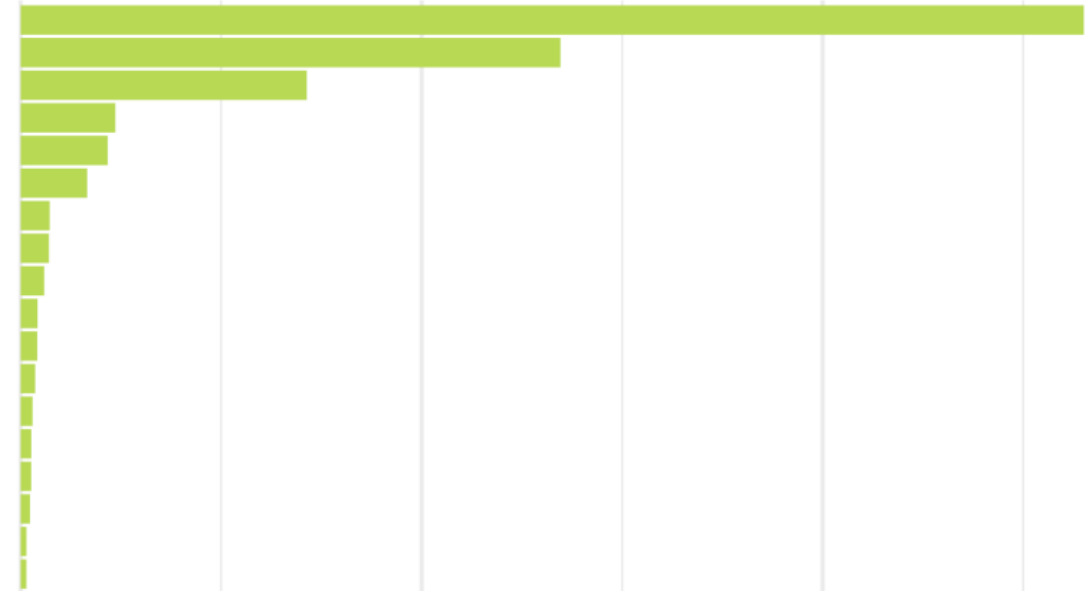
Descriptors of genomic architecture and regulation explain V_{NA}



Random Forest

- ❖ total gene length .
- ❖ transcript length
- ❖ number of exons
- ❖ total number of transcription factors binding sites (TFBS)
- ❖ TF- specific TFBS
- ❖ Within sweep area or not
- ❖ Differential gene expression between populations
- ❖ Gene mean F_{st}
- ❖ π of parental populations
- ❖ $tajD$ of parental populations
- ❖ D_{xy}
- ❖ SNP Density of the transcript

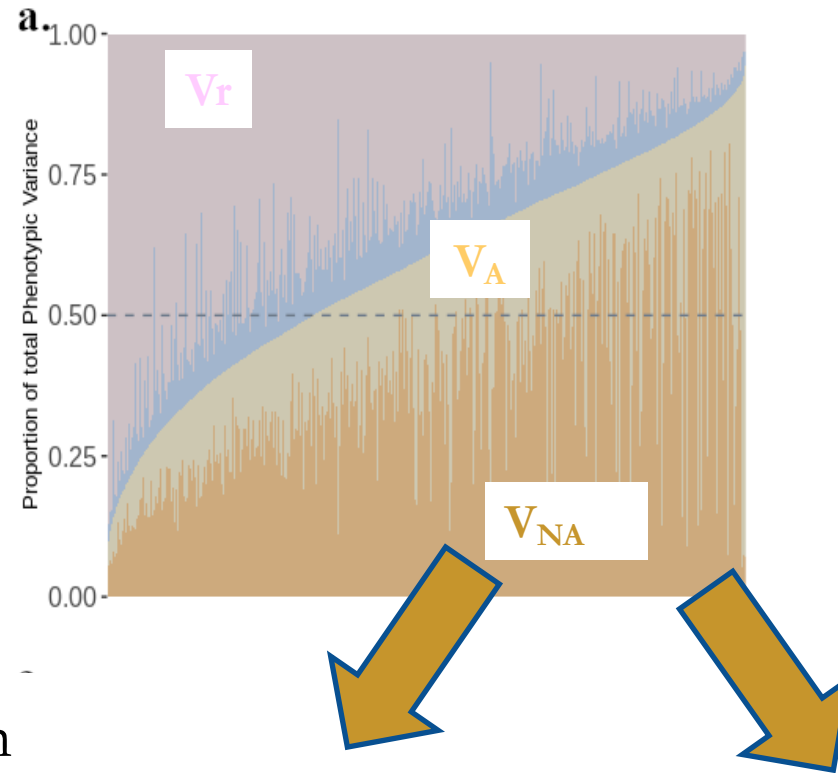
→ Exons
→ Length
transcript.length
DOF5.3
→ total
Density
AT3G24120
AHL12
AGL55
ATHB.16
GenesDEG
PL.pi
DOF2.4
KAN1
DOF5.6
AGL42
AHL20
REF6



$R^2 = 0.18$

- ❖ Gene length,
- ❖ exon number,
- ❖ Binding transcription factor stem thickening

V_{NA} and biology



Fraction V_{NA} increases when

⇒ expression variation more polygenic

⇒ V_G lower

⇒ longer transcripts

⇒ Larger coexpression clusters

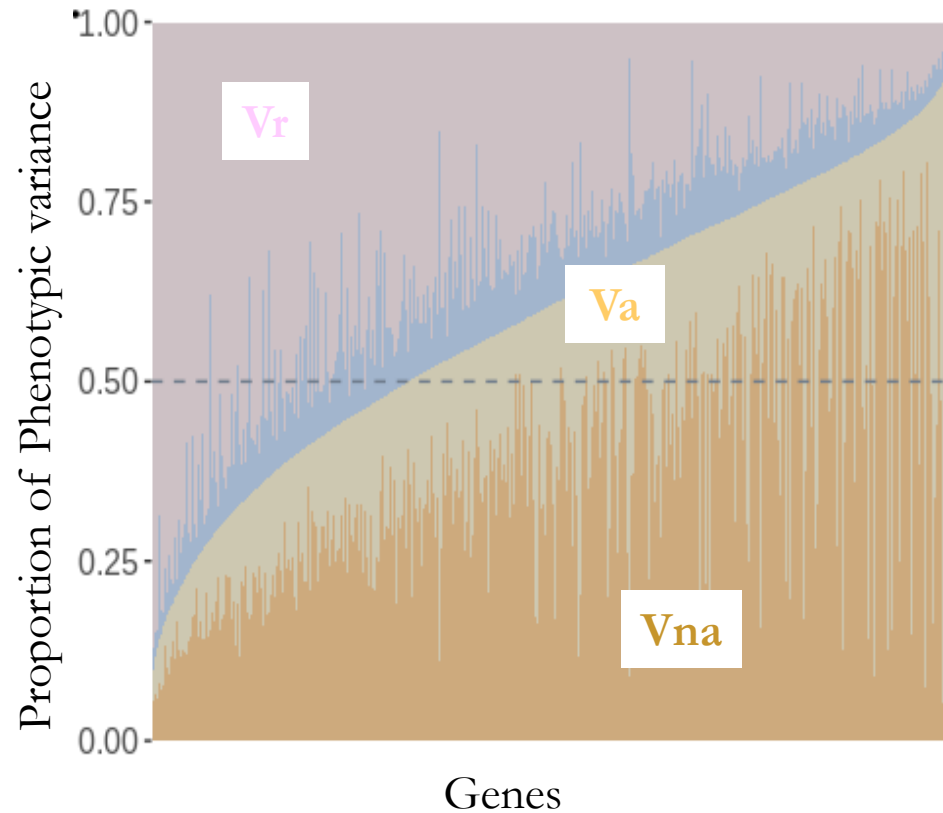
⇒ gene regulation associated with cell differentiation

Function (GO enrichment):

Epigenetic modifiers

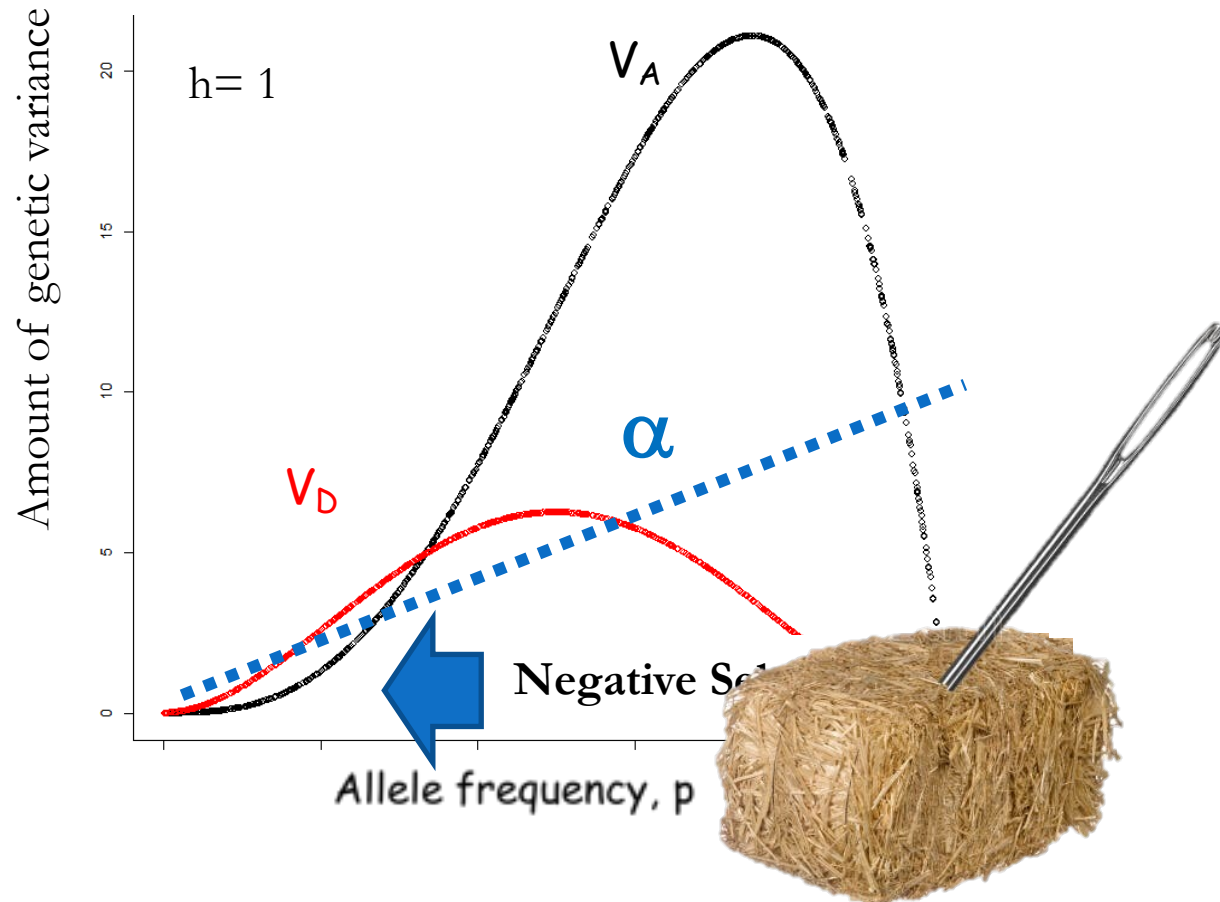
- “chromatin silencing” ($p = 4.5e-16$),
- “DNA methylation” ($p = 4.3e-11$),

Conclusion: Inheritance of *Arabidopsis lyrata* gene expression



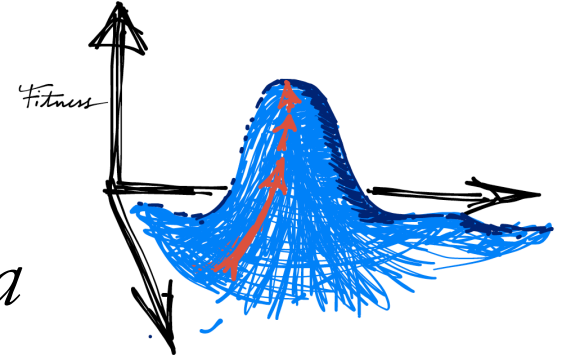
- V_{NA} predominates for 25% of transcripts in inter-population families
- V_{NA} increases in genes subjected to stronger negative selection
- High V_{NA} genes enriched among epigenetic modifiers

Purifying selection shapes genetic variance in expression



- Interpopulation cross = more heterozygotes
- Excess heterozygosity makes dominance variance easier to detect
- Purifying selection increases non-additive genetic variance in expression
- (at least in outcrossing populations)
- **The hay stack shapes the needle!**

Outline



1. Our model system: *Arabidopsis lyrata ssp petraea*
2. Load is not yet affected by postglacial bottlenecks
3. Selection against deleterious variation shapes genetic variation for expression
4. **Can gene expression reveal the effect of polygenic selection?**
5. Can we determine the adaptive relevance of plastic response to stress?

