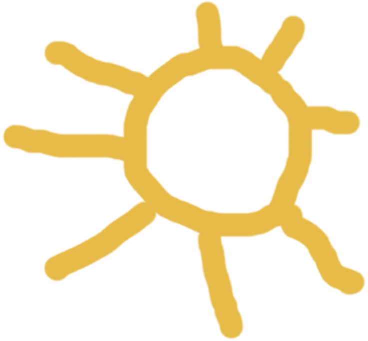
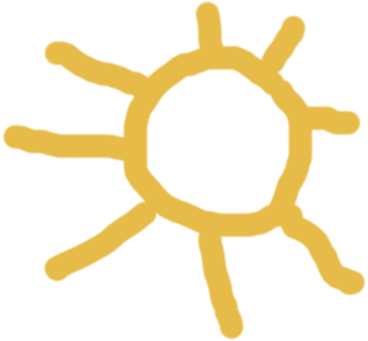


Cytonuclear interactions in *Arabidopsis thaliana*

Françoise Budar

IJPB INRA Versailles

July 21st 2016



SPS
SACLAY PLANT SCIENCES

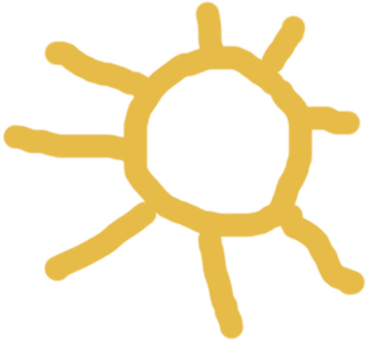
My name is ... Françoise Budar

I work at ... Institut Jean-Pierre Bourgin, Versailles

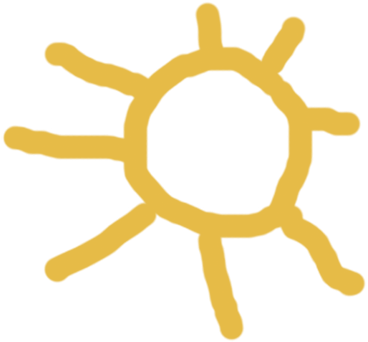
ijpb

I am good at ... Genetics

I am interested in ... Cytonuclear interactions

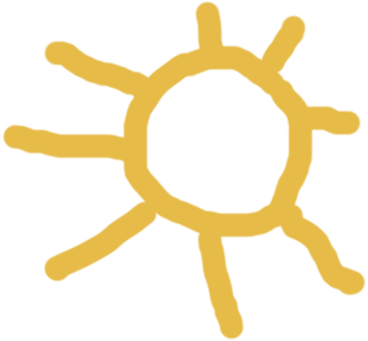


I come mainly for ... Learning about analysis of omic data

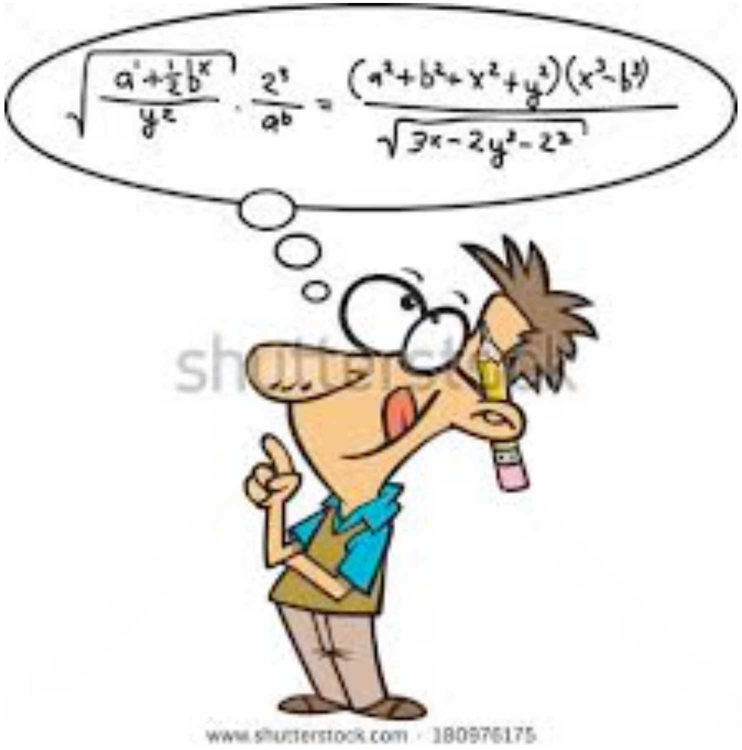


I come mainly for ... Entertaining you with a nice (?) biological story

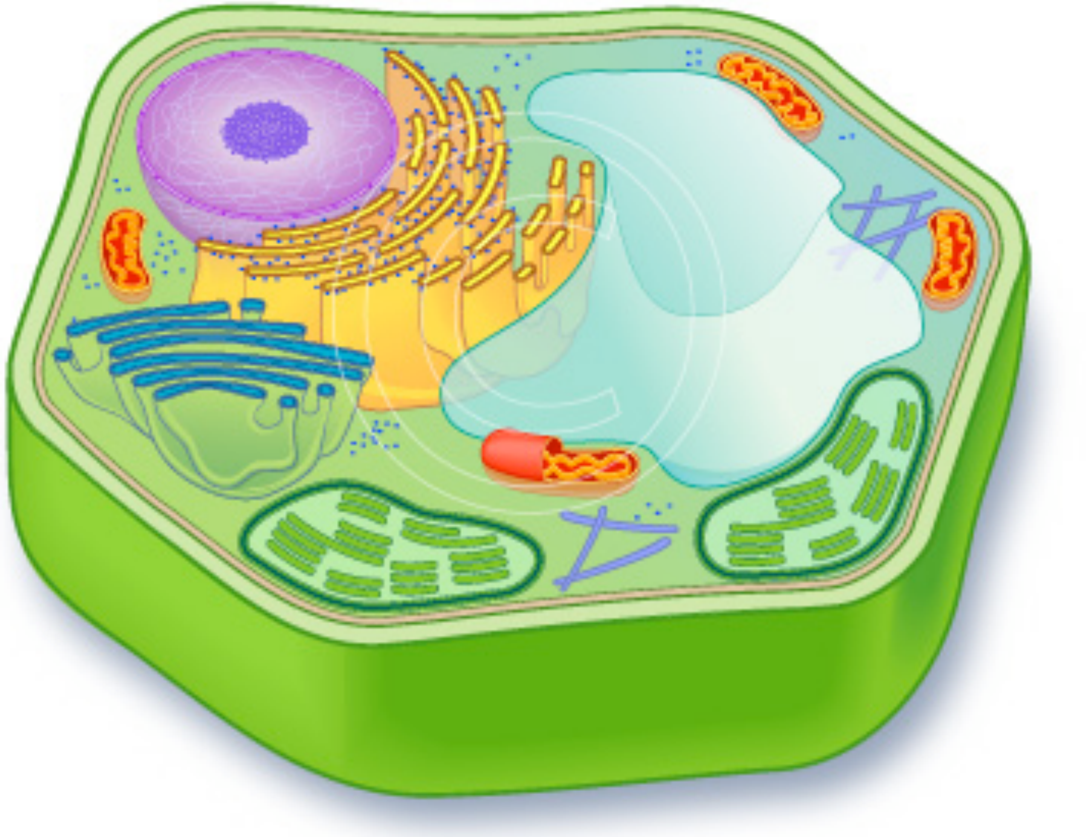
after a hard mathematical day

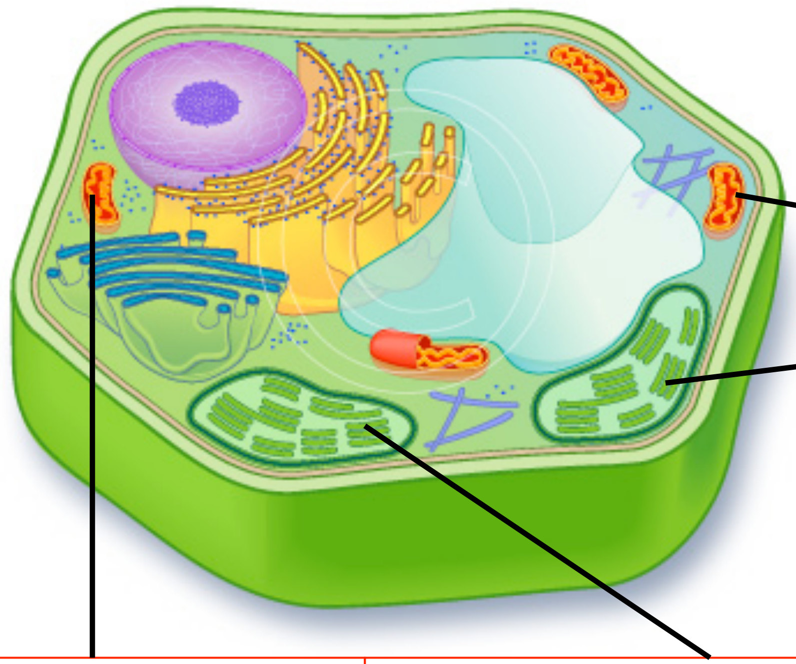


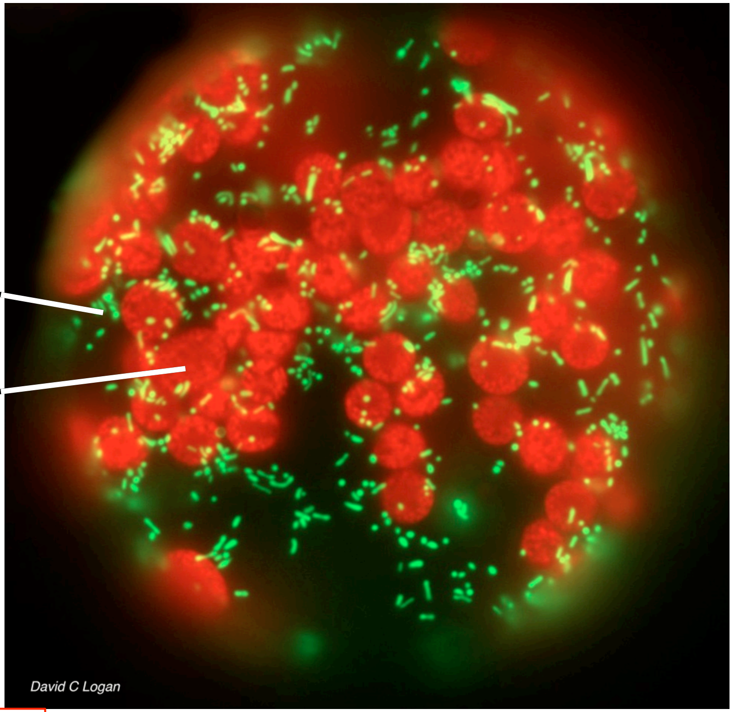
I come mainly for ... Telling you a story about interactions between biologists and statisticians



The plant cell metabolism is compartmentalized





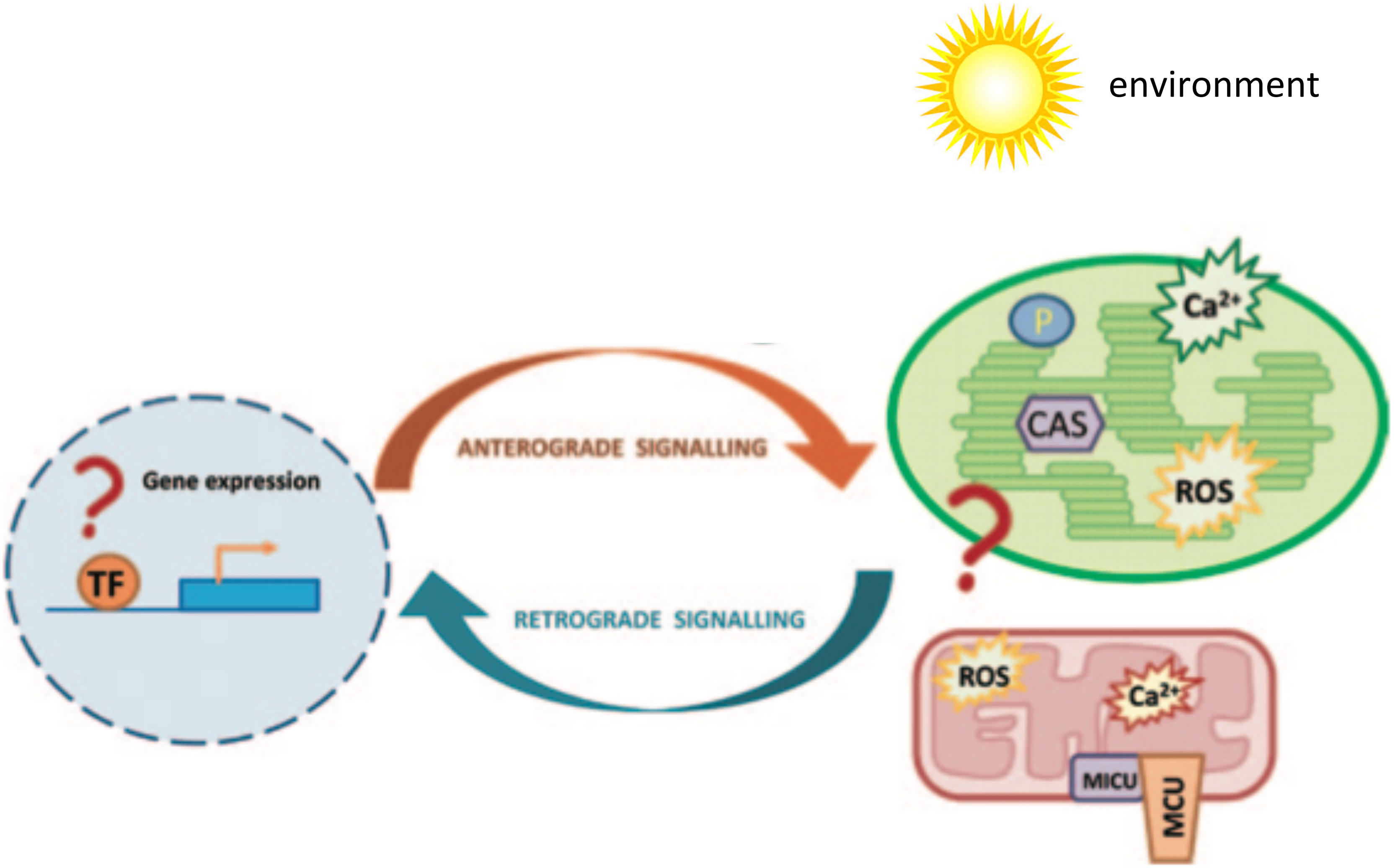


Confocal microscopy by David Logan
Red: chloroplasts (autofluorescence)
Green: mitochondria (GFP)

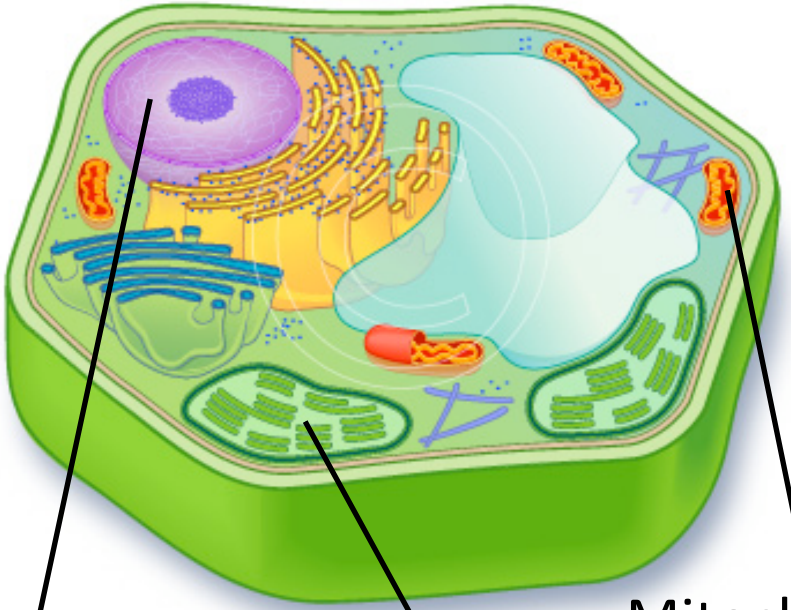
<table>
<tr><td>Respiration
Stress responses
Biosynthetic pathways:
Purines, vitamins
redox metabolism</td><td>Photosynthesis
Environment sensing
Biosynthetic pathways:
Vitamins, hormones, lipids
Nitrogen & sulfur assimilation</td></tr>
<tr><td colspan="2">Energy metabolism</td></tr>
</table>