Complex trait: drought stress tolerance



- ✓ Key relevance in climate change
- ✓ Major limitation of food security
- ✓ Urgent need for sustainable solutions in plant crop breeding

Drought survival: a complex trait



Survival to drought depends on

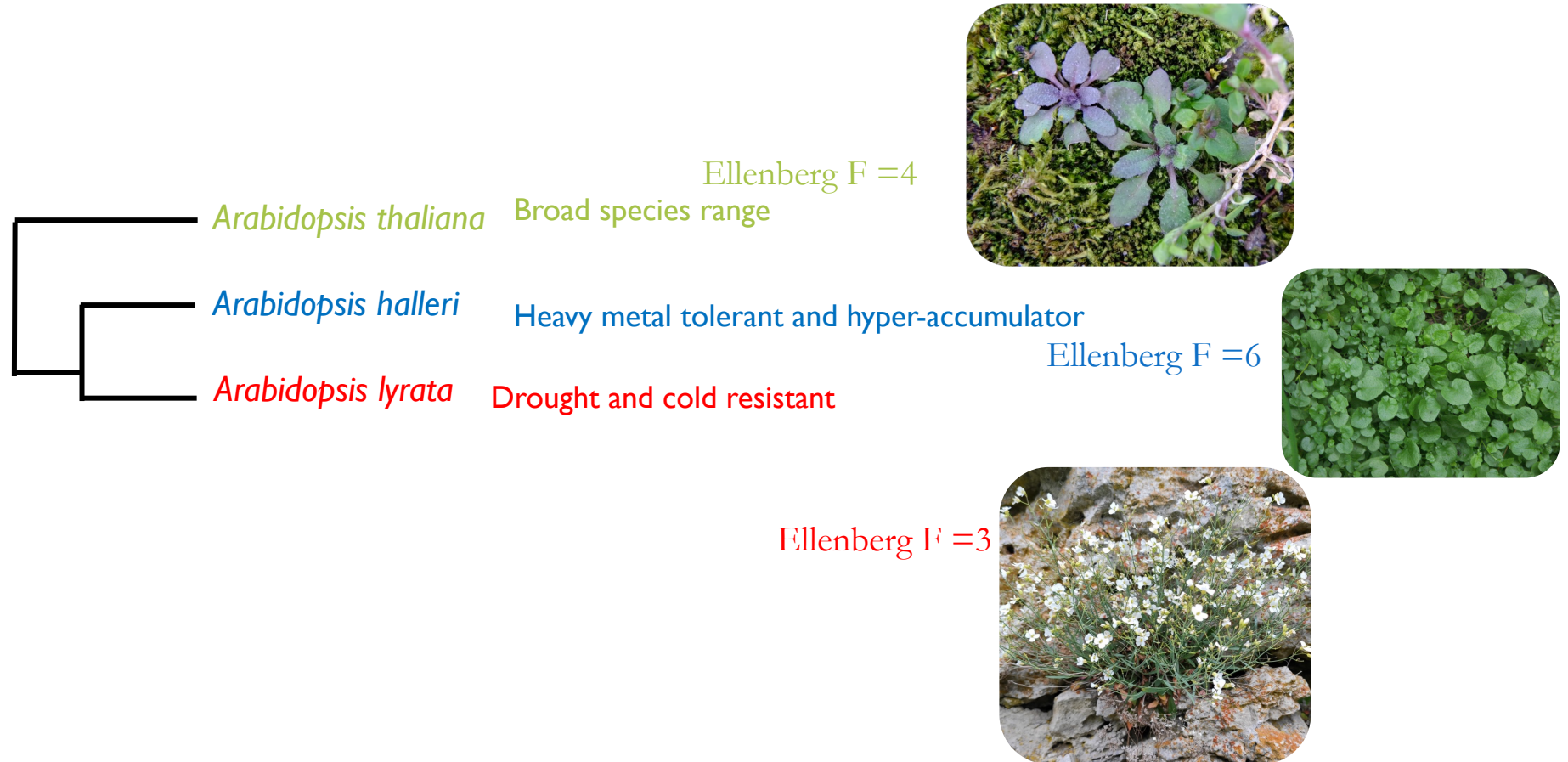
- Constitutive physiology and morphology
- Short term reaction to stress and homeostatic capacity
- Growth vigor in conditions of stress

Three major strategies:

ESCAPE - AVOIDANCE - TOLERANCE

Arabidopsis lyrata: ecologically distinct from congeneric species

Arabidopsis species display distinct life history and ecological characteristics:



Sampling

Plant material

A. thaliana
A. lyrata
A. halleri

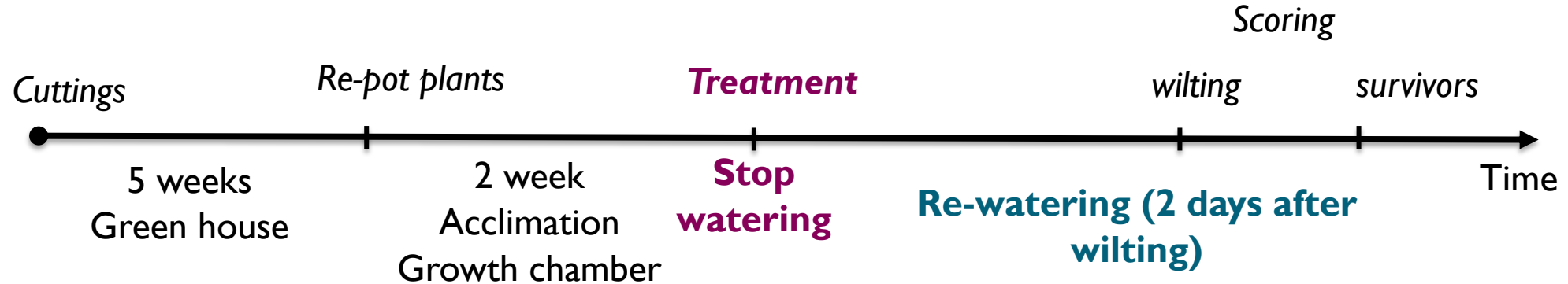


Geographical distribution of populations

Mimicking missing precipitations

Protocol: Measuring reactions to missing precipitations

Assess the ability to deal with **severe water stress** due to long-lasting no rain period.



Phenotypic monitoring:

- ✓ soil water content,
- ✓ wilting status,
- ✓ leaf thickness,
- ✓ PAM
- ✓ survival,
- ✓ damage

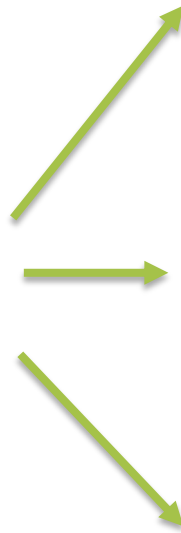
drought stress: *A. lyrata* displays avoidance and tolerance

A. halleri

A. lyrata

A. thaliana

3 main strategies



Dehydration tolerance

Survival to wilting

Limit stress damage

+

+++

-

-

++

--

Dehydration avoidance

Reduce transpiration

Maintain leaf water level

Delay wilting

--

++

++

--

++

++

--

-+

++

+

Drought escape

Fast development

--

--

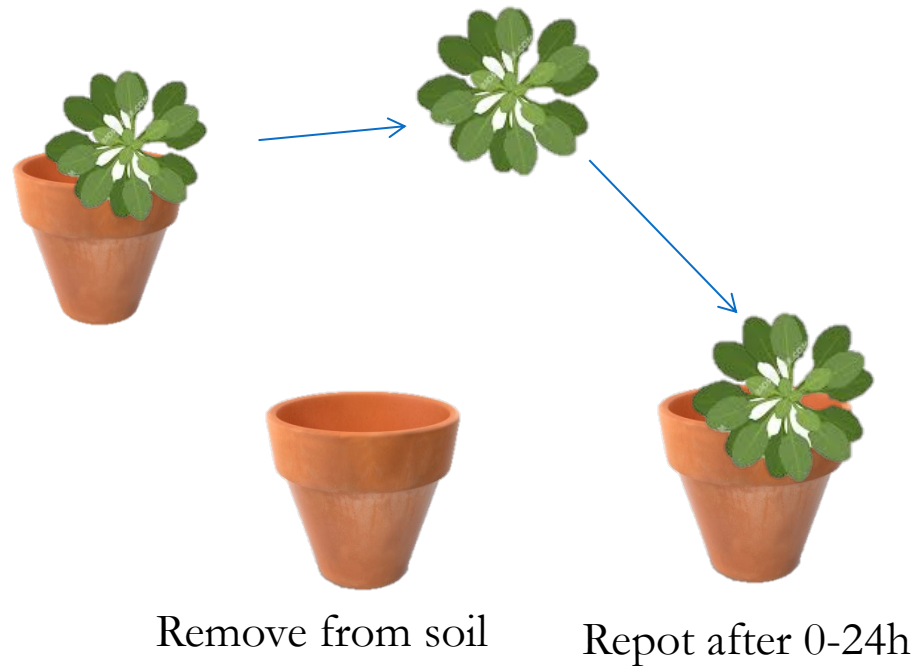
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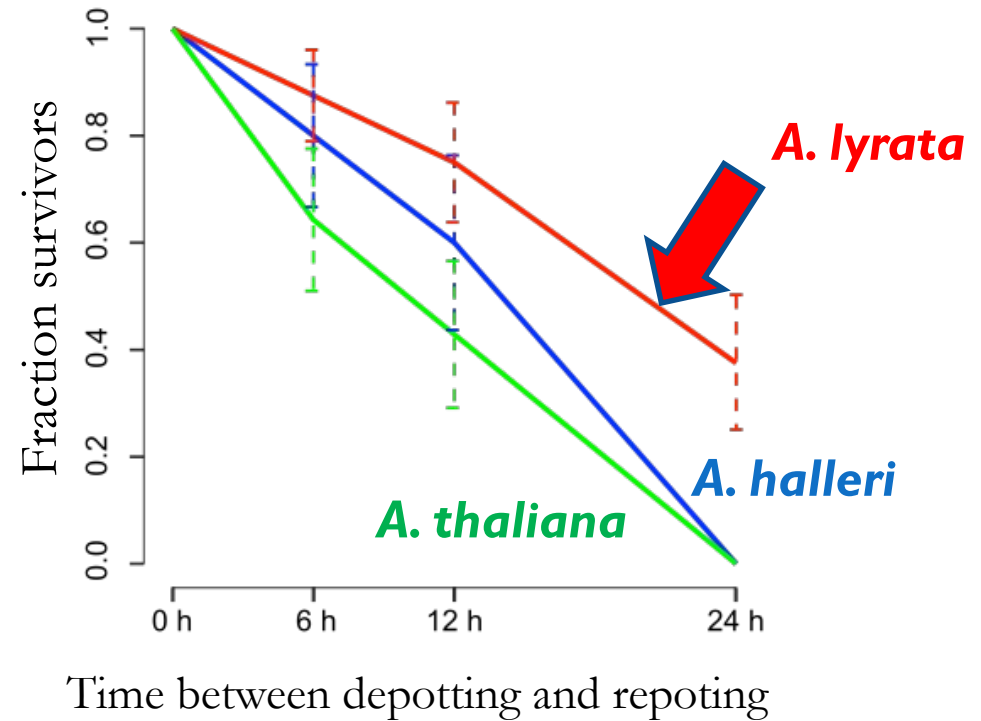
Maroua Bouzid