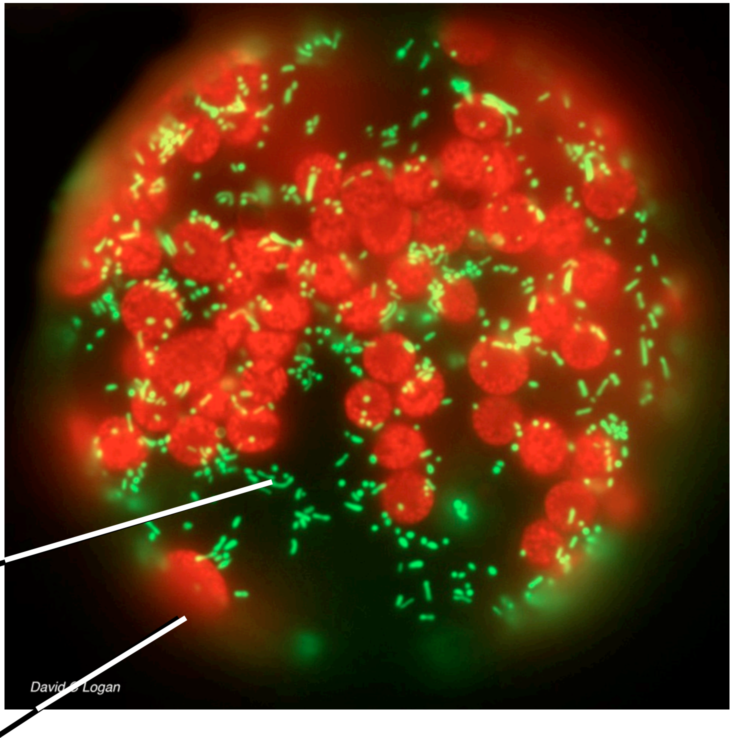
The plant cell metabolism is compartmentalized

The proper function of plant cells relies on interactions between organelles and nucleus



Modified from Kmiecik et al 2016





Mitochondria

~60 genes

Nucleus

~30000 genes

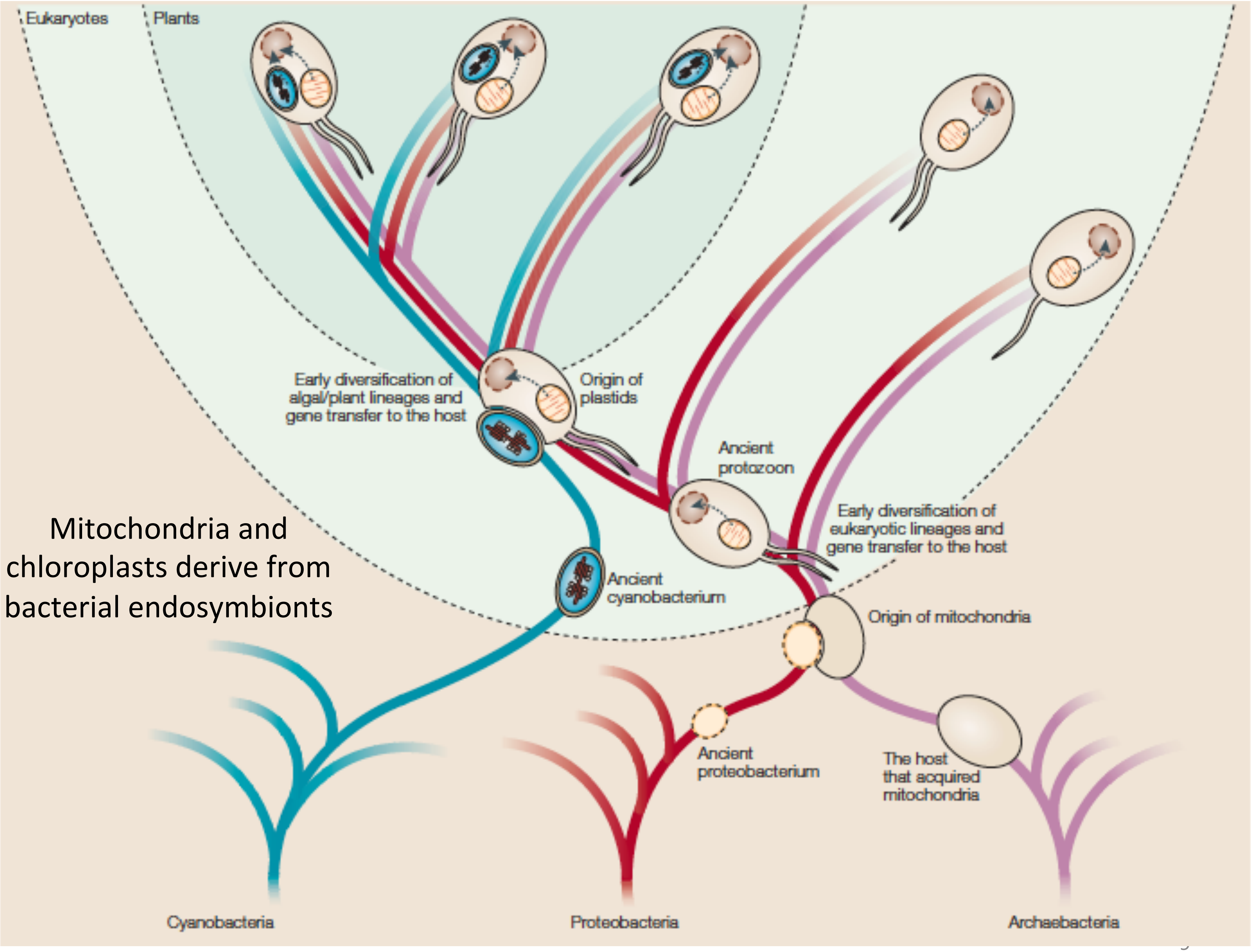
Chloroplasts

~130 genes

The plant cell metabolism is compartmentalized

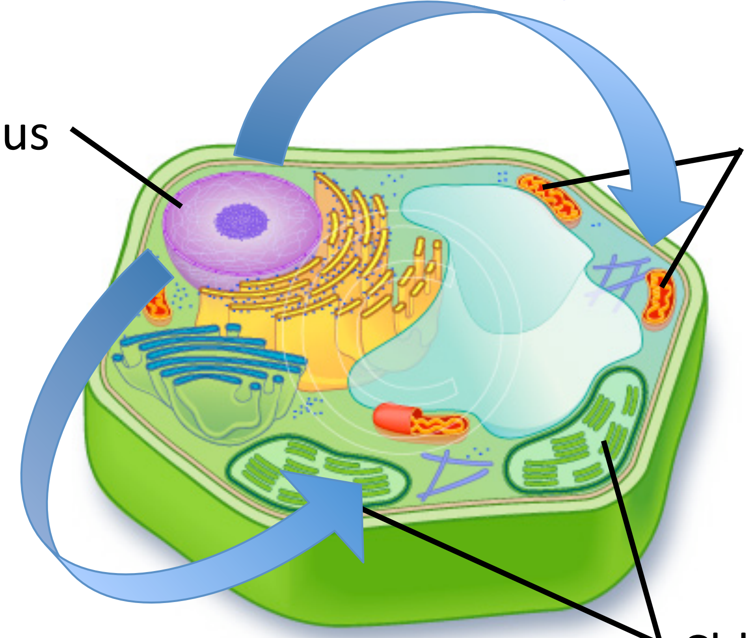
So is its genome

Mitochondria and chloroplasts derive from bacterial endosymbionts



Timmis et al, 2004

Mitochondria and chloroplasts are not functionally autonomous



Import of > 2000 proteins

Nucleus

~30000 genes

Mitochondria
~60 genes

Chloroplasts
~130 genes

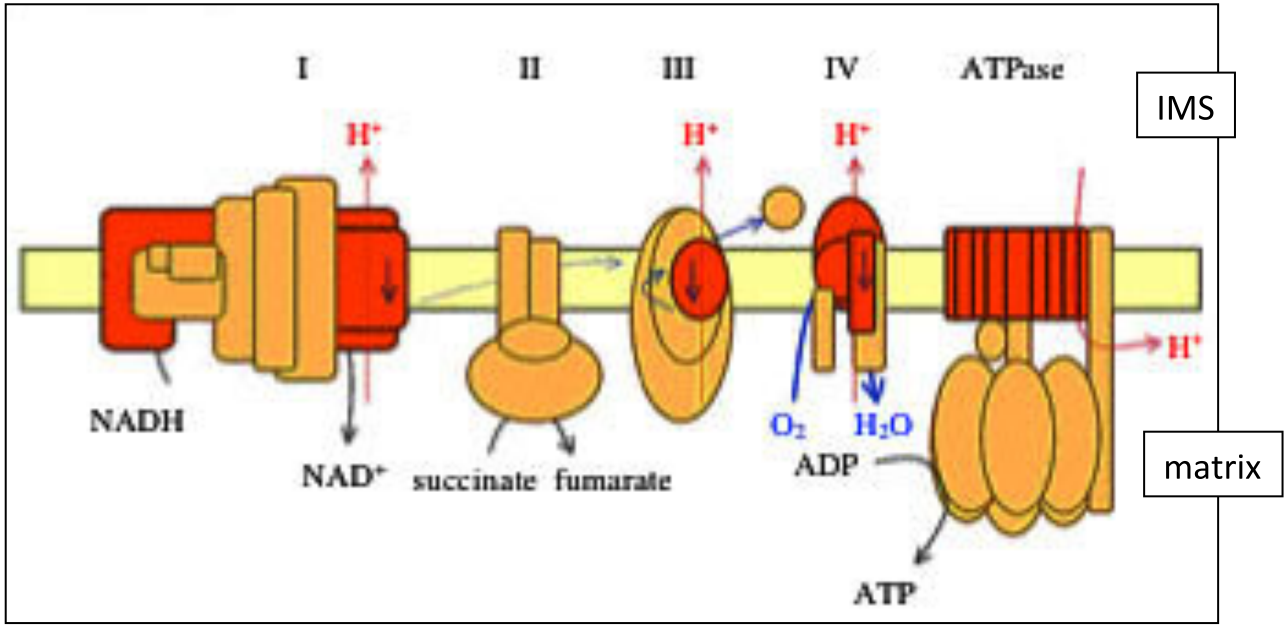
Import of ~3000 proteins

The proper function of plant organelles relies on the interaction between nuclear and organelle genetic units

Mitochondrial electron transport chain

origin of subunits

- ● mitochondrial
- ● nuclear



Cytoplasmic and nuclear genomes are expected to be co adaptated

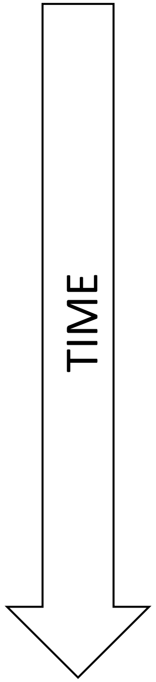
"Co-adaptation occurs when a variation in a factor encoded by one compartment will select for a variation in a factor encoded by the other, due to physical interaction between the two factors."(Rand et al, 2004)

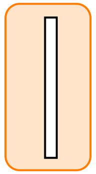
Cytonuclear co adaptation

Genetic level

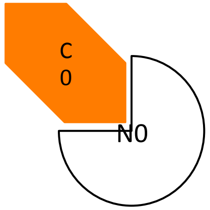
Physiological level

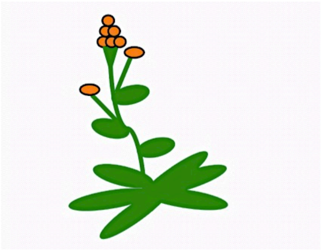
Phenotype level





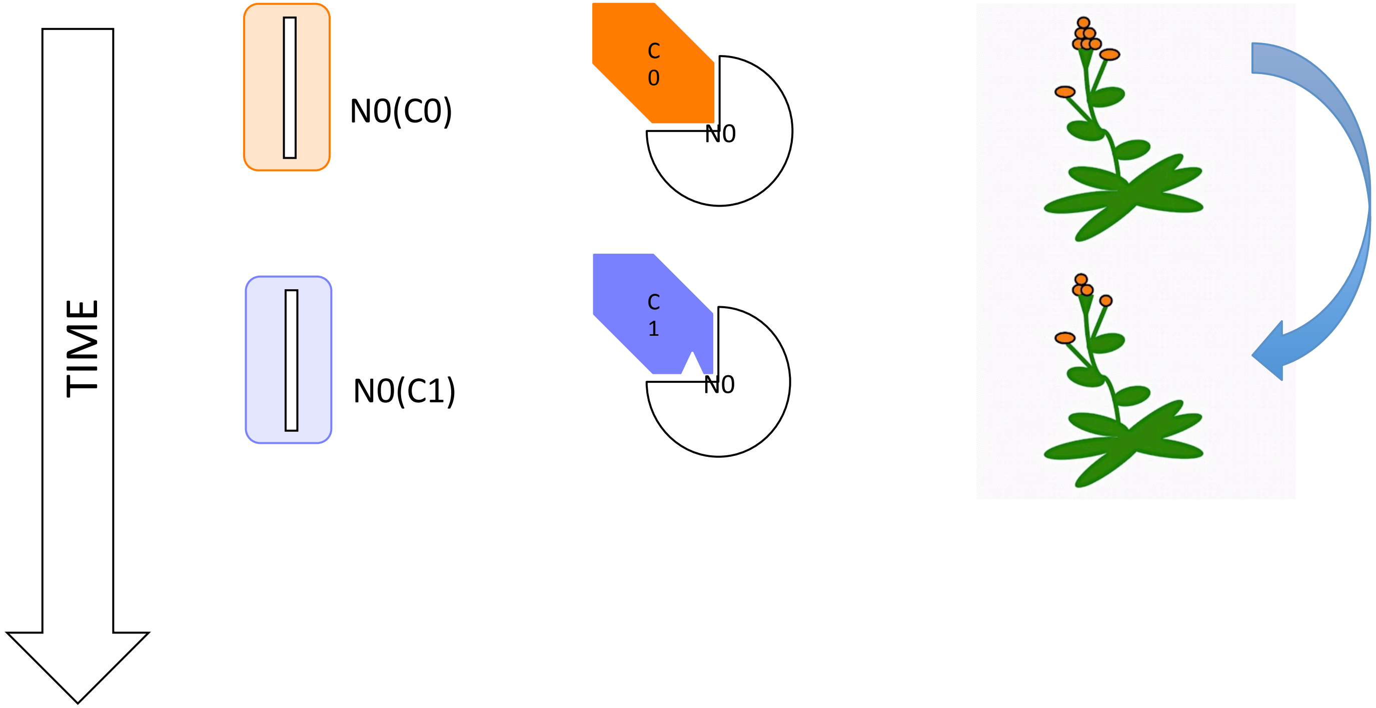
N0(C0)





Cytonuclear co adaptation

Genetic level Physiological level Phenotype level



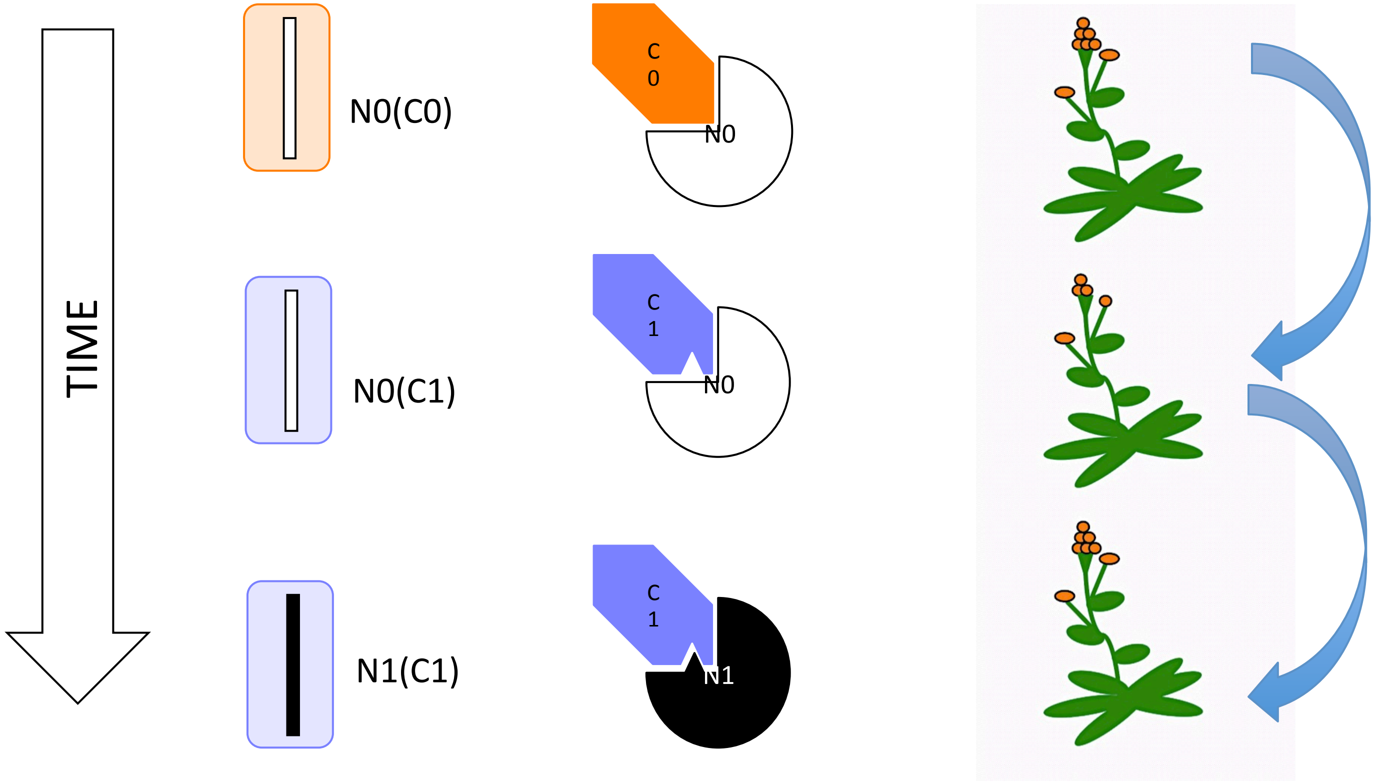
Fixation of a variant in one compartment

Cytonuclear co adaptation

Genetic level

Physiological level

Phenotype level

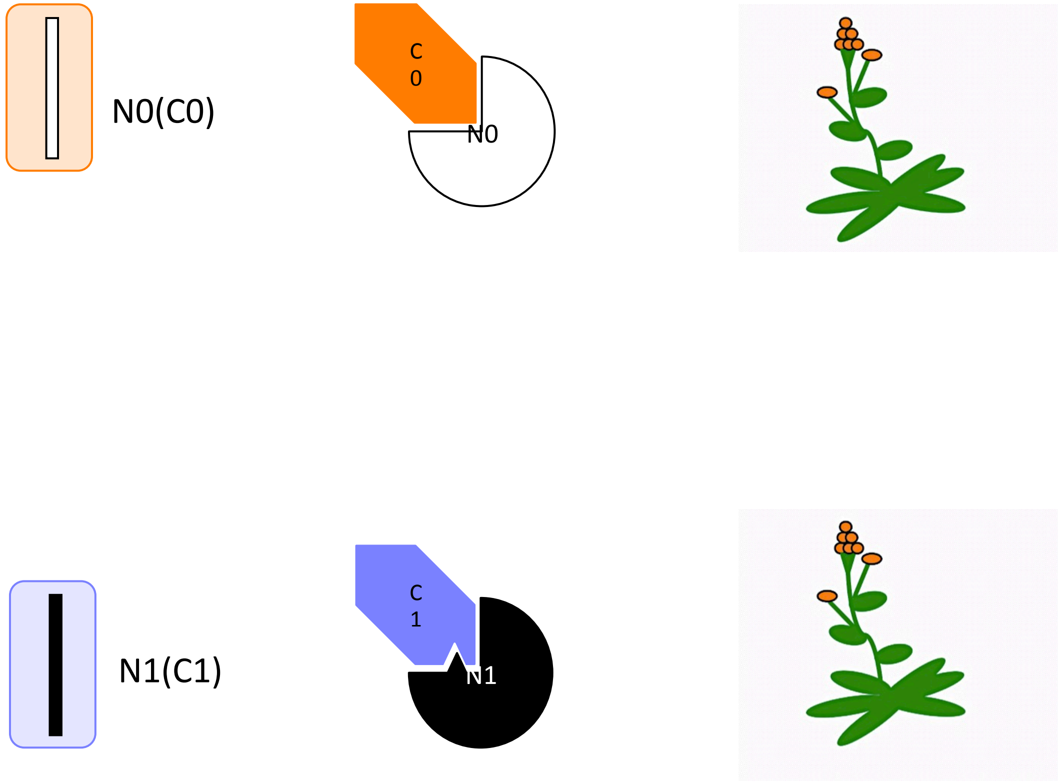


Fixation of a variant in one compartment

selection

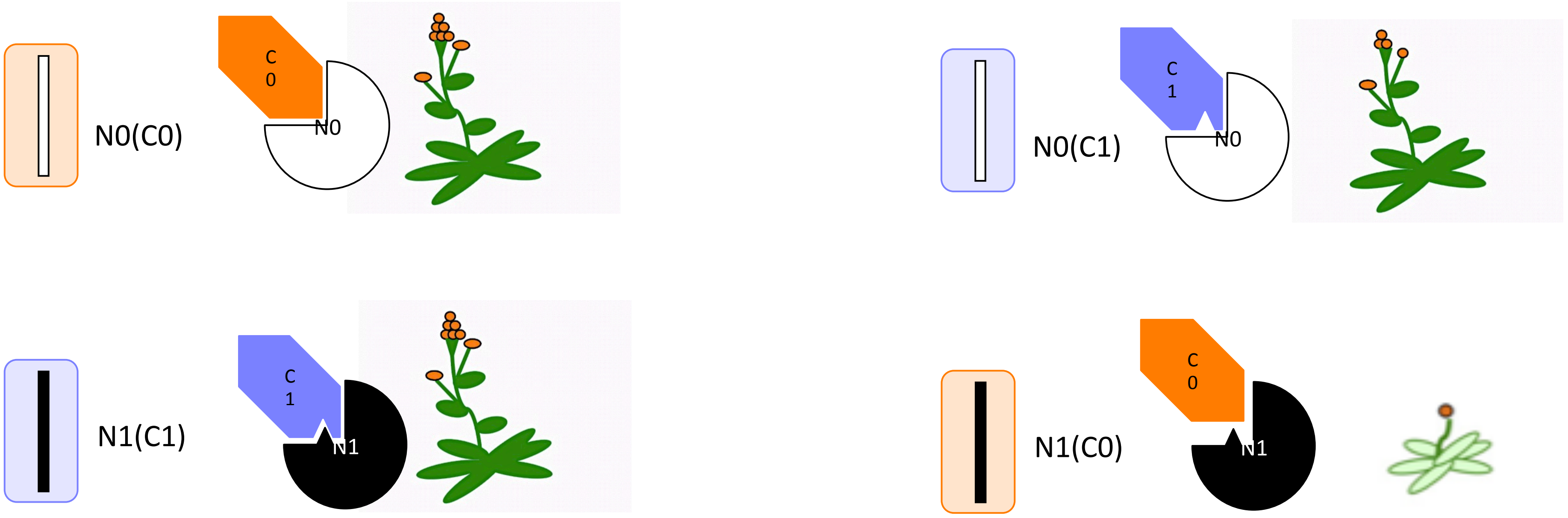
Cytonuclear co adaptation

Genetic level | Physiological level | Phenotype level



Present

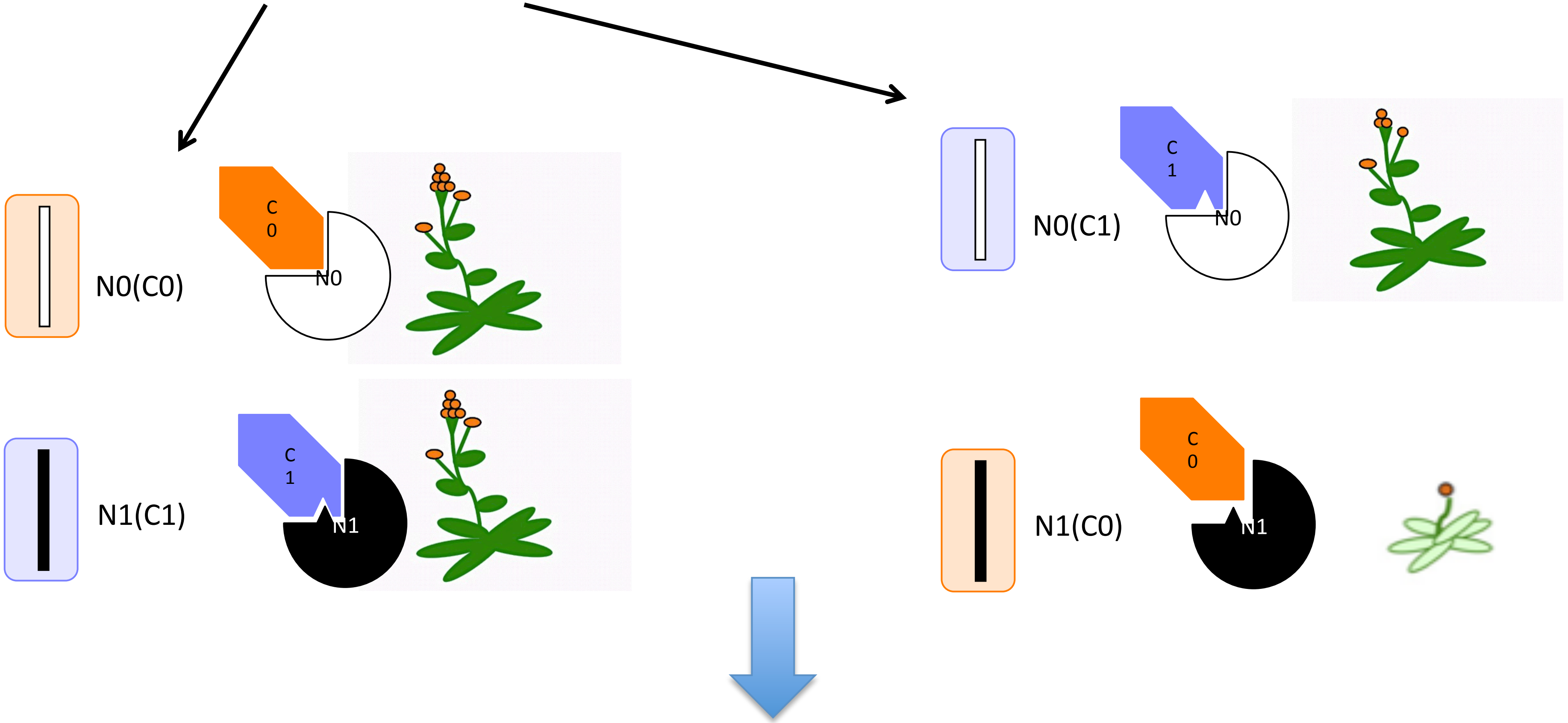
genetic interaction* between genes in the two genetic compartments



*genetic interaction: the phenotypic output of the genotype at one locus depends on the genotype at the other locus

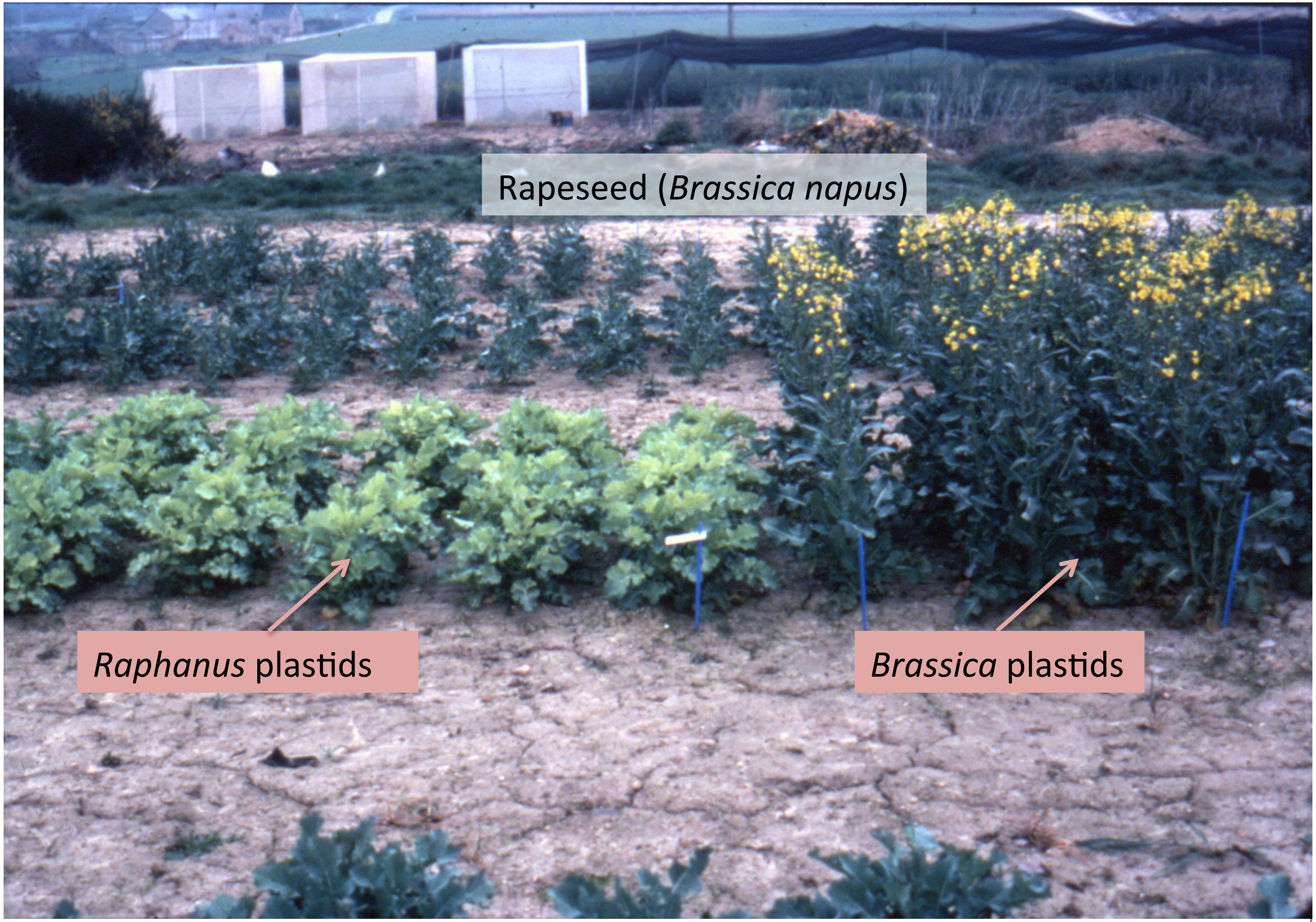
Cytonuclear co adaptation

"We expect variation in phenotypic traits that can contribute to fitness differences between proper and impaired associations of variants." (Rand et al, 2004)



Break it to see it

Cytonuclear co adaptation : break it to see it



Rapeseed (*Brassica napus*)

Raphanus plastids

Brassica plastids

G. Pelletier

Cytonuclear co adaptation : break it to see it

tobacco (*Nicotiana tabacum*)

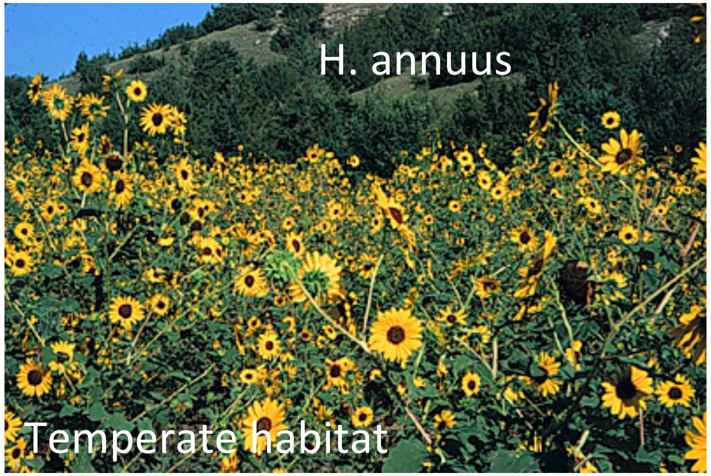
N. debneyi cytoplasm

N. tabacum cytoplasm

Belliard et al 1978

What would drive force the fixation of a mutation in a cytoplasmic genome?