A. lyrata most tolerant to acute dehydration

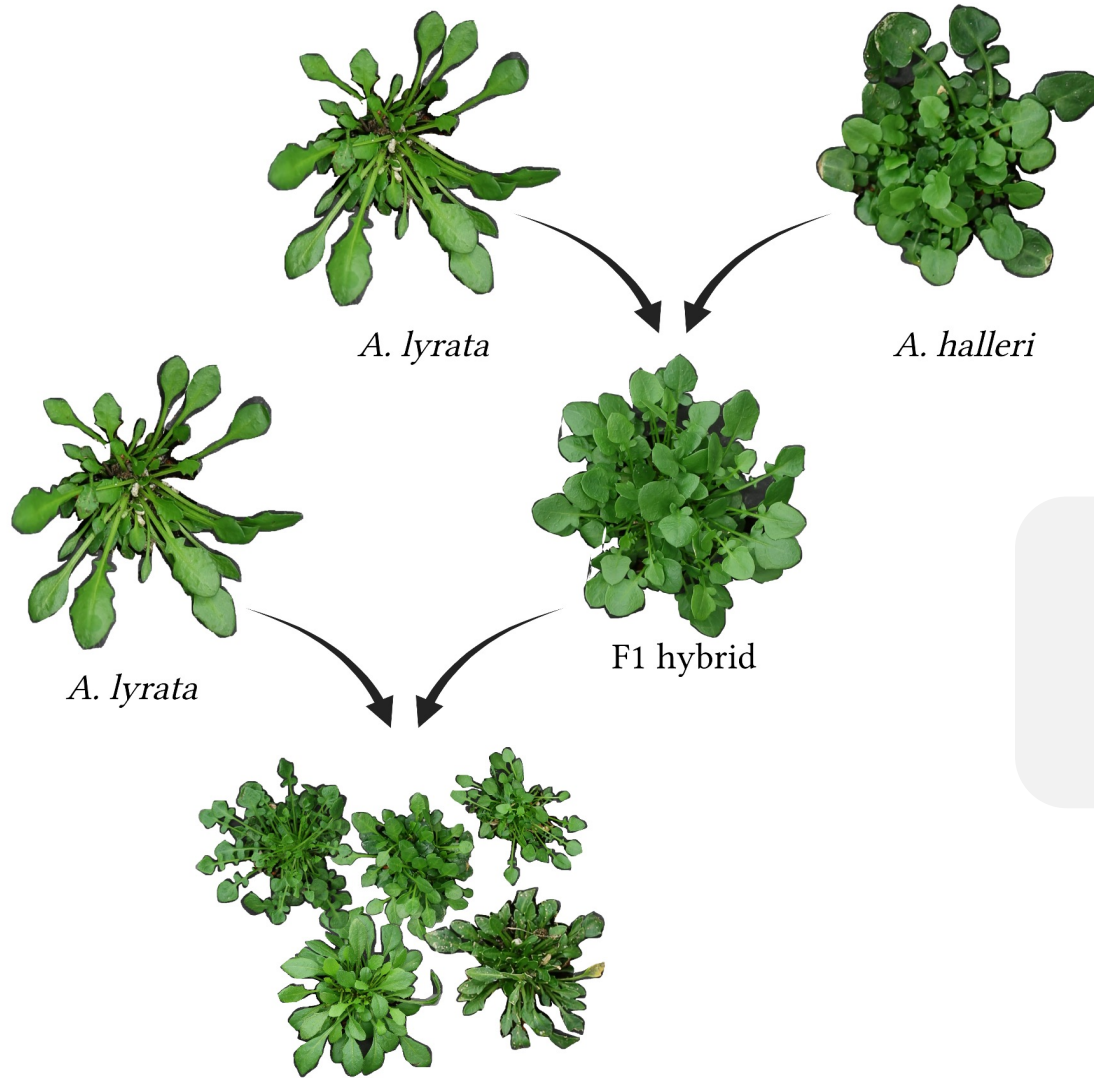


Acute dehydration assay

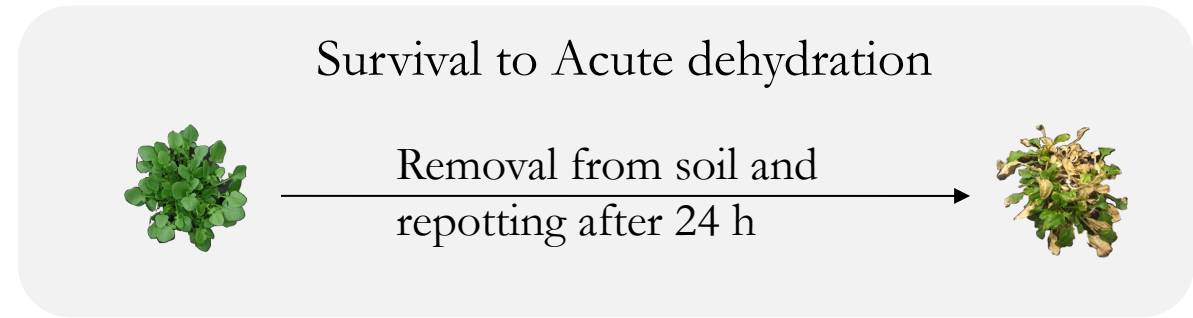
% Survival to depotting/repotting



What is the genetic basis of survival to acute stress?

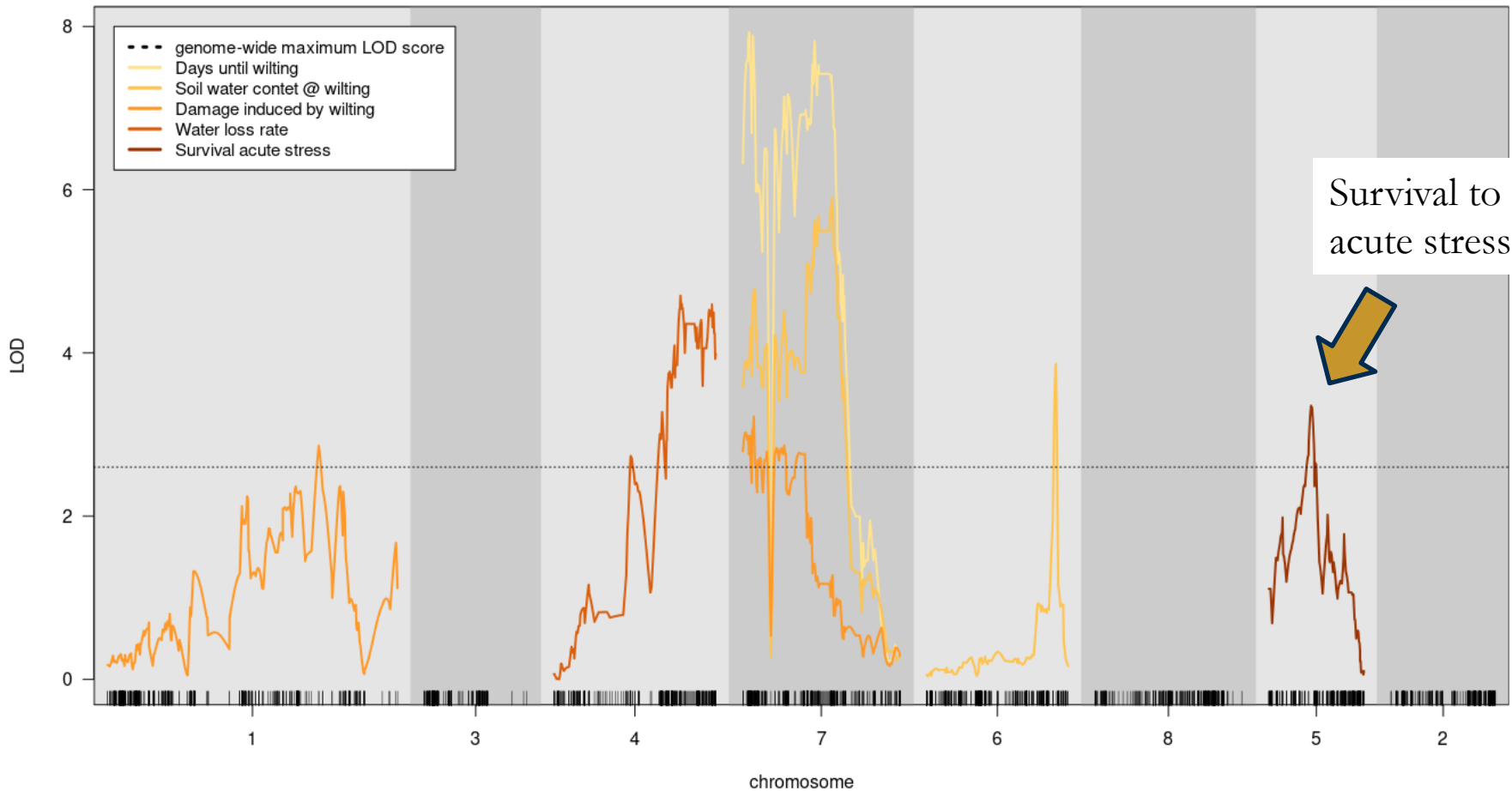


Genetics in
A. halleri × *A. lyrata* fertile BC1



=> QTL Mapping

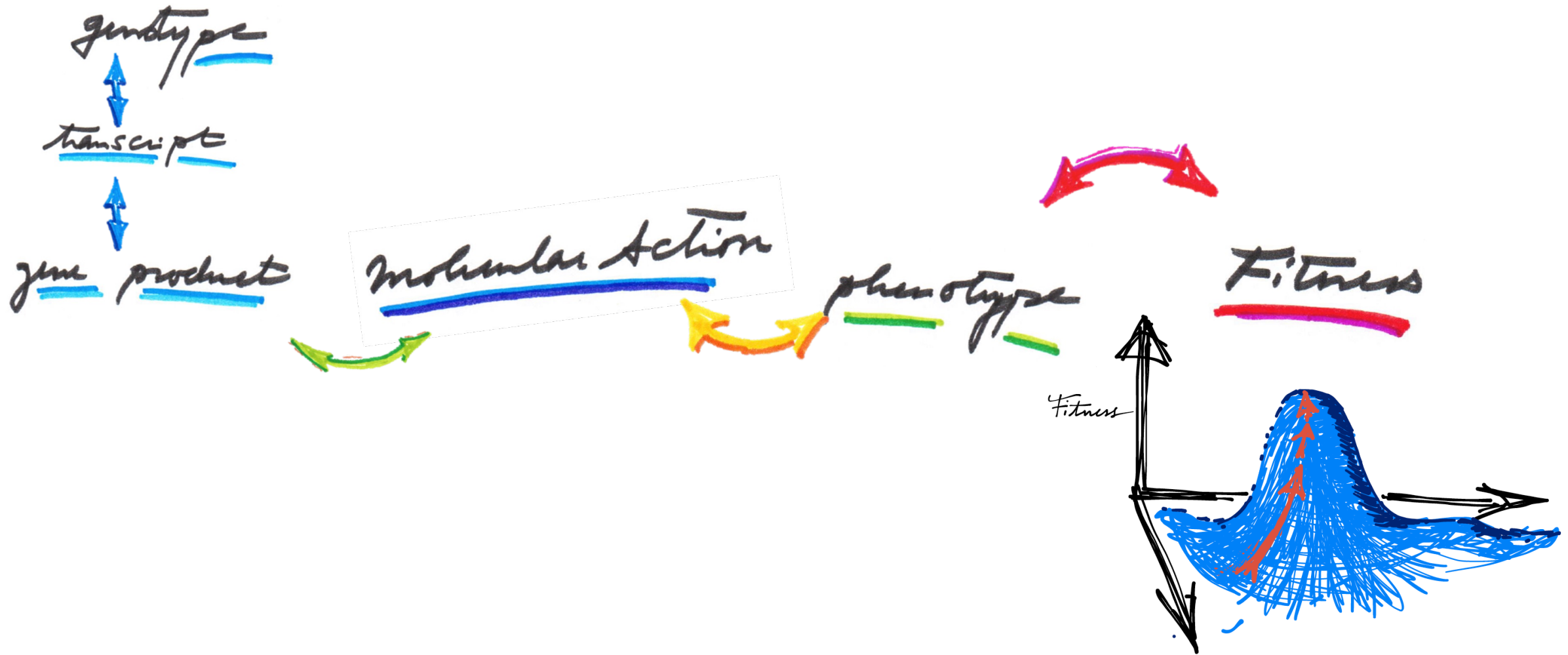
Interspecific differences in stress response enable identification of QTL



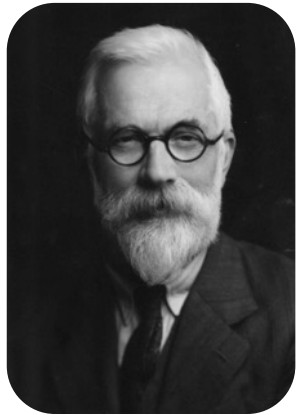
Lea Hördemann

Survival to acute stress
⇒ 1 QTL
⇒ Nevertheless polygenic?

What is the genetic basis of adaptation to wilting?



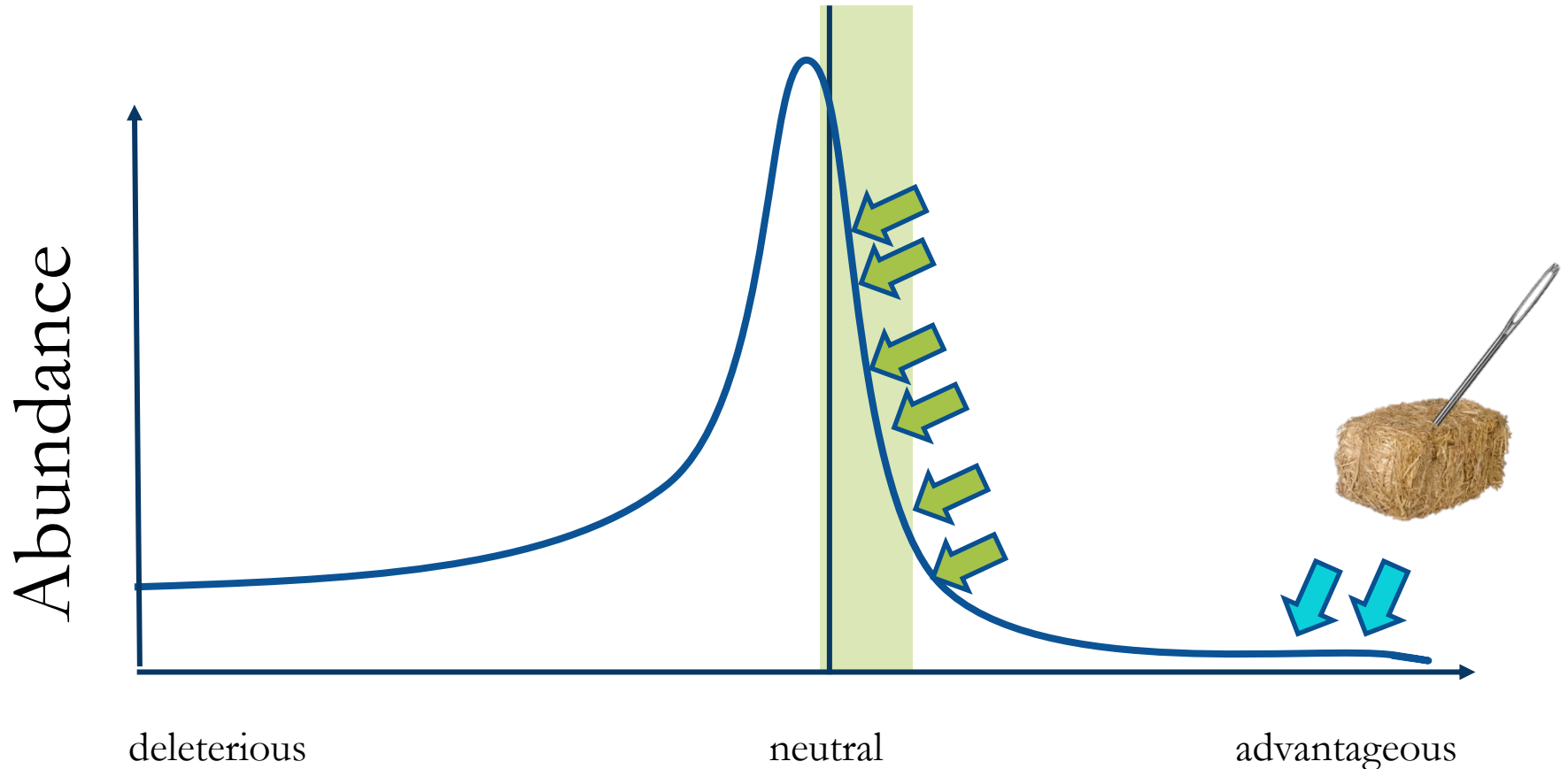
Most traits vary due to (many) small-effect mutations



R.A. Fisher

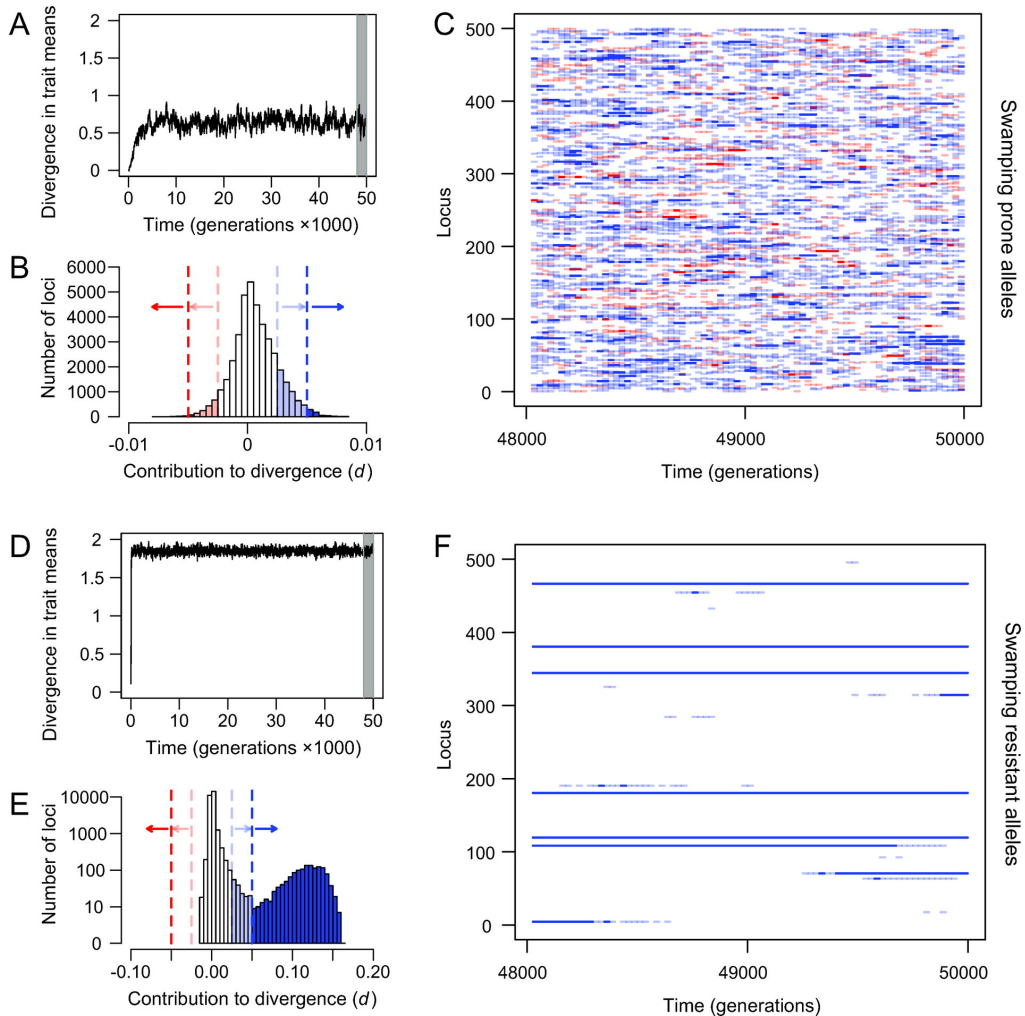
Adaptation

= \sum small effect mutations



Fitness effects of mutations

Polygenic trait change:



- ❖ not sustained by single genes
- ❖ Classical genetics useless
- ❖ (Arguably not informative- Rockmann 2012)

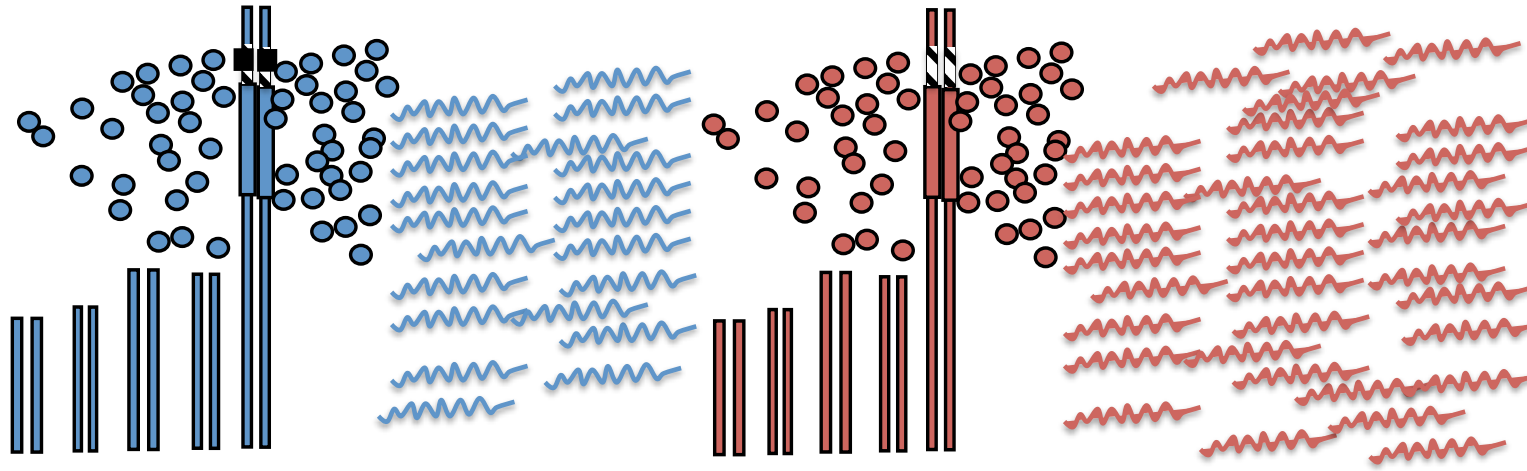
Survival to acute stress: 1 QTL

- ❖ Dissect adaptive changes due to large effect QTL
- ❖ But not representative
- ❖ Knowledge gain is limited

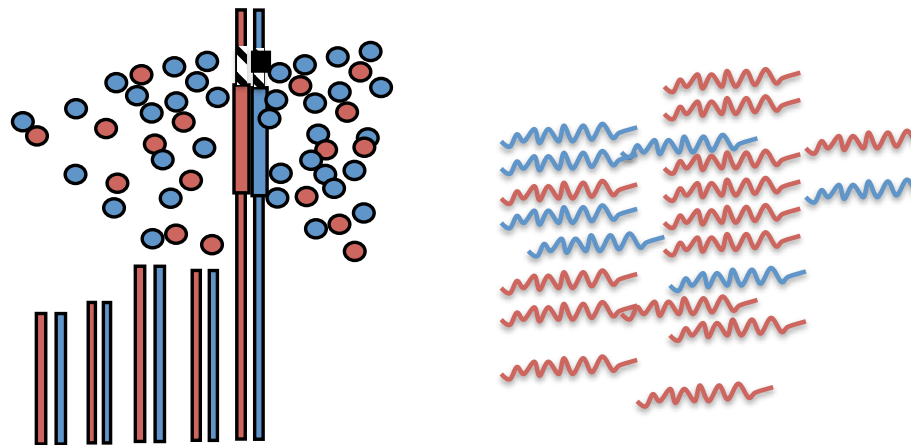
Whatabout the **collective properties** of functional mutations contributing to phenotypic differences?