Variations in cytoplasmic genomes may contribute to plant adaptation to their environment



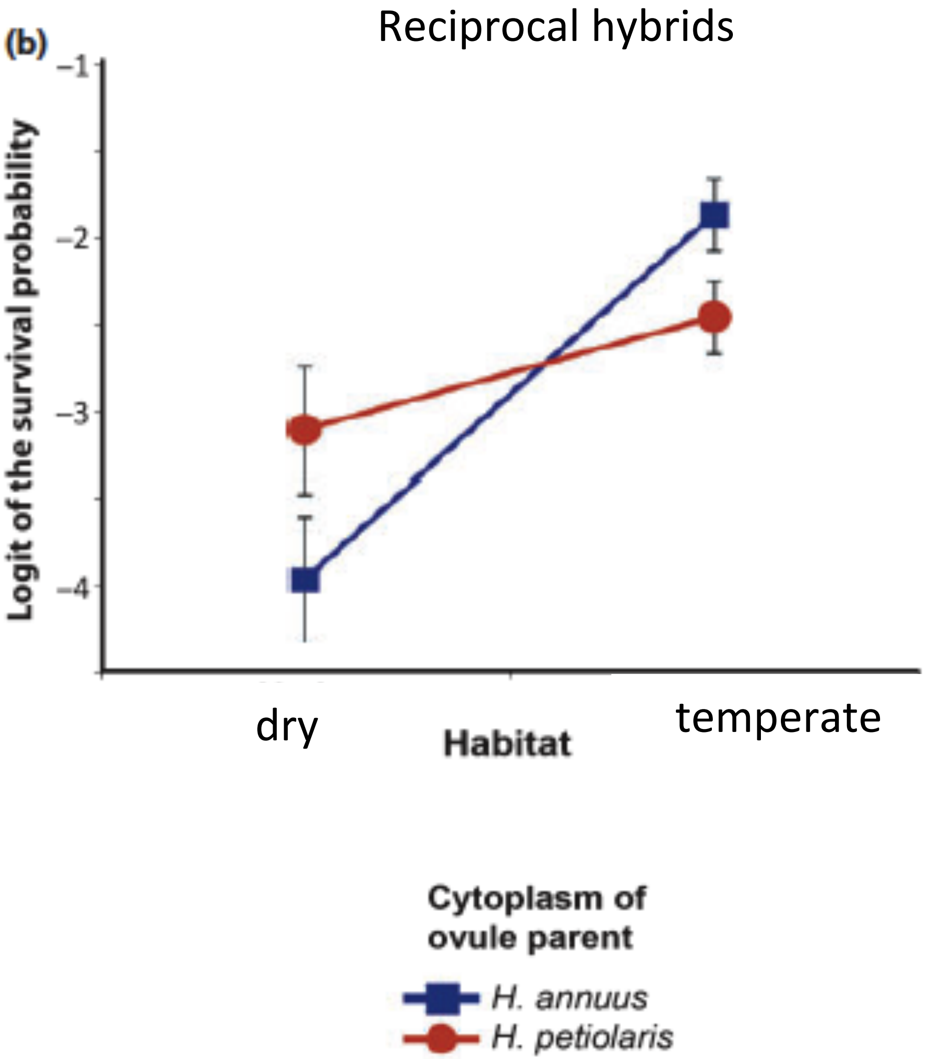
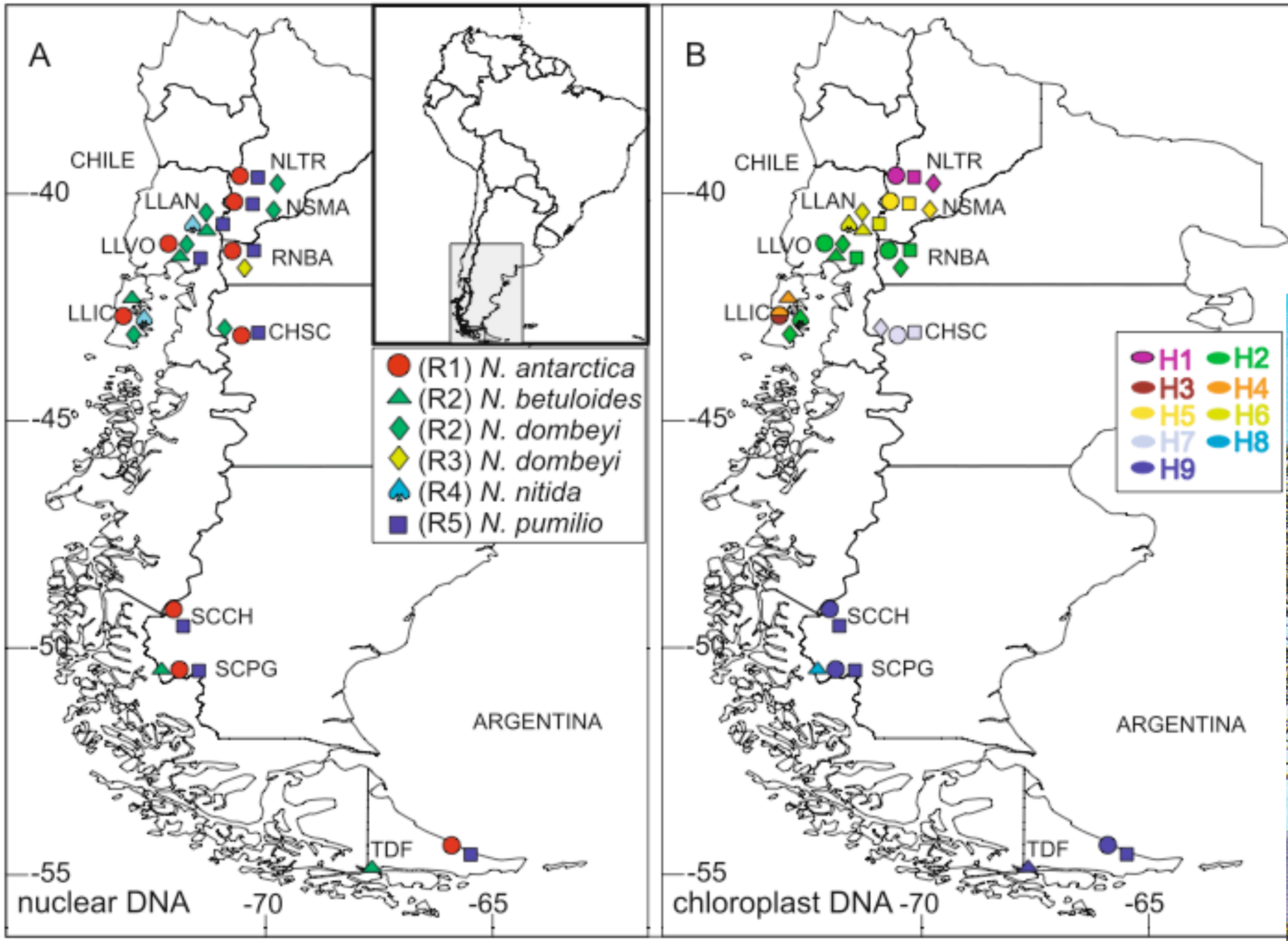
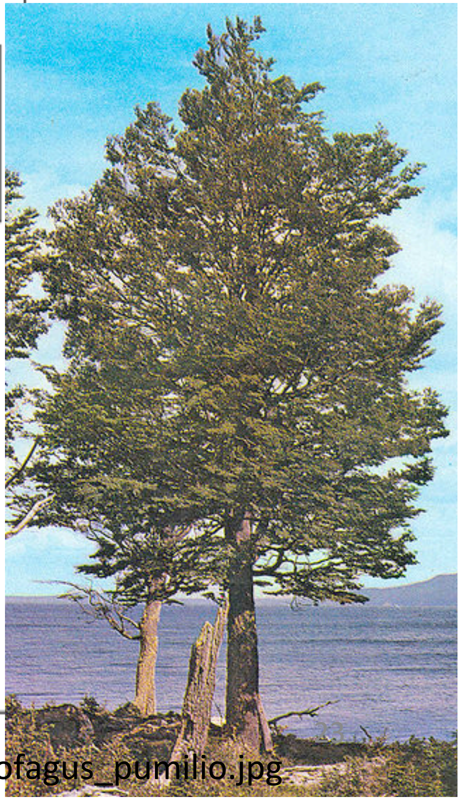


Photo credits: Clarence A. Rechenthin @ USDA-NRCS PLANTS;
http://www.unl.edu/dpilson/sunflower.html

Sambatti et al, 2008

Chloroplast types correlate with latitute, not with specific nuclear marker in populations of the subgenus *Nothofagus*





Acosta & Premoli, 2010

Crédits photo :http://commons.wikimedia.org/wiki/File:Nothofagus_pumilio.jpg

And within species?

If yes

Questions on:

- the traits under selection which drive the co adaptation?
- the genetic variations (in both genetic compartments) involved?
- The routes from genetic variation to phenotypic variation?

But

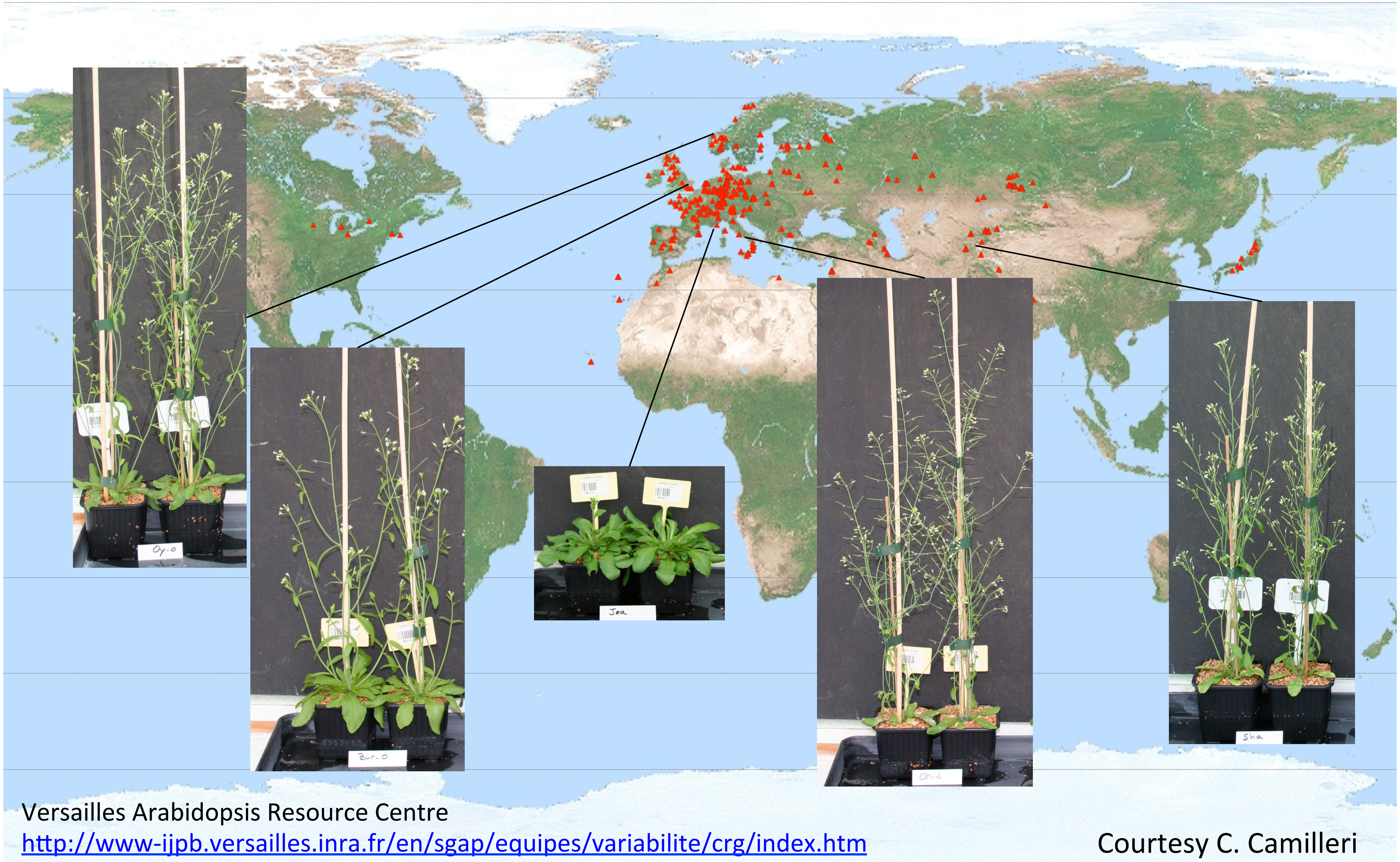
Size of expected genetic effects small or moderate

Address the question in a species where:

- Large genetic resources
- Wide ecological range
- Knowledge on the genetic diversity, ideally in both nuclear and organelle genomes
- Easy for genetic studies (short cycle, autogamy and crosses)….
- ….