1. F1 hybrid enable a genetic view on the distribution of mutations affecting gene expression throughout the genome

A- Expression difference between genotypes is a function of *cis*- and *trans*-acting polymorphisms

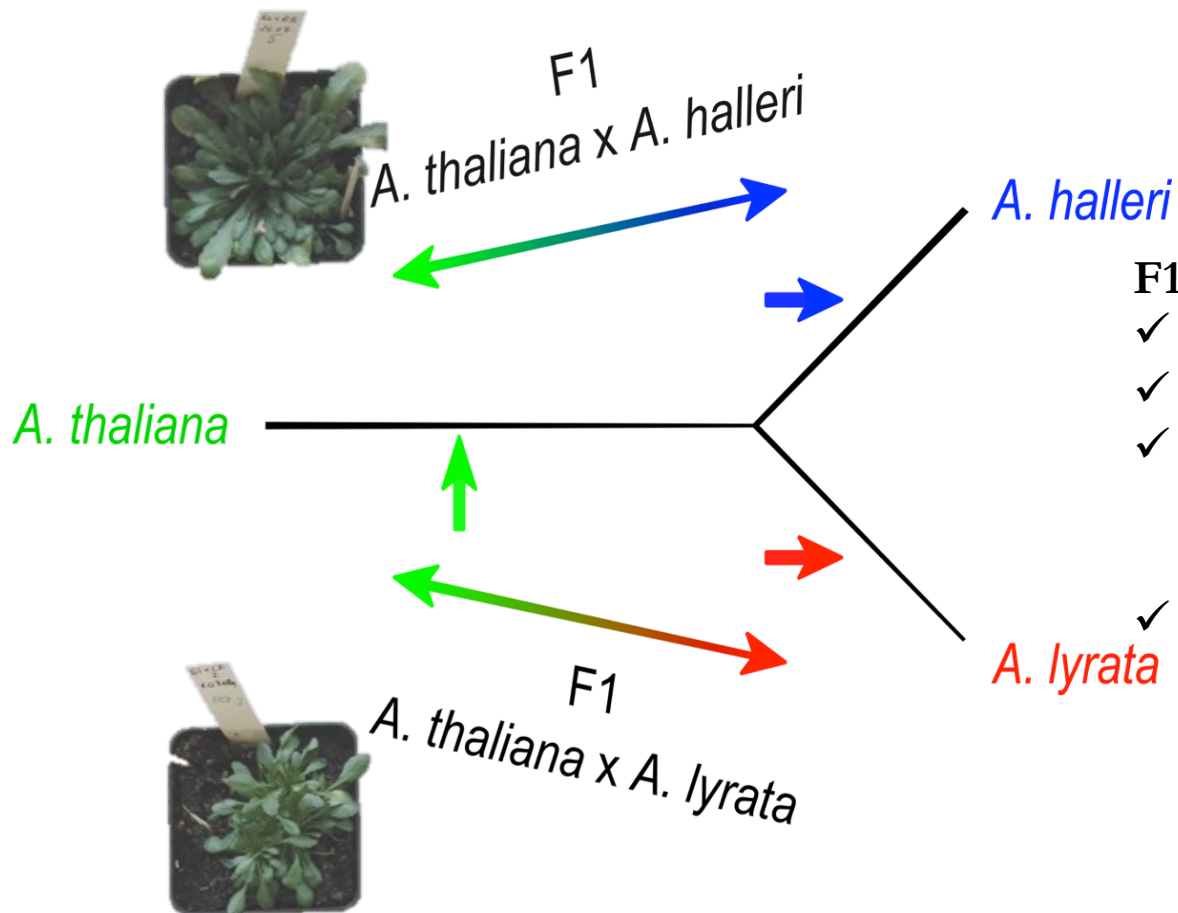


B- Expression difference between alleles within an F1 is a function of *cis*-acting polymorphisms only



de Meaux et al. 2005, 2006, Wittkop et al. (2004)
de Meaux Am J Bot 2018

2. differentiate ancestral and derived genetic changes in expression



F1 hybrids :

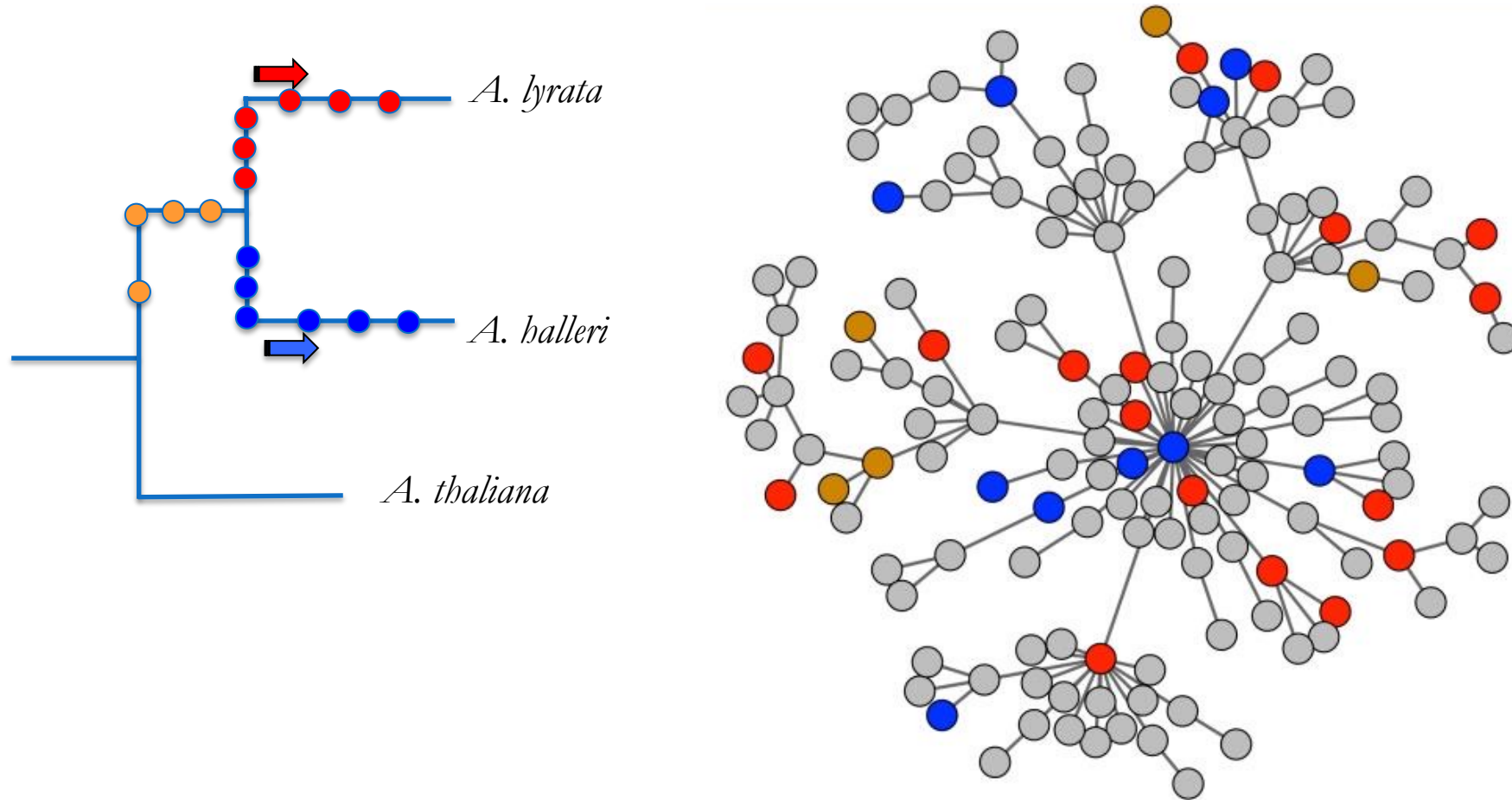
- ✓ Allele-specific expression = independent genetic change
- ✓ Delivers myriads of genetic changes
- ✓ Explains parental differences

(Göbel et al. 2018)

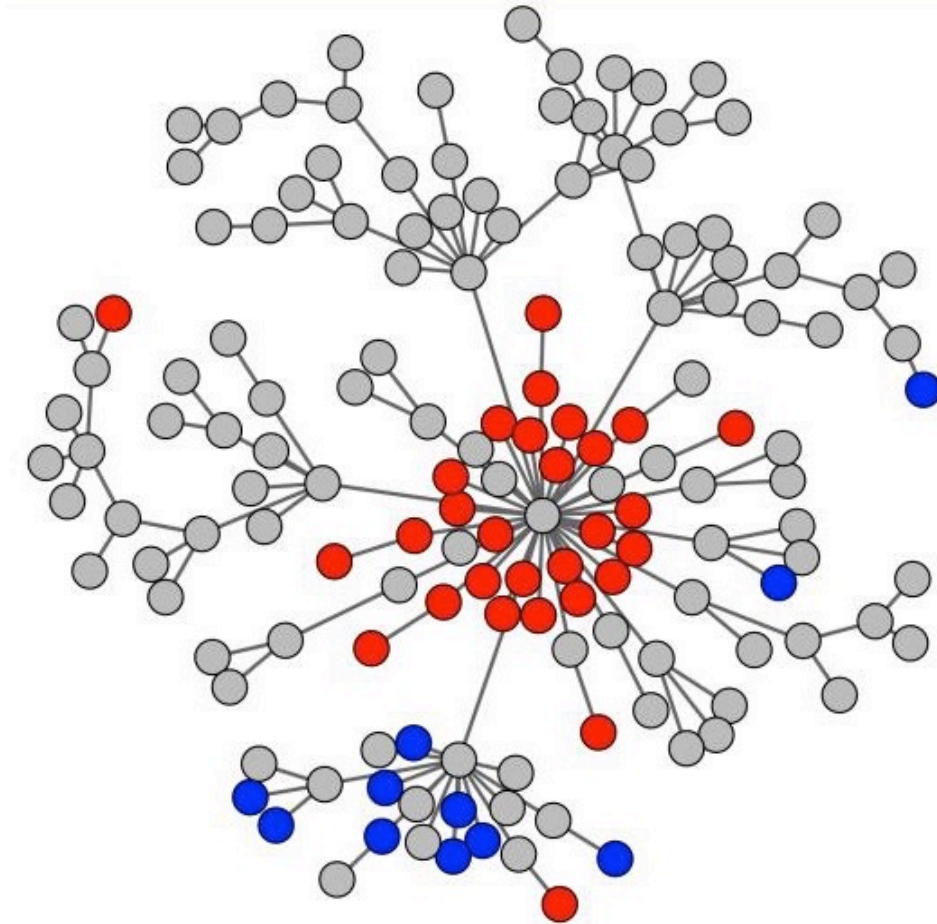
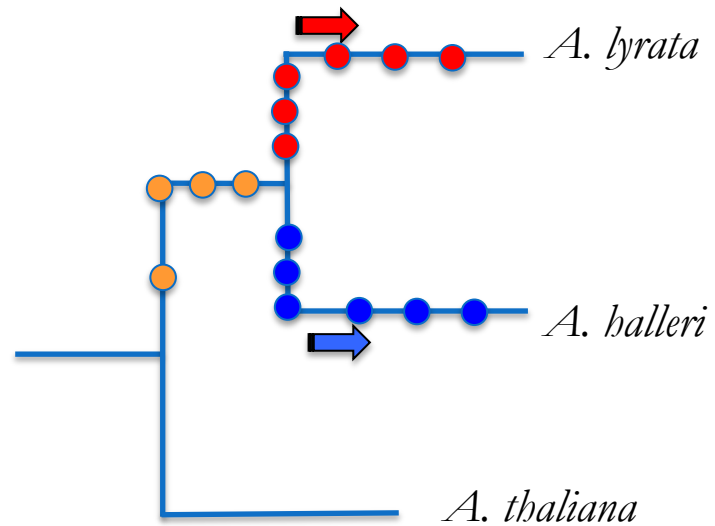
- ✓ Untangles derived & ancestral changes

He et al. 2016, 2021

=>3. Test whether functional mutations are distributed randomly across function



=>3. Test whether functional mutations are distributed randomly across function

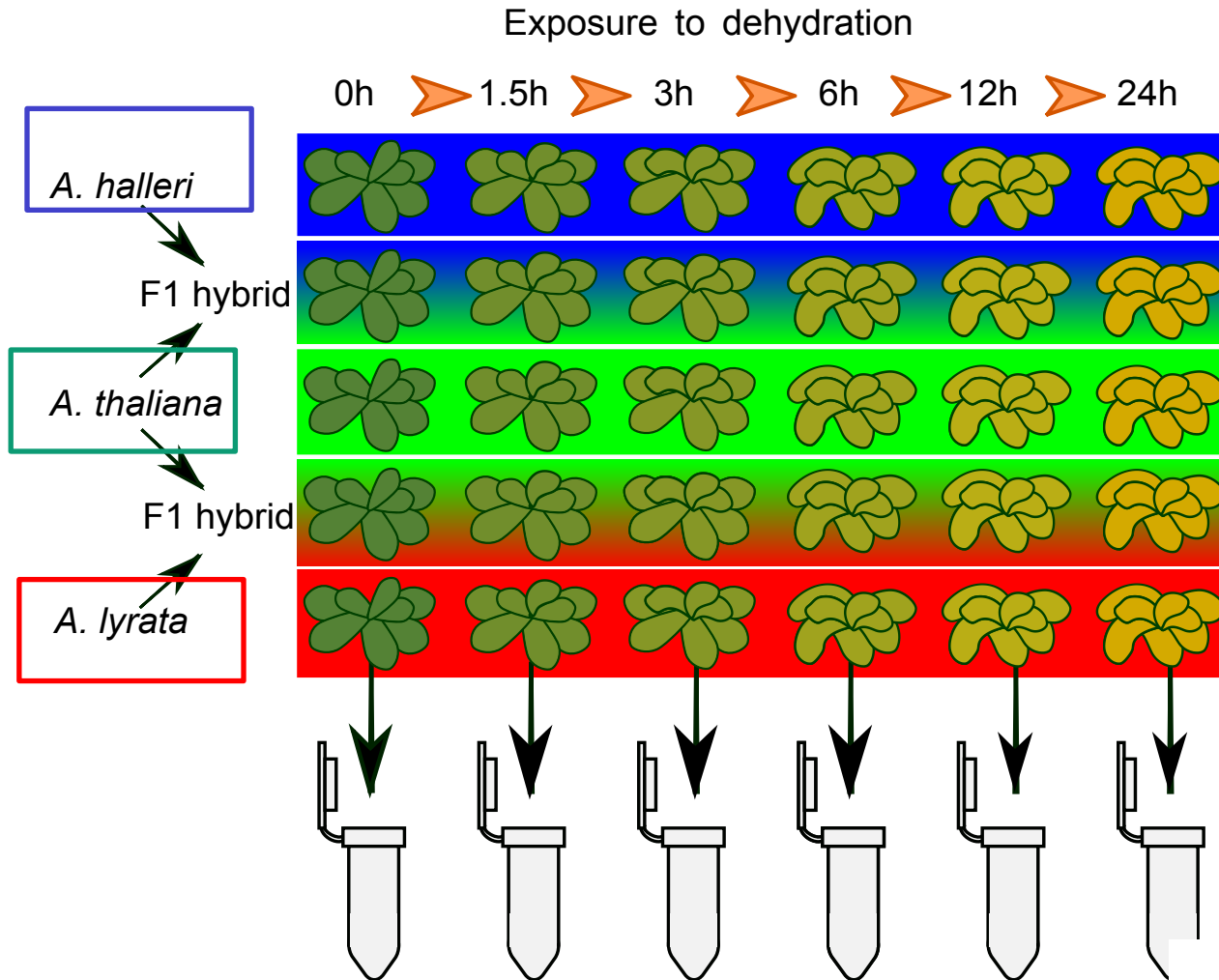


Proof of concept for *A. halleri* in He et al. MBE 2016

Data: gene expression response to dehydration



Fei He



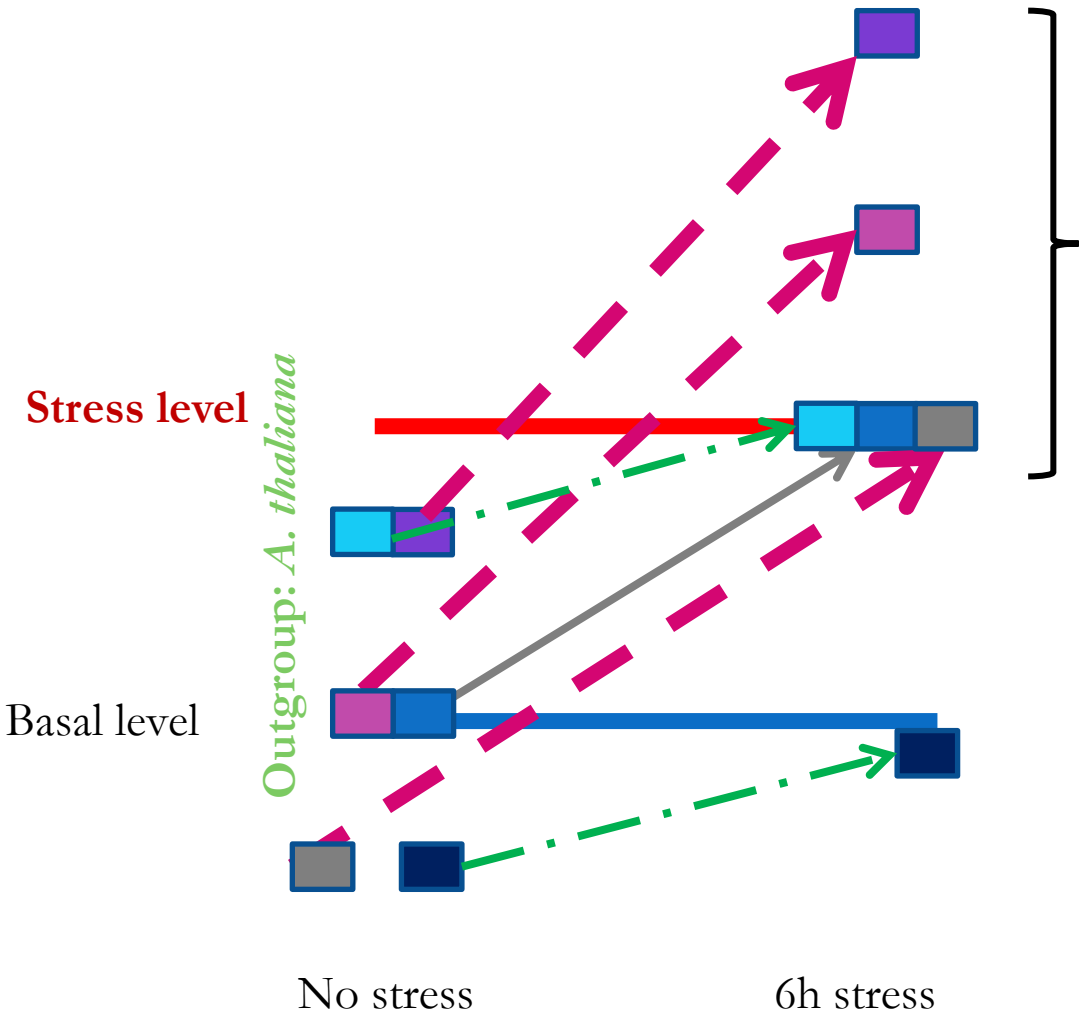
Contrast Allele differences

1. Between Species
2. Within F1 Hybrid



cis-acting basis of changes

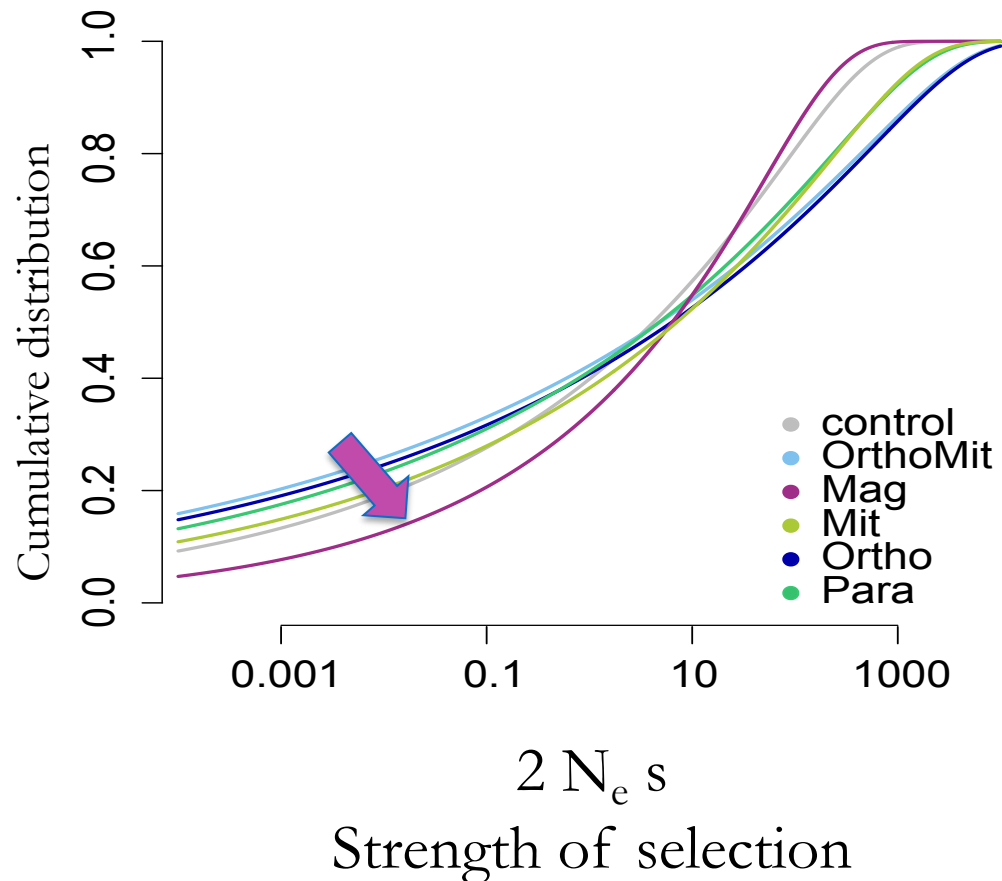
Excess of cis-regulatory change magnifying the stress response in *A. lyrata*



Enriched in cis-regulatory change in *A. lyrata* $p < 2.e^{-16}$

➔ Polygenic selection for increased plasticity of genes responding to acute drought stress

Genes with excess cis-regulatory variants experience specifically stronger constraints in *A. lyrata*

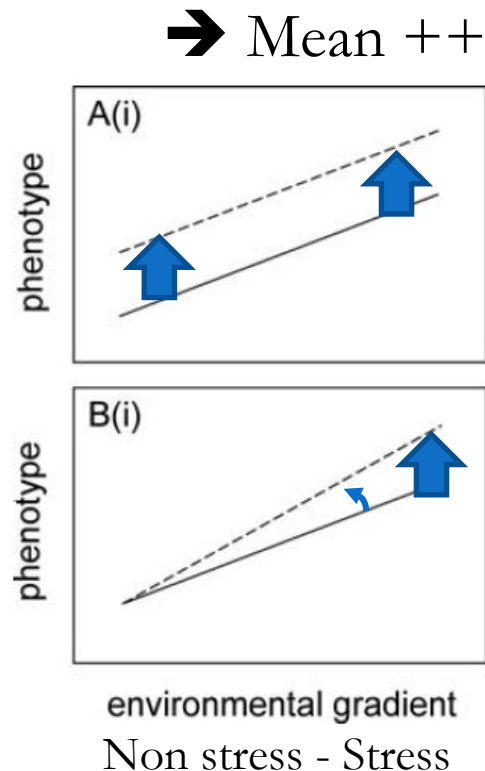


- Shift in **DFE**: mutations in genes with magnified response are more deleterious
- Signature is specific of *A. lyrata*
- **Lower frequency of non synonymous variants in *A. lyrata* confirms special fitness relevance**

**Can we learn something about the evolution of
plasticity in general?**

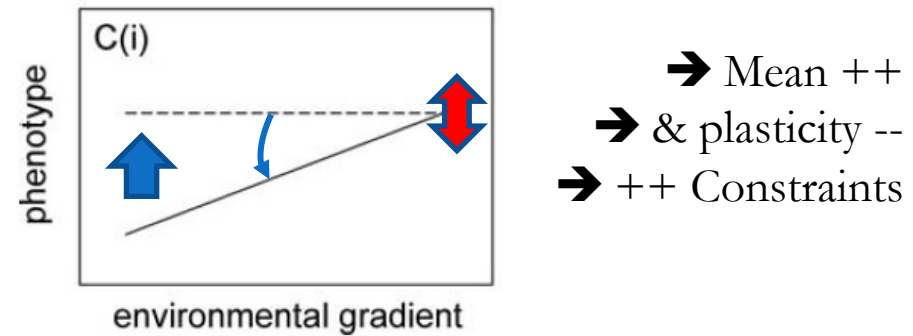
Plasticity & Evolution: 3 major modes proposed