Arabidopsis thaliana = model in functional genomics and evolutionary and adaptive studies

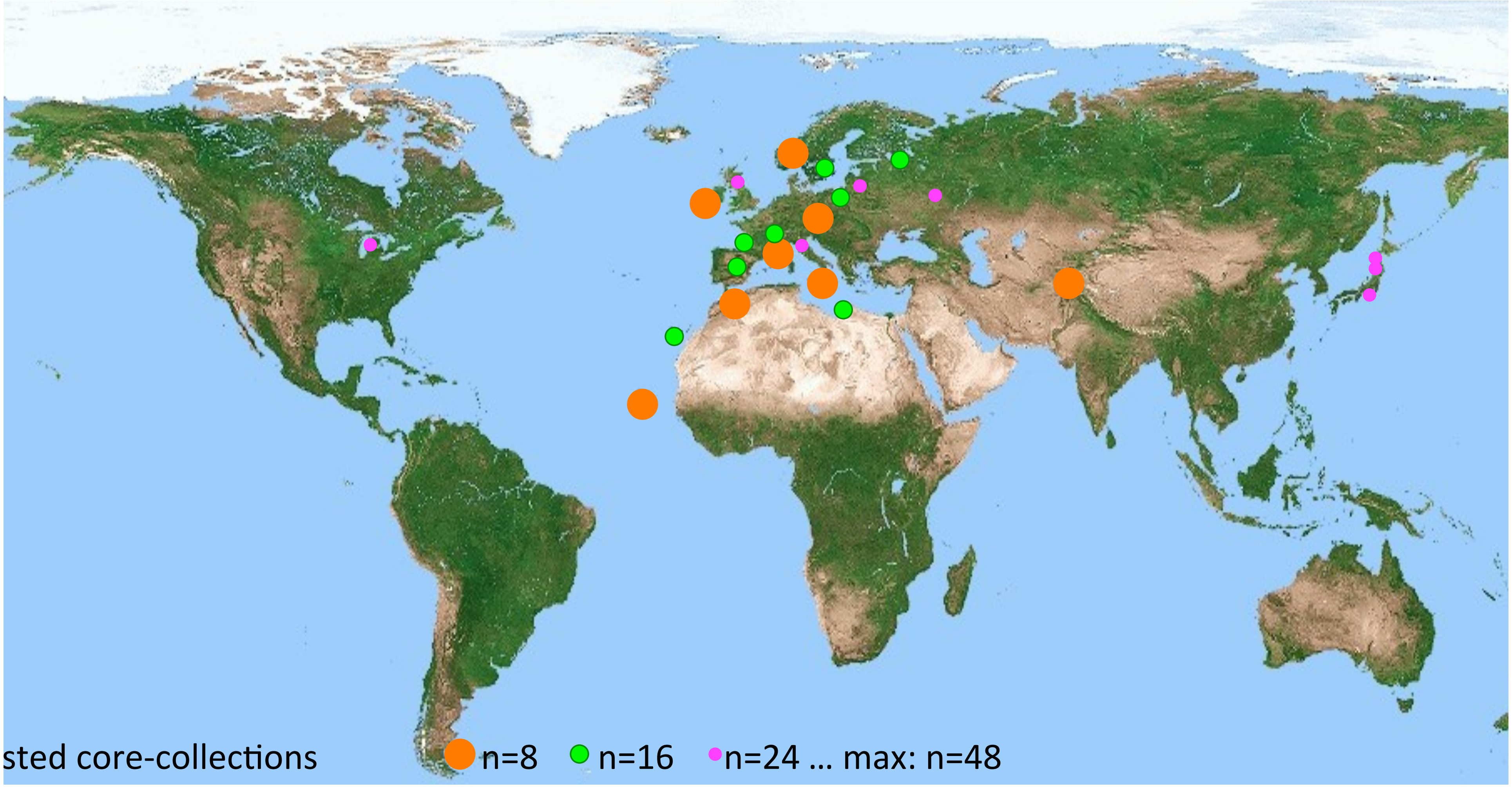
Worldwide collection of >700 Arabidopsis accessions



Versailles Arabidopsis Resource Centre
http://www-ijpb.versailles.inra.fr/en/sgap/equipes/variabilite/crg/index.htm

Courtesy C. Camilleri

Core-collections were designed to maximize the representation of the nuclear diversity present in collections…

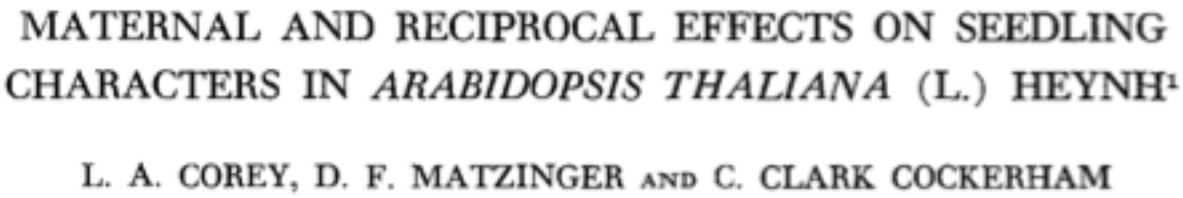


… the smallest core-collection also covers cytoplasmic diversity

McKhann et al, 2004; Moison et al, 2010

Literature suggests cytonuclear co adaptation in *A. thaliana*

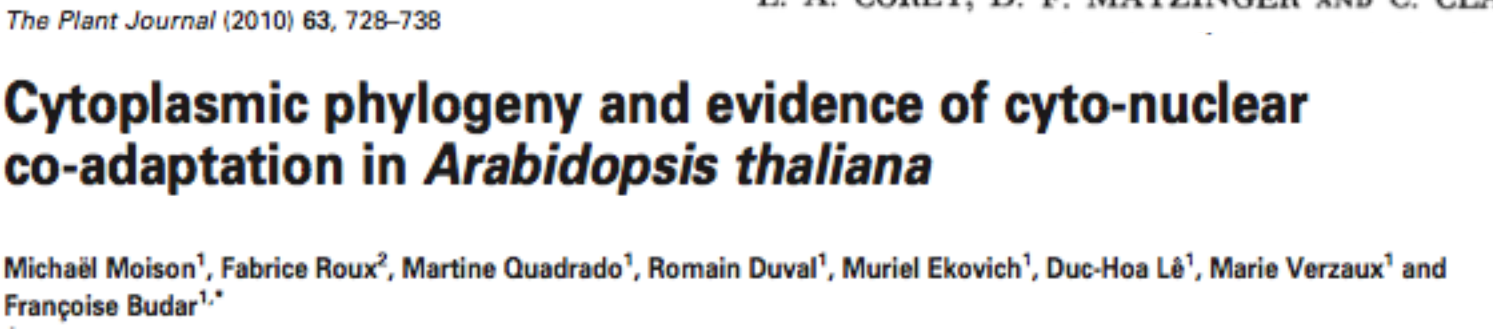
Effects on germination



MATERNAL AND RECIPROCAL EFFECTS ON SEEDLING CHARACTERS IN *ARABIDOPSIS THALIANA* (L.) HEYNH[1]

L. A. COREY, D. F. MATZINGER AND C. CLARK COCKERHAM

Genetics, 1976

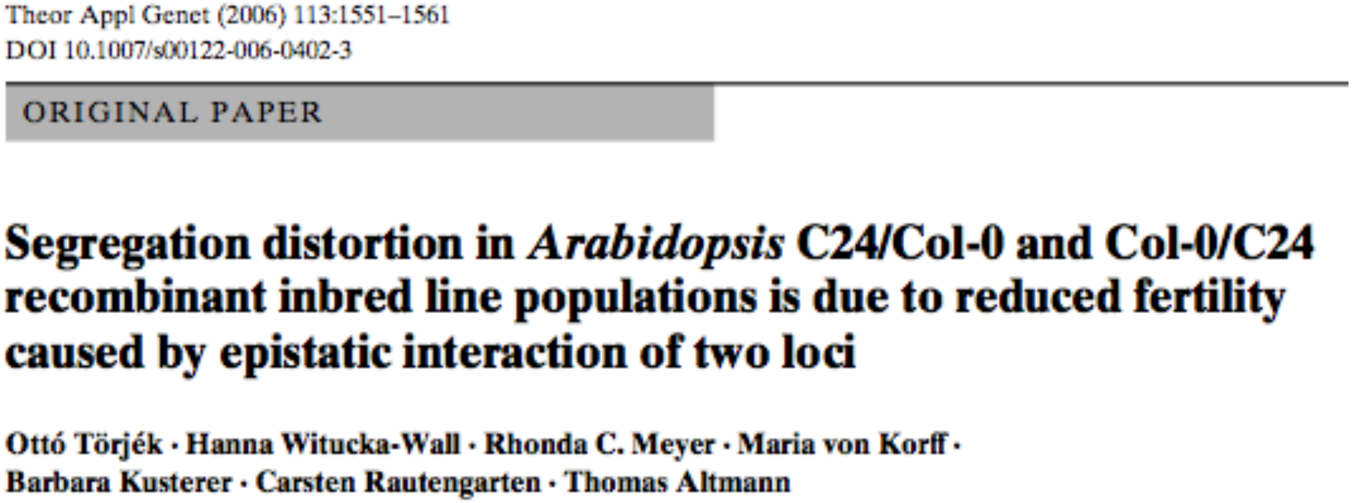


The Plant Journal (2010) 63, 728–738

Cytoplasmic phylogeny and evidence of cyto-nuclear co-adaptation in *Arabidopsis thaliana*

Michaël Moison[1], Fabrice Roux[2], Martine Quadrado[1], Romain Duval[1], Muriel Ekovich[1], Duc-Hoa Lê[1], Marie Verzaux[1] and Françoise Budar[1,*]

Biases in allele distributions in reciprocal RILS



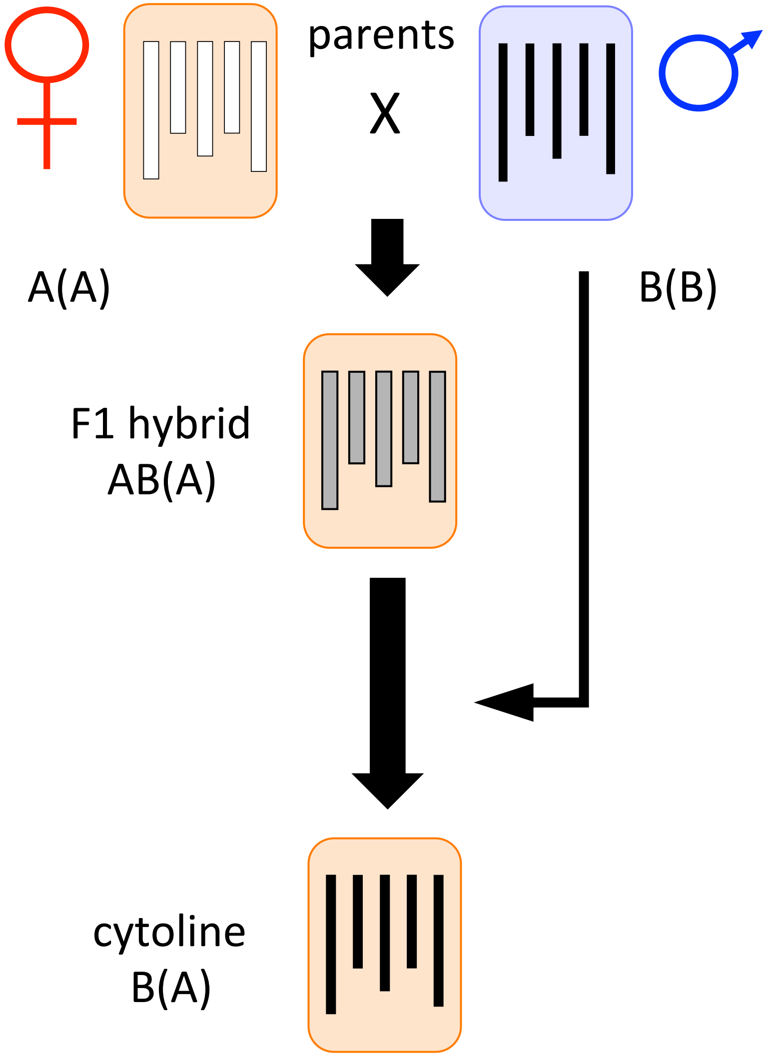
Theor Appl Genet (2006) 113:1551–1561
DOI 10.1007/s00122-006-0402-3

ORIGINAL PAPER

Segregation distortion in *Arabidopsis* C24/Col-0 and Col-0/C24 recombinant inbred line populations is due to reduced fertility caused by epistatic interaction of two loci

Ottó Törjék · Hanna Witucka-Wall · Rhonda C. Meyer · Maria von Korff · Barbara Kusterer · Carsten Rautengarten · Thomas Altmann

Arabidopsis cytolines: cytoplasm exchange between natural accessions



parents

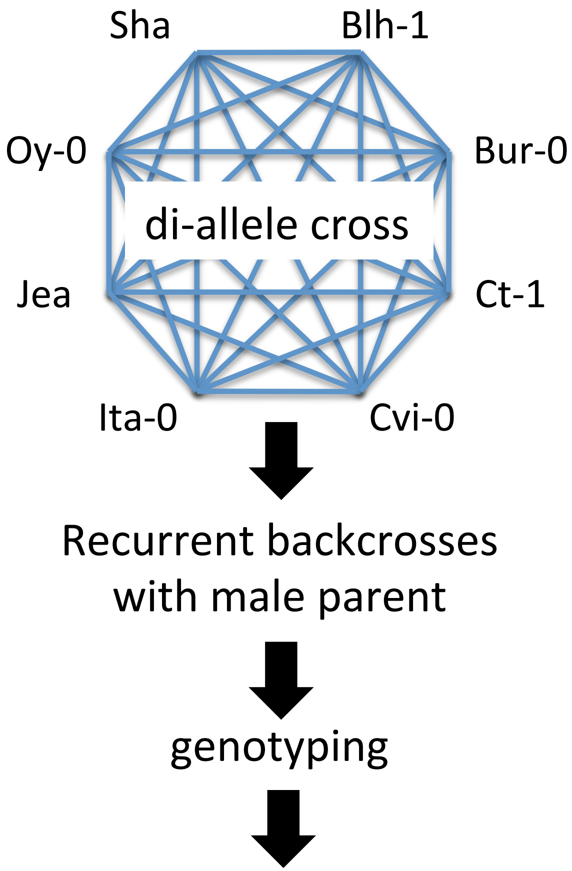
X

A(A)

B(B)

F1 hybrid
AB(A)

cytoline
B(A)



Sha

Blh-1

Oy-0

Bur-0

di-allele cross

Jea

Ct-1

Ita-0

Cvi-0

Recurrent backcrosses
with male parent

genotyping

56 cytolines

Versailles Arabidopsis Resource Centre, C. Camilleri et al

CYTOPHENO ANR 2012-2016

Multi level phenotype evaluation of cytolines and parents

Genetic level → Physiological level → Integrated level

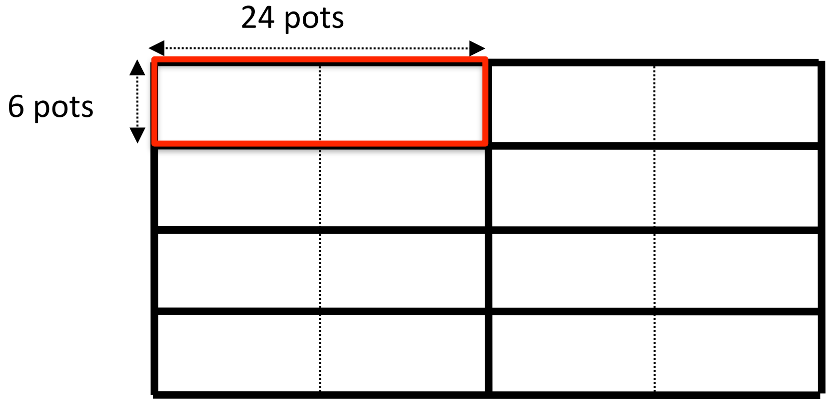
- Nuclear and organellar transcriptomes
- total and organellar proteomes

- metabolome
- photosynthesis
- respiration

- Fitness-related traits in ecological conditions
- Germination and early growth
- Response to Nitrogen starvation

A single seed production for all phenotyping experiments

1 culture chamber (56 m^2) with 8 tables

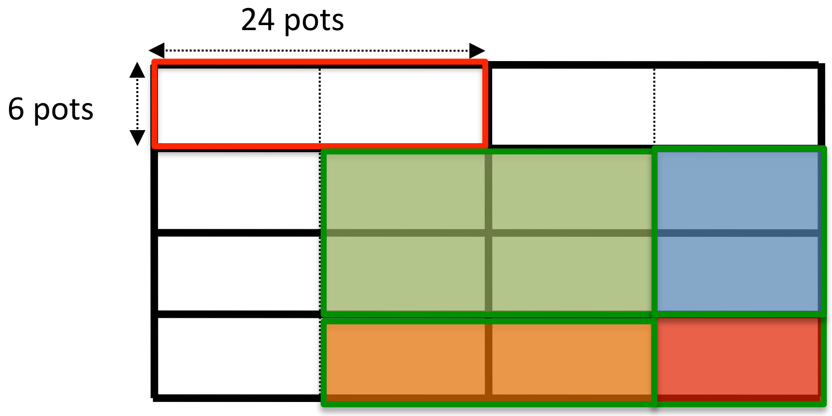


64 genotypes
16 plants per genotype

1024 plants

A single seed production for all phenotyping experiments

But : very strong heterogeneities in the culture chamber (edge effects)



-> definition of blocs with≠ edge effects and pots randomly disposed in each bloc so that 1 plant of each genotype on each half-table.