1. Baldwin effect (Plasticity first)

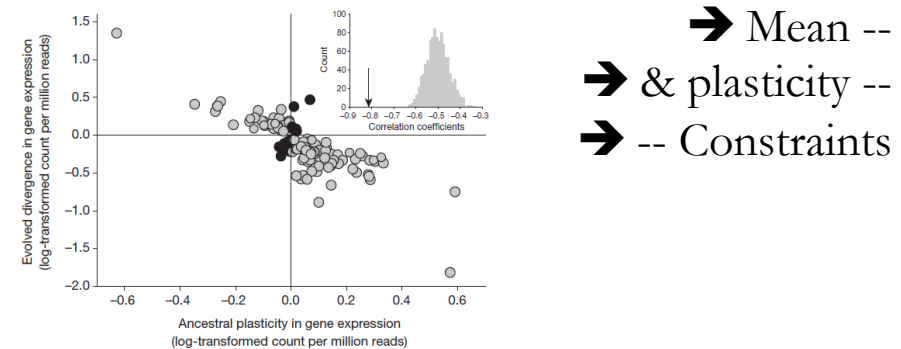


After Crispo et al. 2007

2. Genetic assimilation (Waddington)



3. Correcting maladaptive plasticity



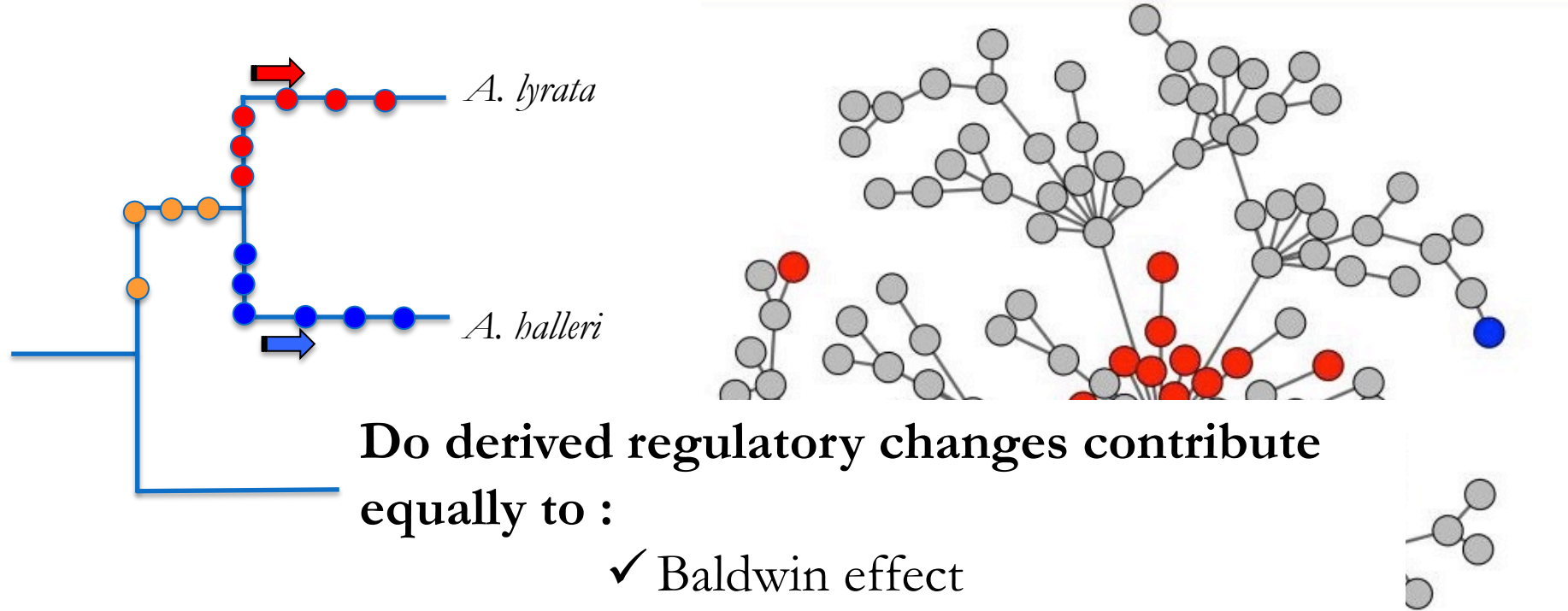
Conover et al. 2009, Ghilambor et al. 2015

Plasticity & Evolution:

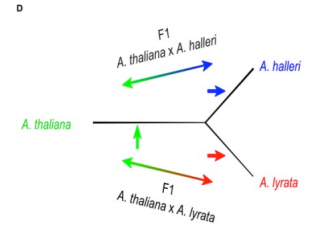
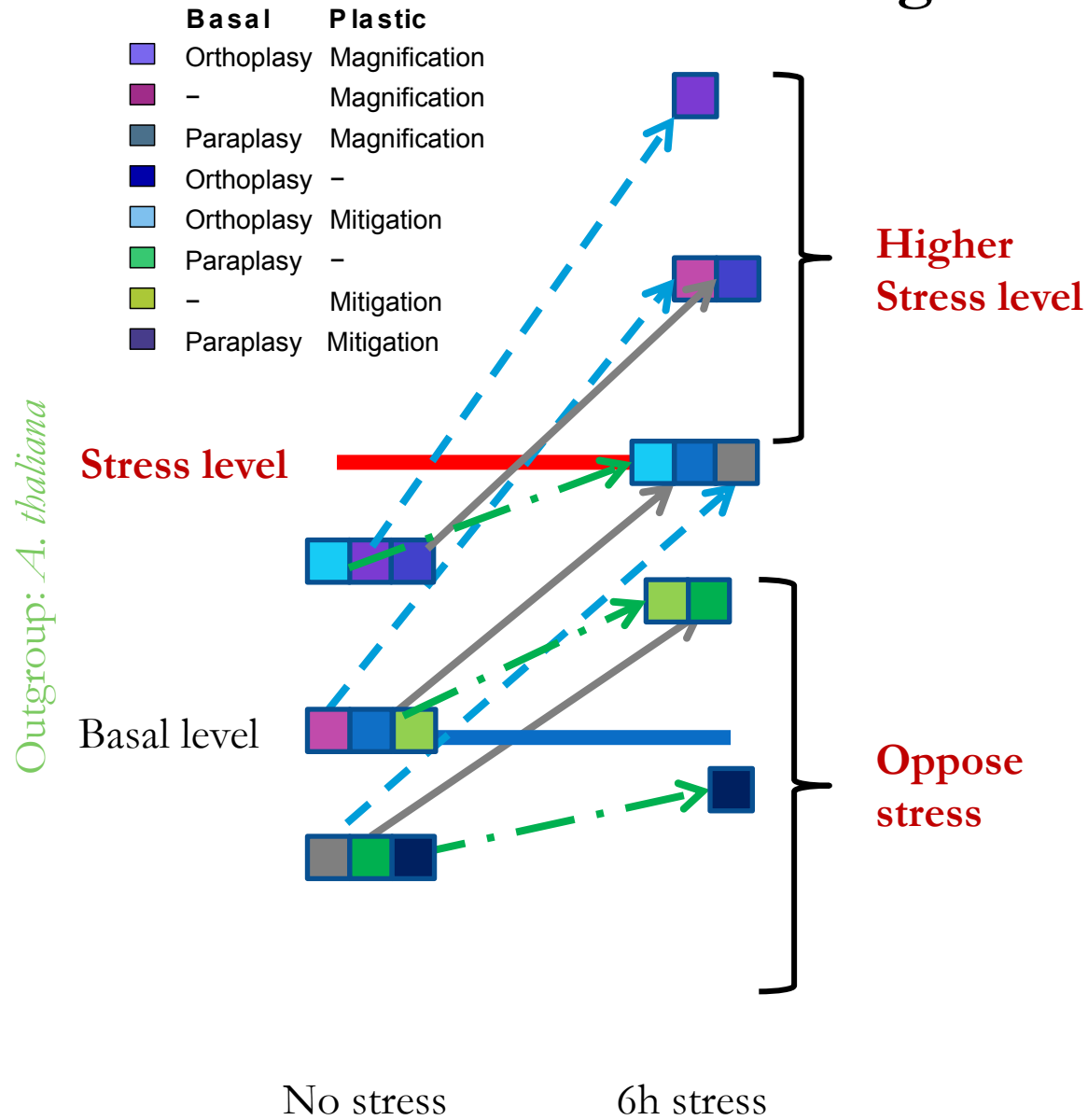
What is the relative importance of these trajectories?

- Baldwin effect & Genetic assimilation (stepping stone)
 - Reduction of maladaptive plasticity
 - Neutral evolution
-
- ❖ **Monitor plasticity evolution in many traits**
 - ❖ **Determine whether mutation modify plasticity randomly or not**









Functional clustering spotlights the action of natural selection on plasticity



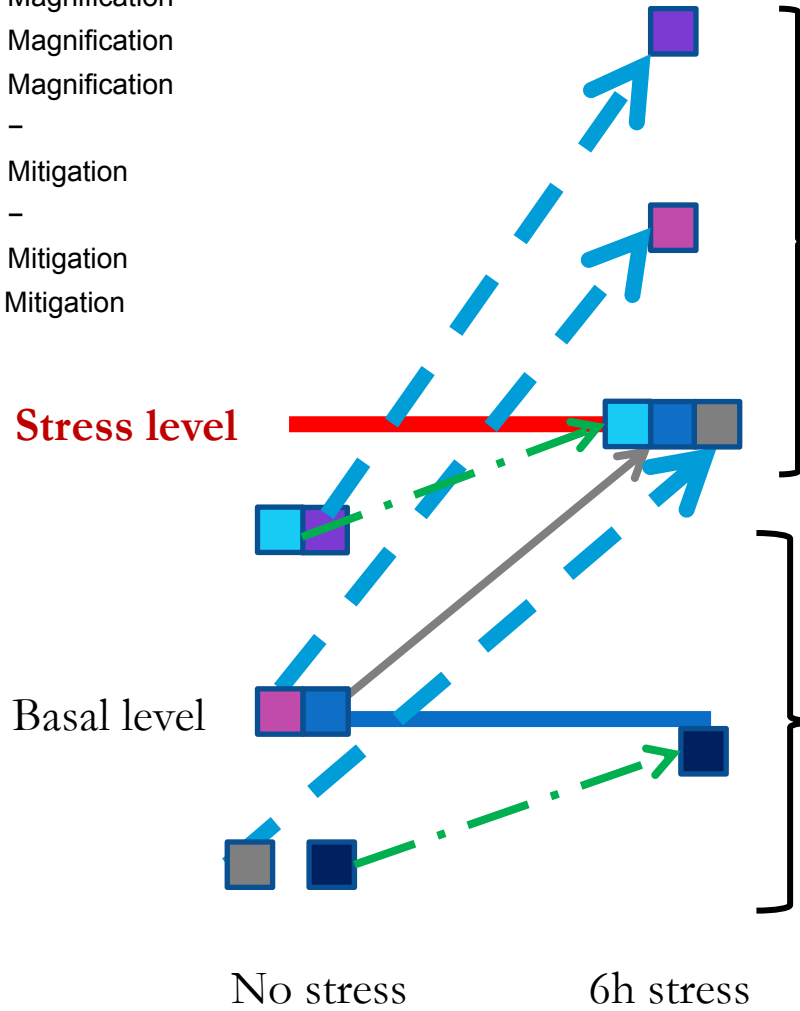
How do basal and plastic (slope) cis-regulatory changes combine?



Adaptive magnification in *A. lyrata*?

- | Basal | Plastic |
|---|---------------|
|  Orthoplasia | Magnification |
|  - | Magnification |
|  Paraplasia | Magnification |
|  Orthoplasia | - |
|  Orthoplasia | Mitigation |
|  Paraplasia | - |
|  - | Mitigation |
|  Paraplasia | Mitigation |

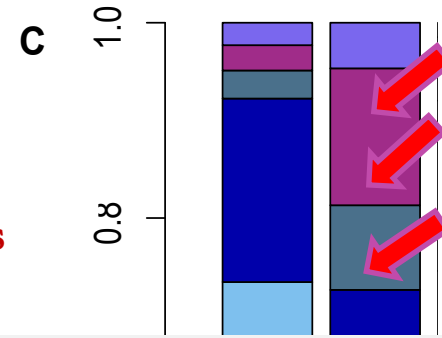
Outgroup: *A. thaliana*



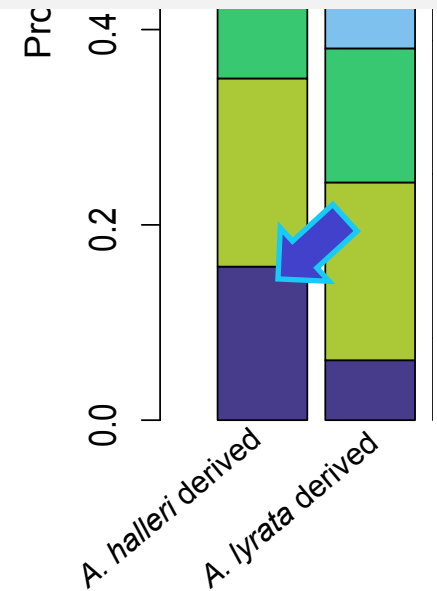
In excess in *A.*

→ Polygenic selection targets distinct evolutionary trajectory of plasticity

In excess in *A. halleri*



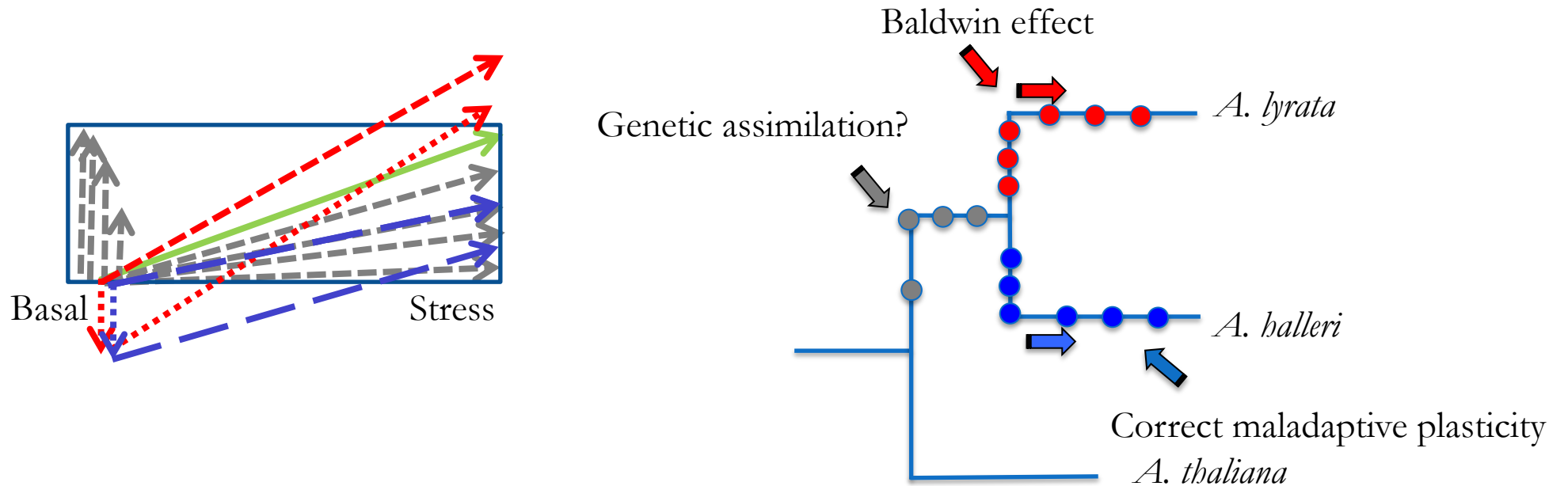
Plastic genes become more plastic! => Baldwin effect!



Plastic genes become less plastic! => Loss of maladaptive plasticity

$P < 2 \cdot 10^{-16}$

Lineage specific evolution of plastic reactions



Cis-regulatory changes do not contribute equally to :

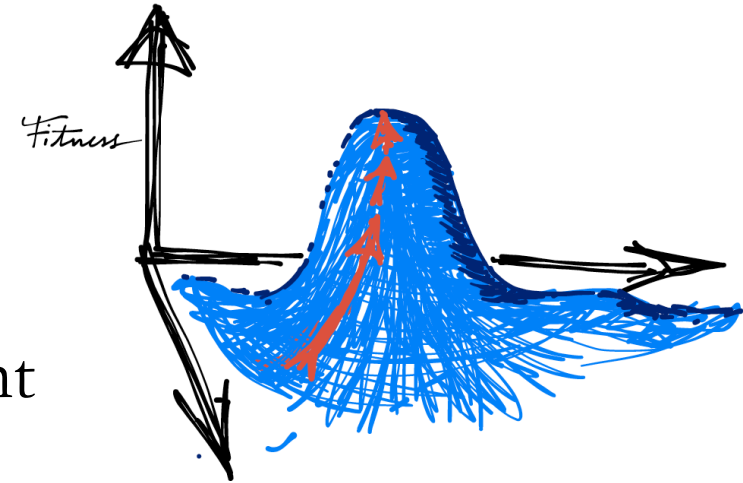
- ✓ Baldwin effect
- ✓ Genetic assimilation?
- ✓ Correct maladaptive plasticity

Conclusion-Complex trait

- *Arabidopsis* species differ in their strategy to deal with drought
 - *A. lyrata* is best able to survive wilting, *A. halleri* has no avoidance strategy
 - Survival to wilting explained by one QTL
 - *A. lyrata* evolved to respond faster and more strongly to acute drought
 - Validation by modified DFE
-
- Baldwin effect: Plasticity serves as a basis of optimized stress response in *A. lyrata*
 - Plasticity is maladaptive in *A. halleri*

Wrap up

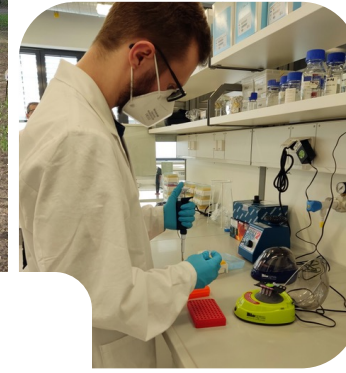
1. No increase in mutational load despite bottleneck
2. Non-additive variance in gene expression increases for genes subject to purifying selection
3. Non-additive variance can be predominant
4. Cis-regulatory variants show lineage specific enrichment in the molecular networks
5. These enrichments coincide with sharpening of purifying selection
6. The evolutionary trajectory of plasticity differs between lineages



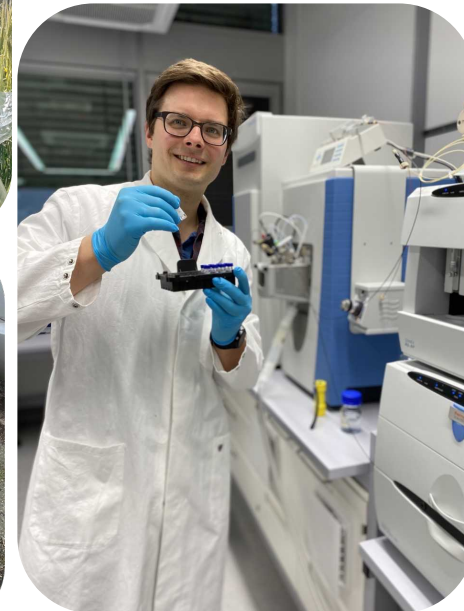
Outlook

1. Deleterious variation helps understand selection on complex traits
2. Gene expression variation is shaped by natural selection against deleterious variation
3. Cis-regulatory variants reveal the complex networks targeted by natural selection
4. The collective properties of small effect mutations need to be investigated





From lab to nature





Tahir Ali
Margarita Takou (Stetter Group)
Holger Schielzeth (Uni Jena)
Dan Balick, Evan Koch and Shamil
Sunyaev (Harvard Med School)
Josselin Clo (CNRS, Lille)
Kim Steige (Uni Hohenheim)
Fei He (Uni Bonn)

Mercator fellowship foster international
collaborations!

trr_341 plant
ecological
genetics

DFG Deutsche
Forschungsgemeinschaft



Bundesministerium
für Bildung
und Forschung



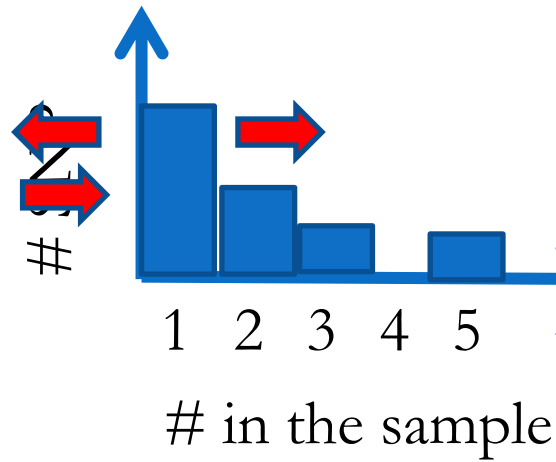
European Research Council
Established by the European Commission

Why did the load not increase? Bottleneck



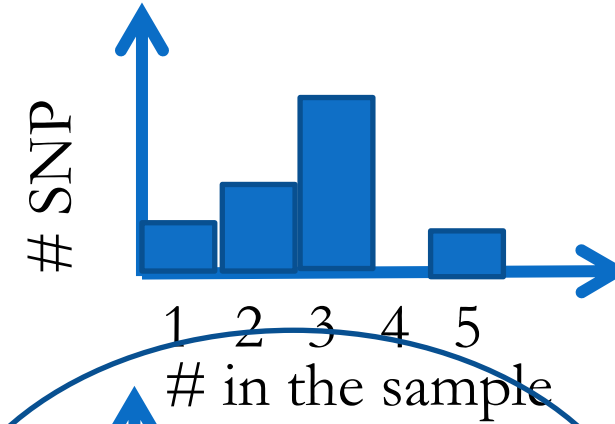
Load increase

Site frequency spectrum

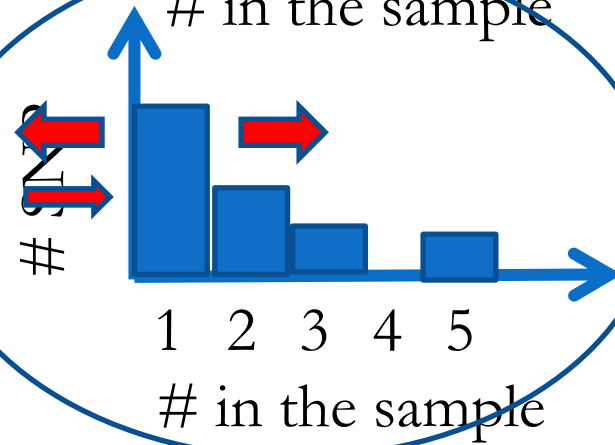


Bottleneck

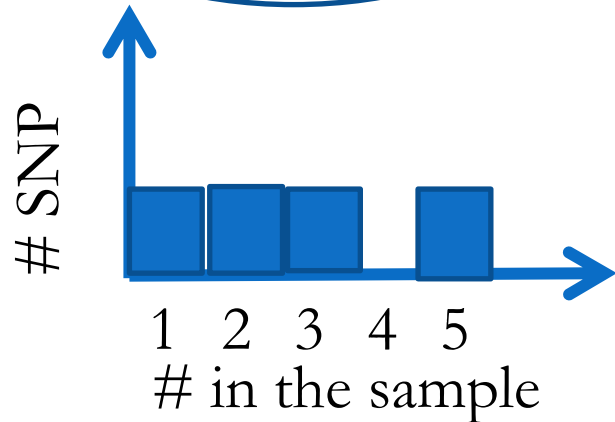
A



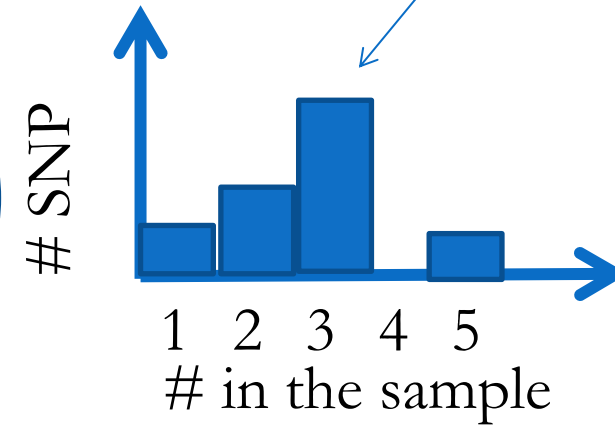
B



C



Old mutations



After the bottleneck

Site frequency spectrum

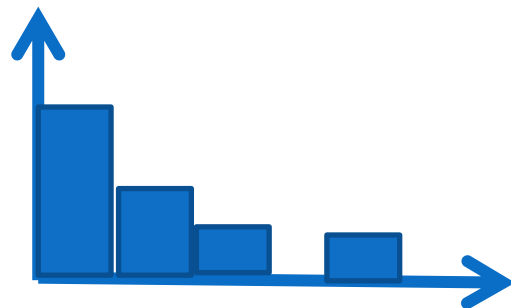
	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6	SNP 7	SNP 8
Sample 1	0	1	0	0	0	0	1	0
Sample 2	1	0	1	0	0	0	1	0
Sample 3	0	1	1	0	0	1	0	0
Sample 4	0	0	0	0	1	0	1	1
Sample 5	0	0	1	0	0	0	1	0
Sample 6	0	0	0	1	0	1	1	0
Total	1	2	3	1	1	2	5	1

Distribution of frequencies before bottleneck – Population at equilibrium

Site frequency spectrum

	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 6	SNP 7	SNP 8
Sample 1	0	1	0	0	0	0	1	0
Sample 2	1	0	1	0	0	0	1	0
Sample 3	0	1	1	0	0	1	0	0
Sample 4	0	0	0	0	1	0	1	1
Sample 5	0	0	1	0	0	0	1	0
Sample 6	0	0	0	1	0	1	1	0
Total	1	2	3	1	1	2	5	1

SNP



4 SNPs have 1 occurrence in the sample

in the sample

Site frequency spectrum