Versailles Arabidopsis Resource Centre, C. Camilleri et al
Experimental design by ML Martin-Magniette

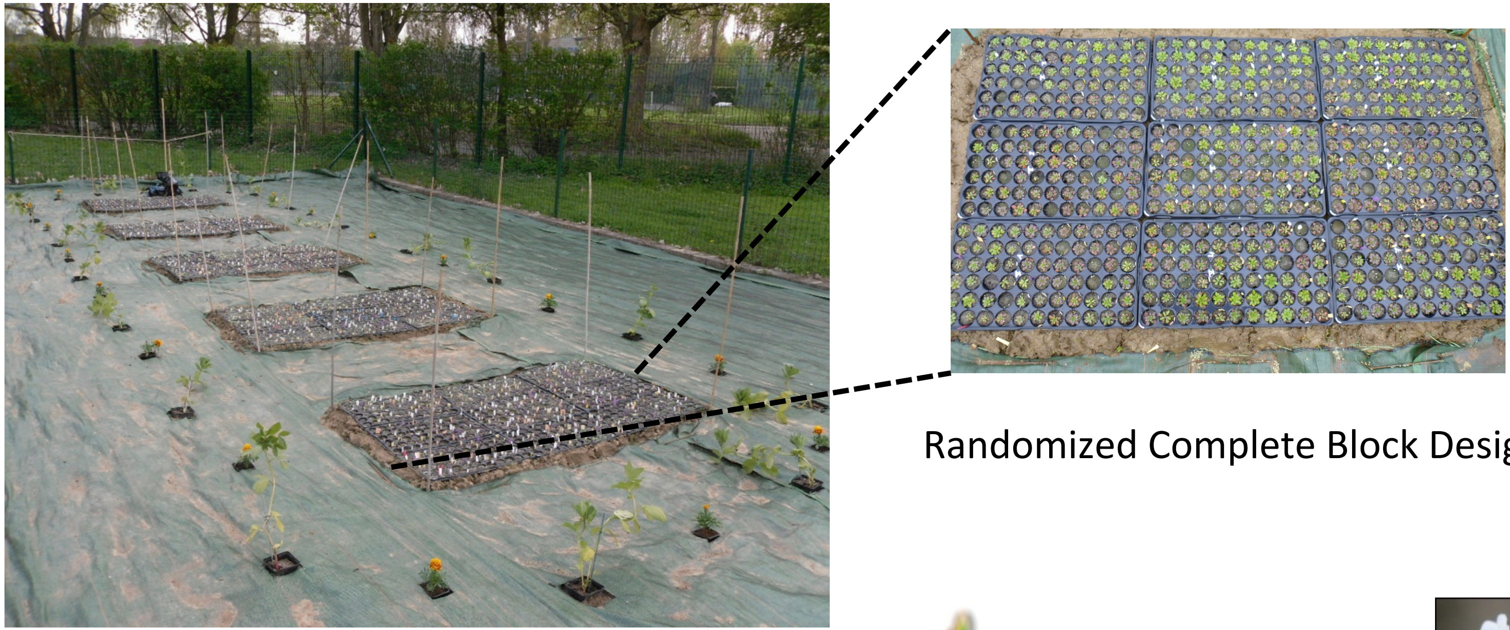
Other choices to make :

-> sowing dates

-> management of different life cycle lengths

First phenotyping of cytolines: fitness-related traits in the field

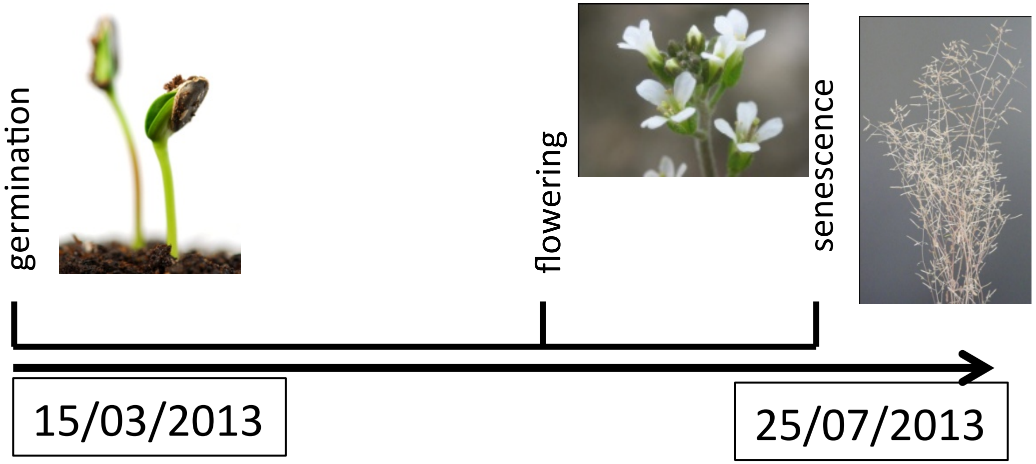
Common garden, University of Lille (North of France)



2700 plants

Randomized Complete Block Design

27 quantitative traits
+ survival

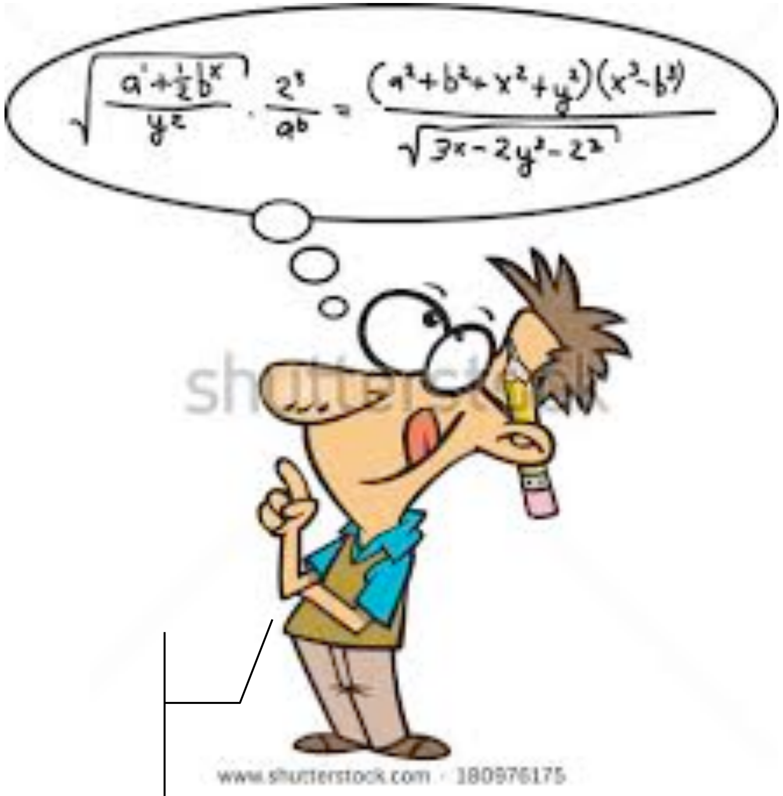


Roux et al, 2016

F. Roux, E. Barillot, C. Glorieux

Statistical analysis : discussion between biologists and statisticians

I wish to know if cytonuclear (genetic) interaction influences the traits that I measured



- What's the experimental design?
- What do you know about the environmental heterogeneities?
- What do you expect as interactions between genotypes and environment?

For each trait: what significant effects?

Mixed model. $Y \sim \underbrace{\text{block} + \text{nucleus} + \text{cytoplasm} + \text{nuc.*cyt.} + \text{block*nuc.} +} [\text{ENV}]$

Nominal FDR 5% (for each effect)

T. Mary-Huard

For each trait: what significant effects?

Mixed model. $Y \sim \underbrace{\text{block} \boxed{+ \text{nucleus} + \text{cytoplasm} + \text{nuc.*cyt.}} + \text{block*nuc.}}_{} + [\text{ENV}]$

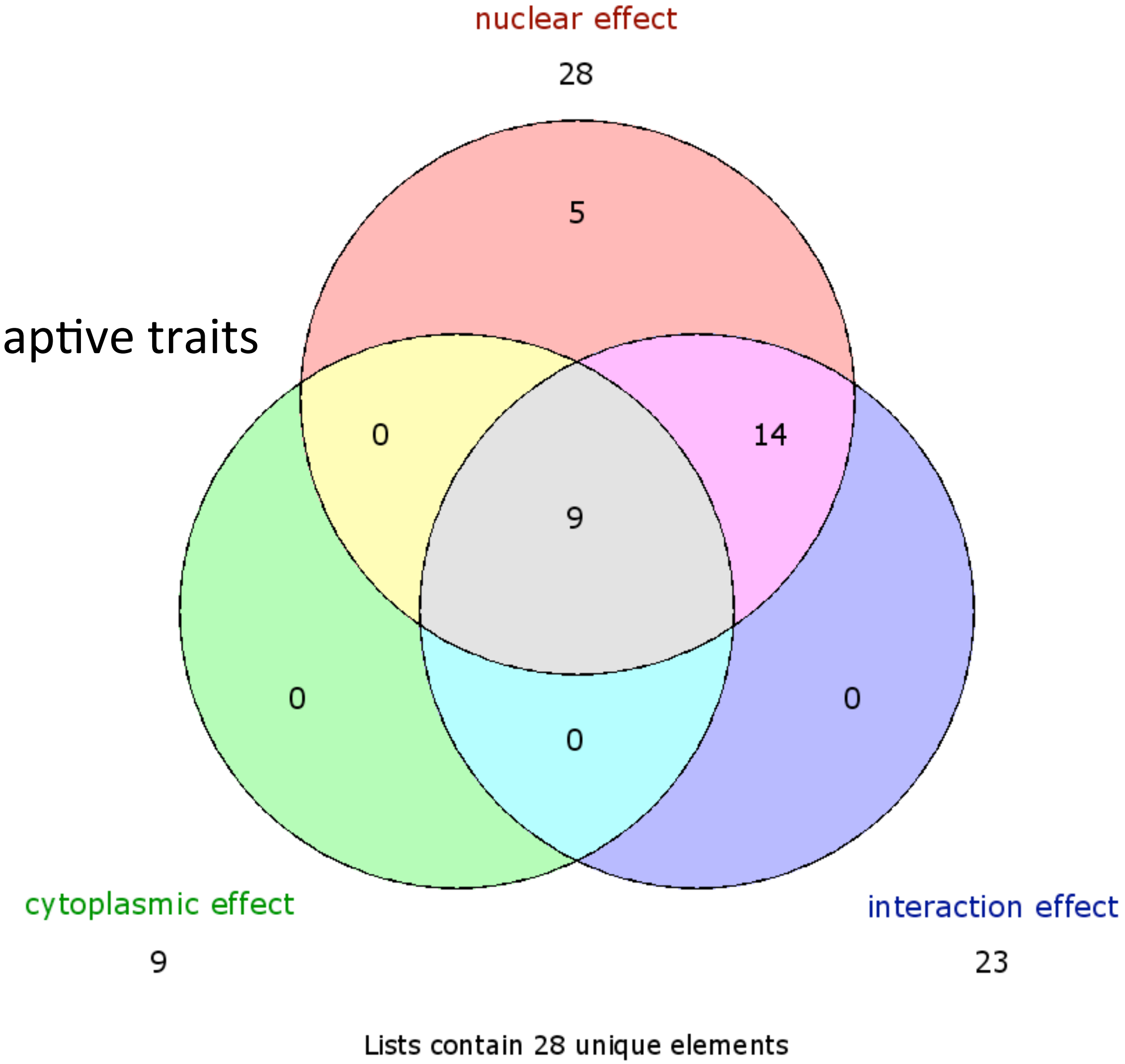
Nominal FDR 5% (for each effect)

For each trait: what significant effects?

Mixed model. Y ~ block + nucleus + cytoplasm + nuc.*cyt. + block*nuc. + [ENV]

Nominal FDR 5% (for each effect)

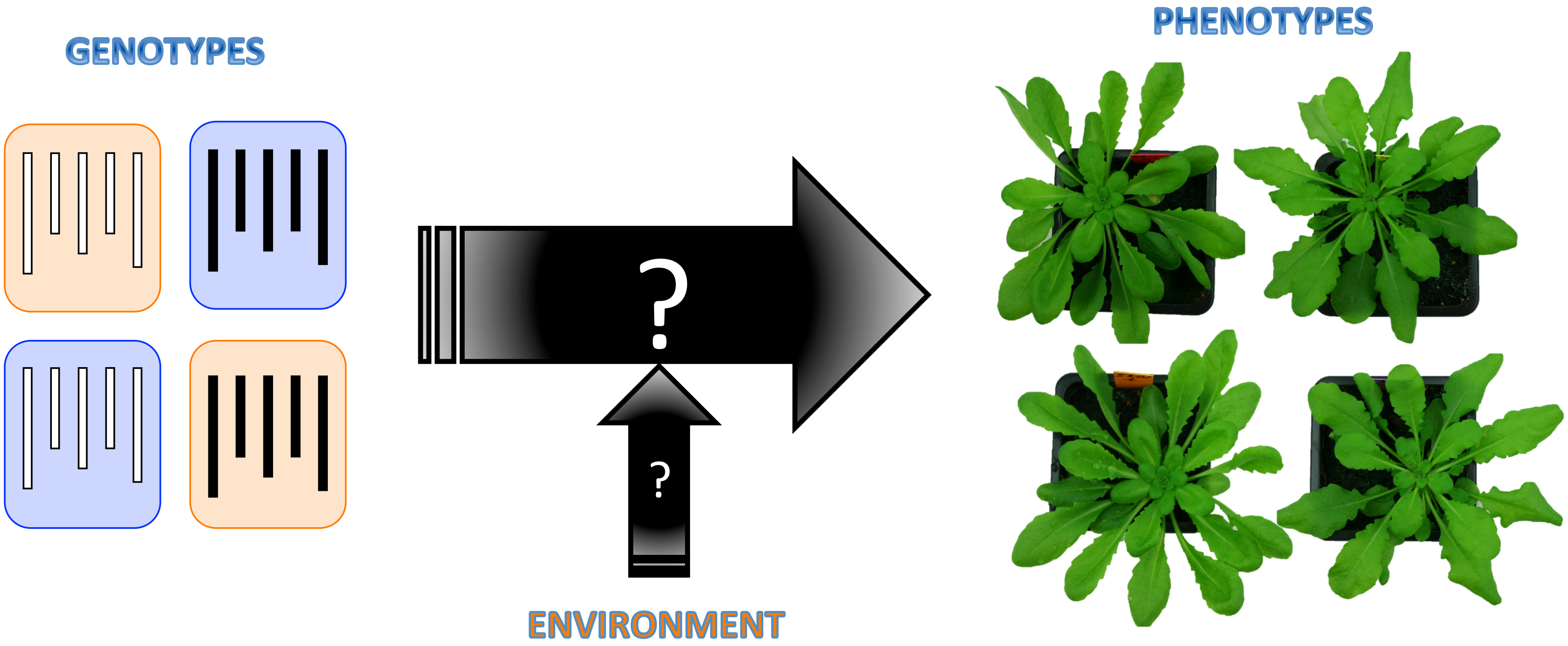
cytonuclear interactions affect adaptive traits



Roux et al, 2016

Towards a molecular understanding of the effects of cytoplasmic exchange

From genotype to phenotype



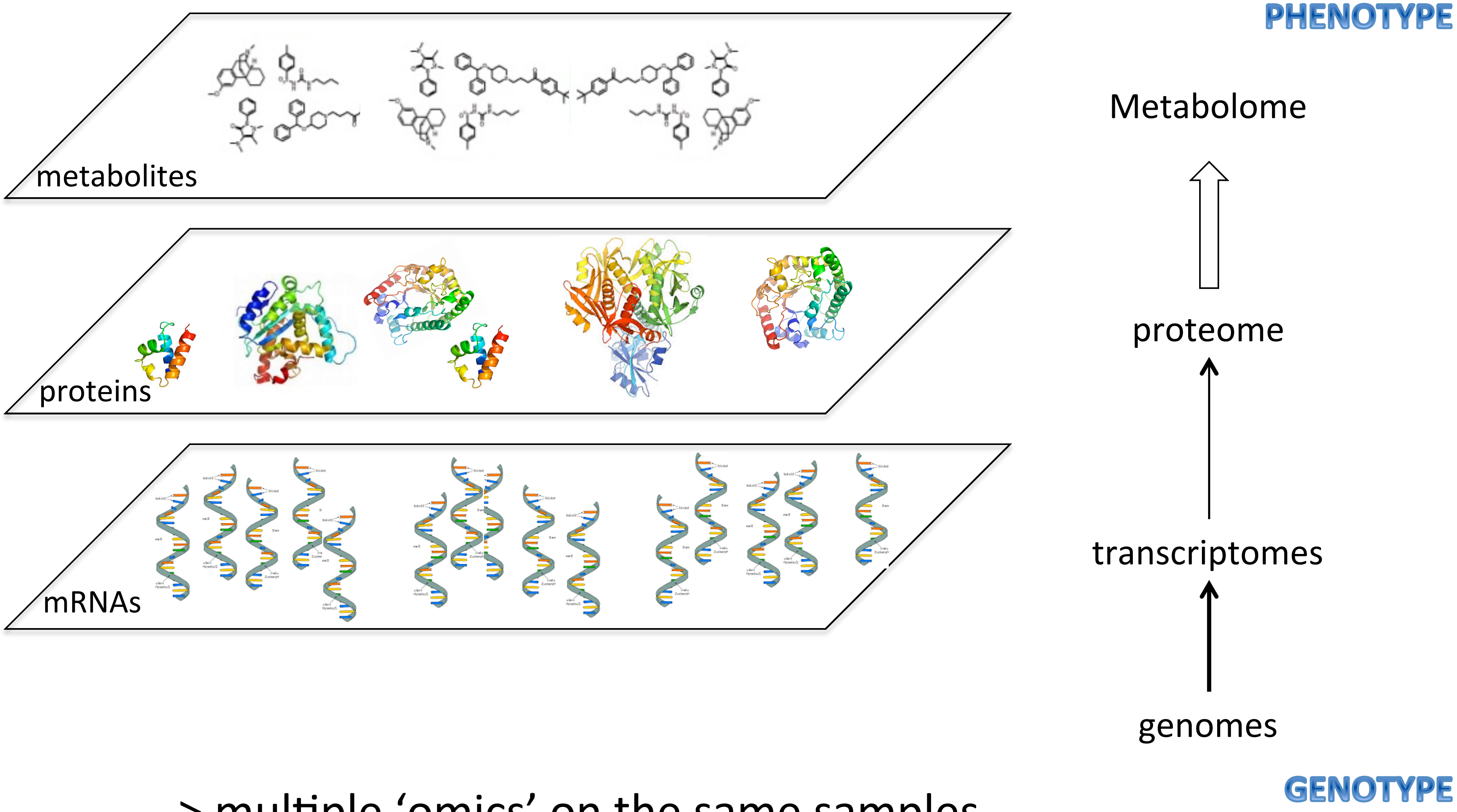
GENOTYPES

ENVIRONMENT

PHENOTYPES

Towards a molecular understanding of the effects of cytoplasmic exchange

Multilevel molecular phenotyping



metabolites

proteins

mRNAs

PHENOTYPE

Metabolome

proteome

transcriptomes

genomes

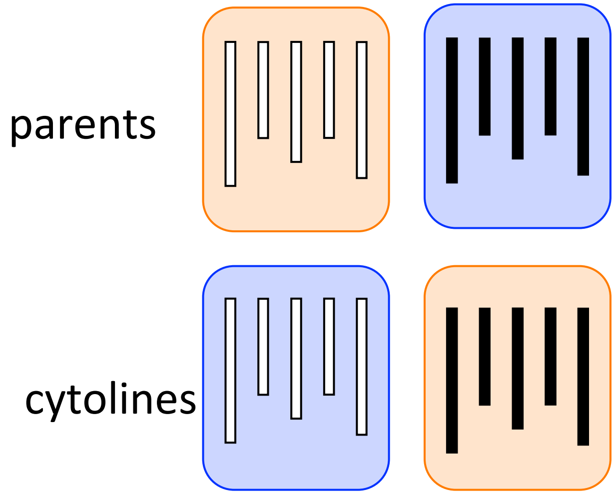
GENOTYPE

-> multiple ‘omics’ on the same samples

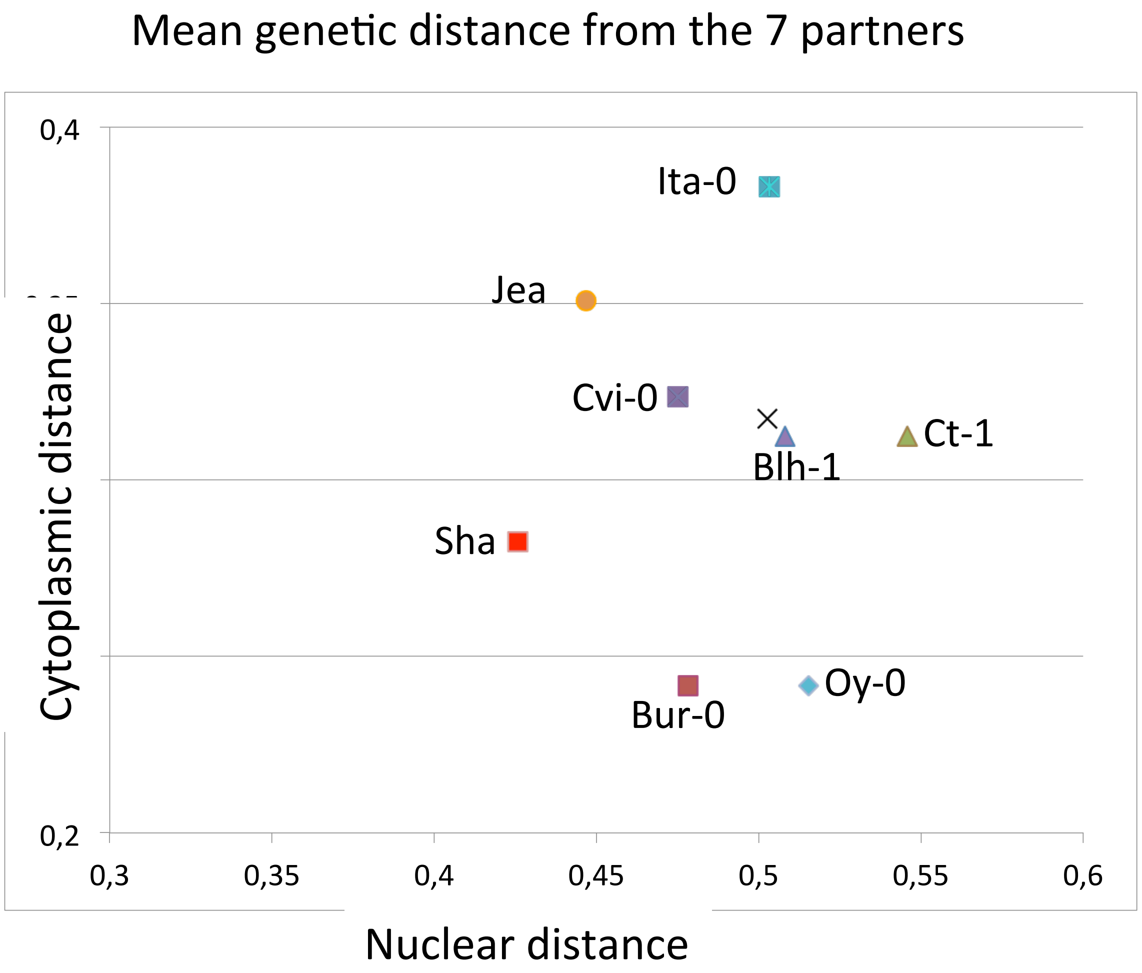
Limitation of genotypes for some of the phenotyping experiments

Which ones??

Minimal set:
4 genotypes

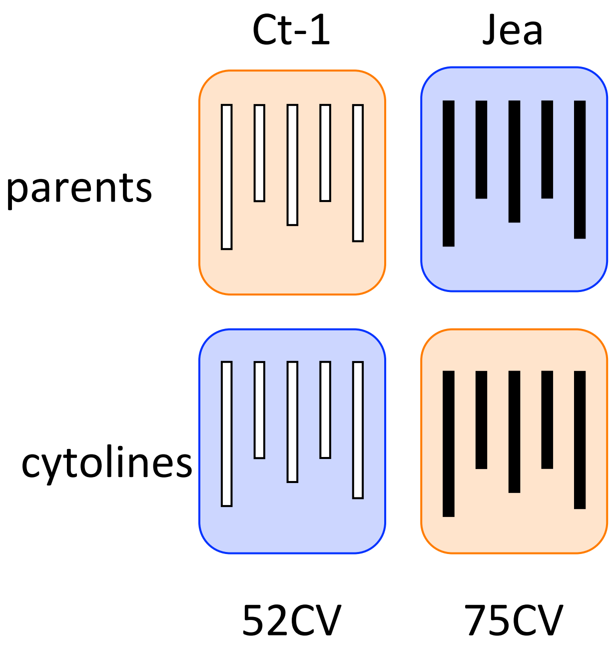


From partial genotyping information

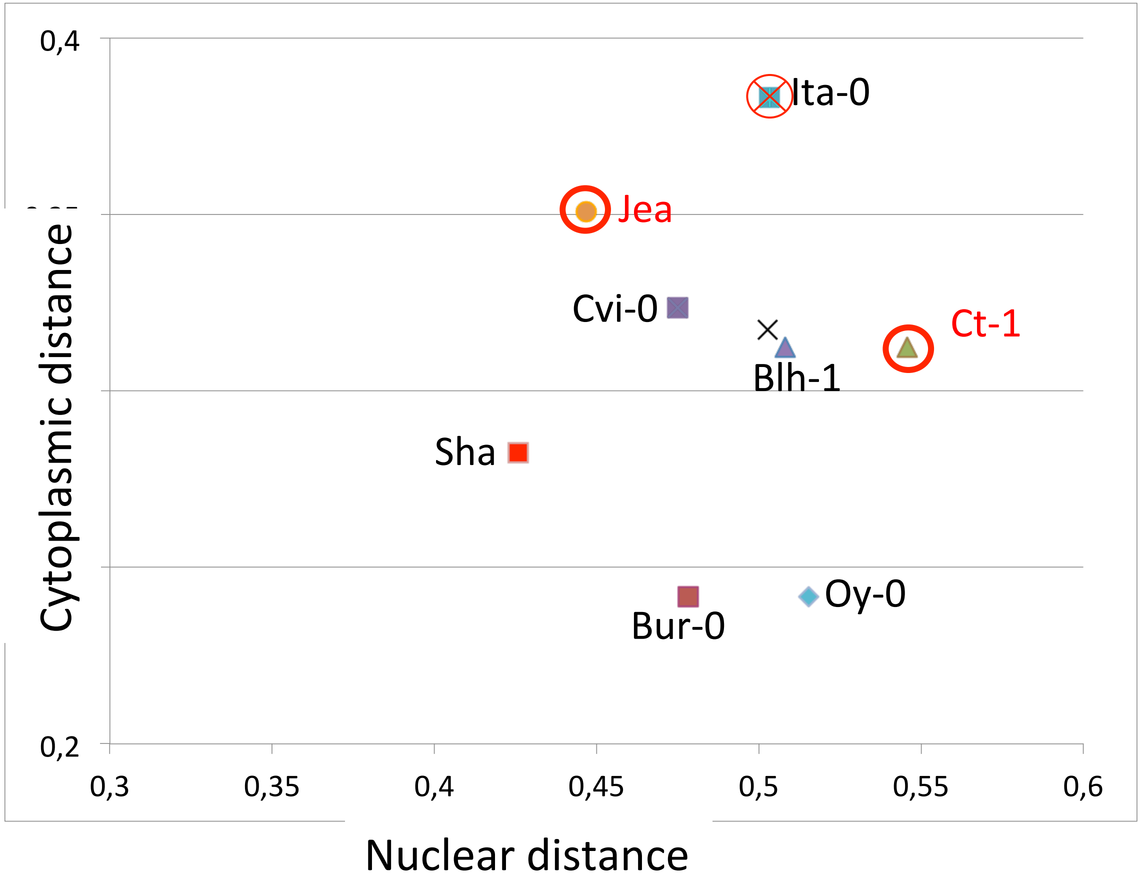


Limitation of genotypes for some of the phenotyping experiments

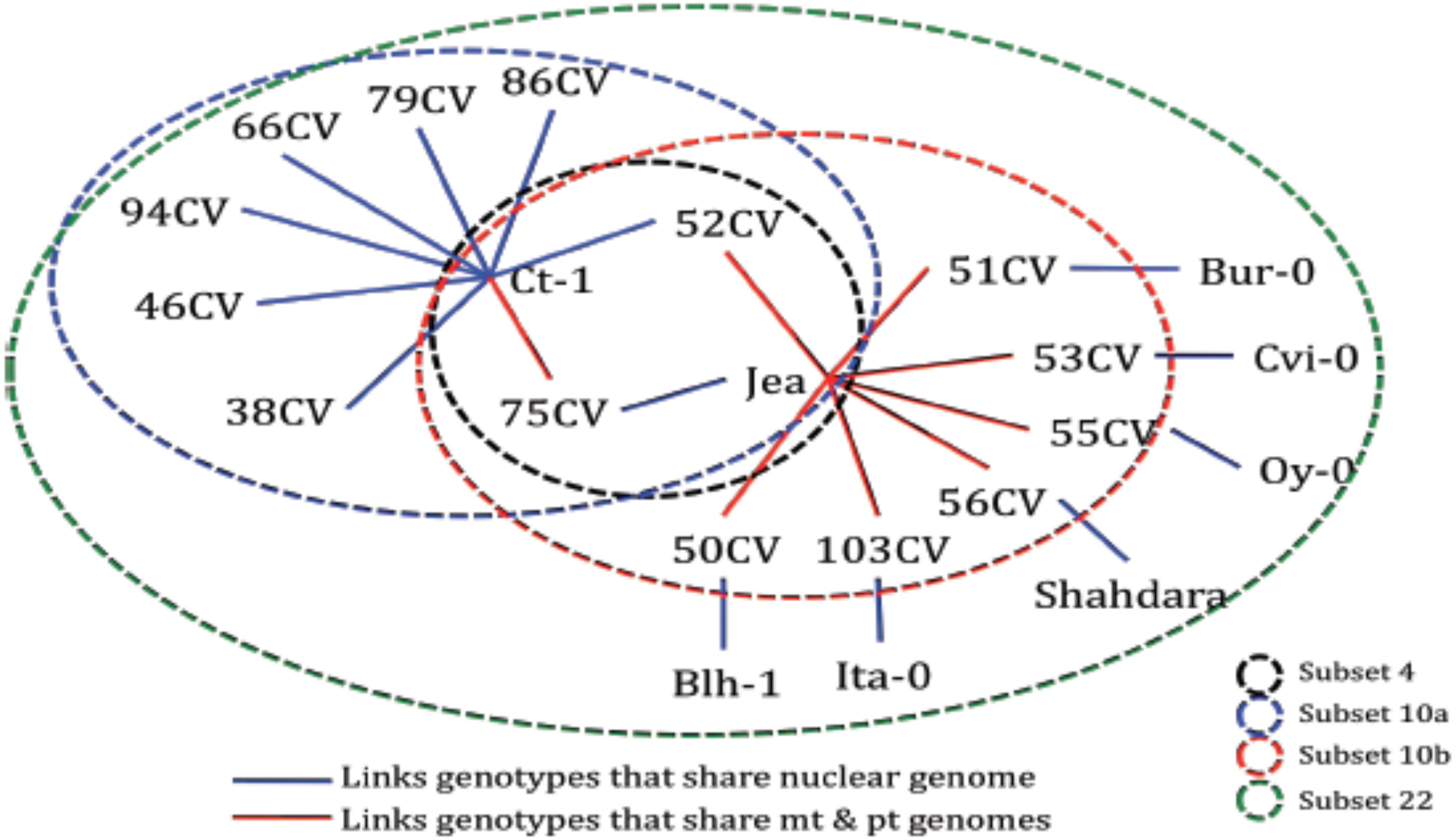
choice of genotypes



Mean genetic distance from the 7 partners



subsets of genotypes



Towards a molecular understanding of the effects of cytoplasmic exchange

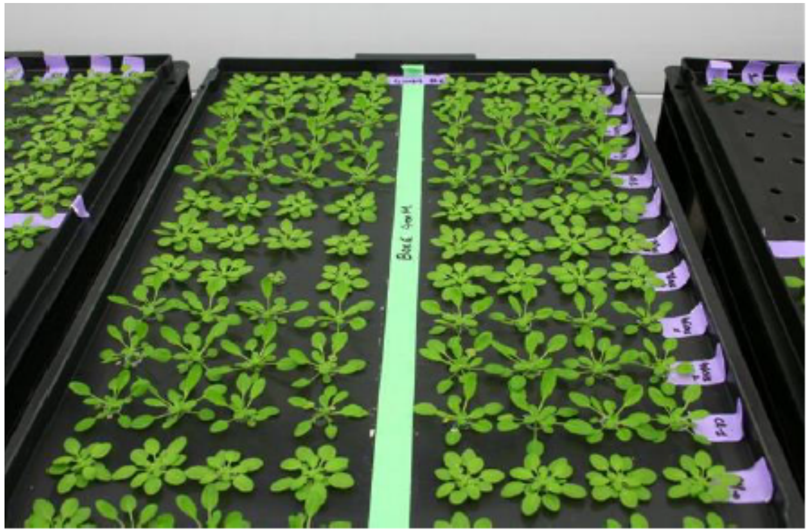
Multilevel molecular phenotyping:
Production of samples

Requirements :

- All measures on same sample -> at least 400mg of fresh weight/sample needed (1 starved rosette <100 mg)
- All samples at the same physiological state
- Three biological repetitions

Multilevel molecular phenotyping:
Production of samples

Hydroponic growth of plants in culture chamber (short days- 5 weeks)



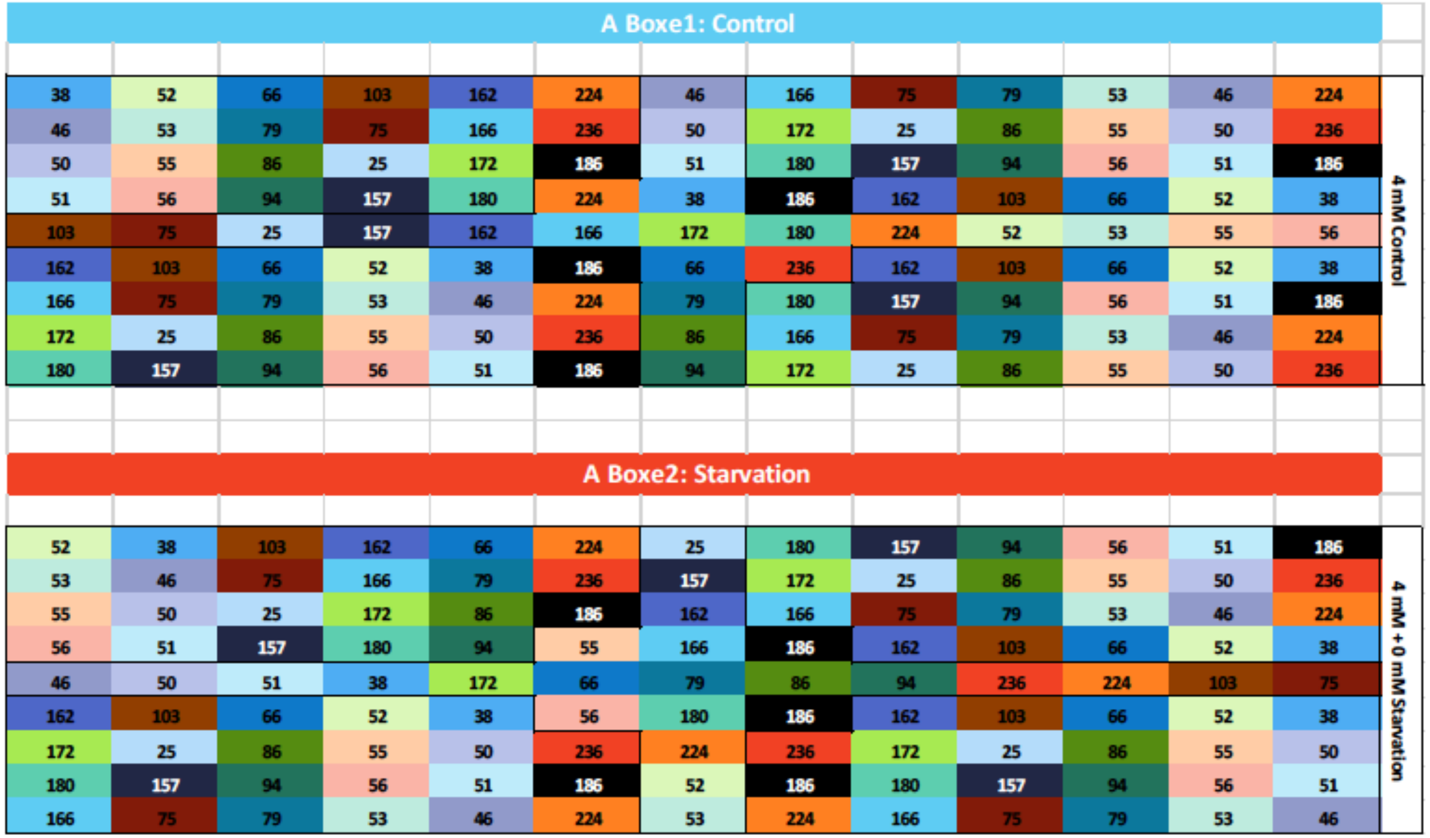
Two conditions of nitrogen nutrition:
N+: 4mM nitrate
N0: nitrate starvation (0mM) 1 week before harvest

F. Chardon

Multilevel molecular phenotyping:
Production of samples

1 experiment

Genotypes	Genotype	nucléaire	cyto
38	38Cv1b1	Ct-1	Blh-1
46	46CV1b1	Ct-1	Ita-0
50	50CV1b1	Blh-1	Jea
51	51CV1b1	Bur-0	Jea
52	52CV1b1	Ct-1	Jea
53	53CV1b1	Cvi-0	Jea
55	55CV1b1	Oy-0	Jea
56	56CV1b1	Shahdara	Jea
66	66CV1b1	Ct-1	Oy-0
79	79CV1b1	Ct-1	Cvi-0
86	86CV1b1	Ct-1	Bur-0
94	94CV1b1	Ct-1	Shahdara
103	103CV1b1	Ita-0	Jea
75	75CV1b1	Jea	Ct-1
25	Jea	Jea	Jea
157	Ita-0	Ita-0	Ita-0
162	Ct-1	Ct-1	Ct-1
166	Cvi-0	Cvi-0	Cvi-0
172	Bur-0	Bur-0	Bur-0
180	Blh-1	Blh-1	Blh-1
224	Oy-0	Oy-0	Oy-0
236	Shahdara	Shahdara	Shahdara
186	Col-0	Col-0	Col-0



A Boxe1: Control

38	52	66	103	162	224	46	166	75	79	53	46	224
46	53	79	75	166	236	50	172	25	86	55	50	236
50	55	86	25	172	186	51	180	157	94	56	51	186
51	56	94	157	180	224	38	186	162	103	66	52	38
103	75	25	157	162	166	172	180	224	52	53	55	56
162	103	66	52	38	186	66	236	162	103	66	52	38
166	75	79	53	46	224	79	180	157	94	56	51	186
172	25	86	55	50	236	86	166	75	79	53	46	224
180	157	94	56	51	186	94	172	25	86	55	50	236

4 mM Control

A Boxe2: Starvation

52	38	103	162	66	224	25	180	157	94	56	51	186
53	46	75	166	79	236	157	172	25	86	55	50	236
55	50	25	172	86	186	162	166	75	79	53	46	224
56	51	157	180	94	55	166	186	162	103	66	52	38
46	50	51	38	172	66	79	86	94	236	224	103	75
162	103	66	52	38	56	180	186	162	103	66	52	38
172	25	86	55	50	236	224	236	172	25	86	55	50
180	157	94	56	51	186	52	186	180	157	94	56	51
166	75	79	53	46	224	53	224	166	75	79	53	46

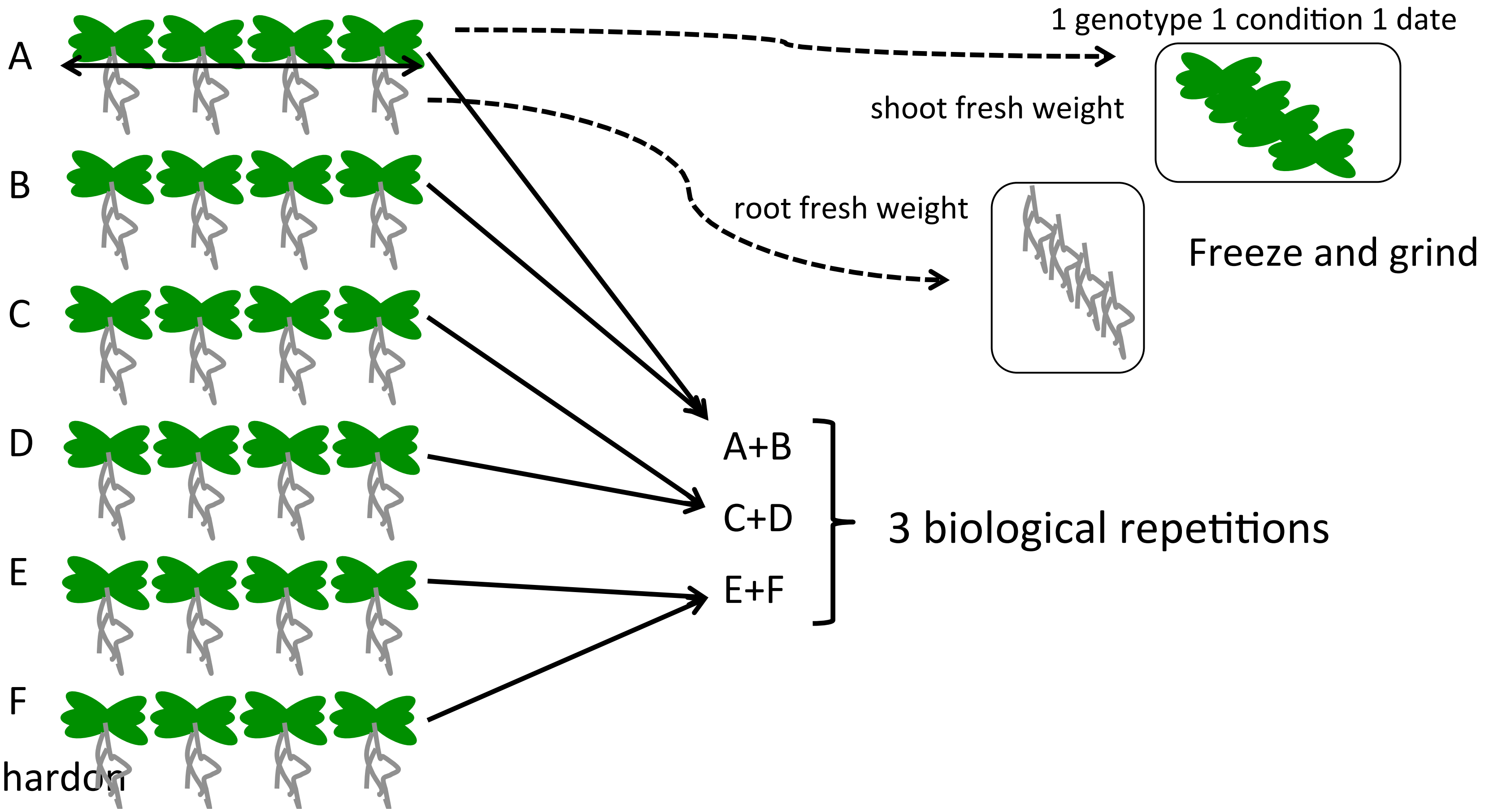
4 mM +0 mM Starvation

F. Chardon

Towards a molecular understanding of the effects of cytoplasmic exchange

Multilevel molecular phenotyping:
Production of samples

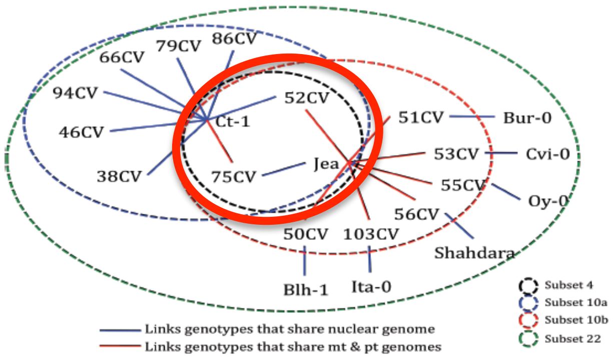
- ✓ 6 independent productions (randomised designs)
- ✓ 4 plants /production/genotype/ N cond
- ✓ All harvests at 11:00 am
- ✓ 1 sample = pool of 2 productions of successive dates

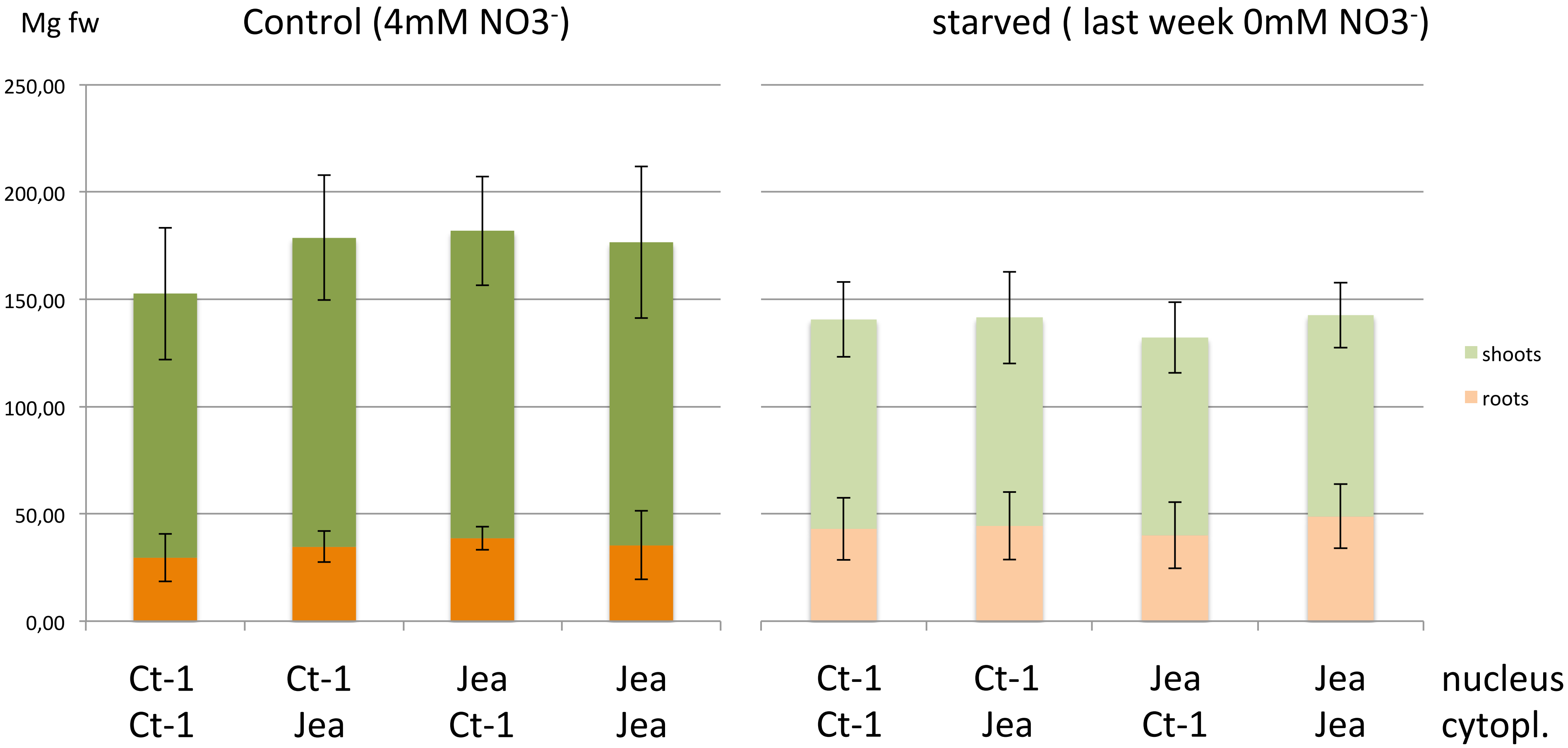


3 biological repetitions

F. Chardon

Biomass production

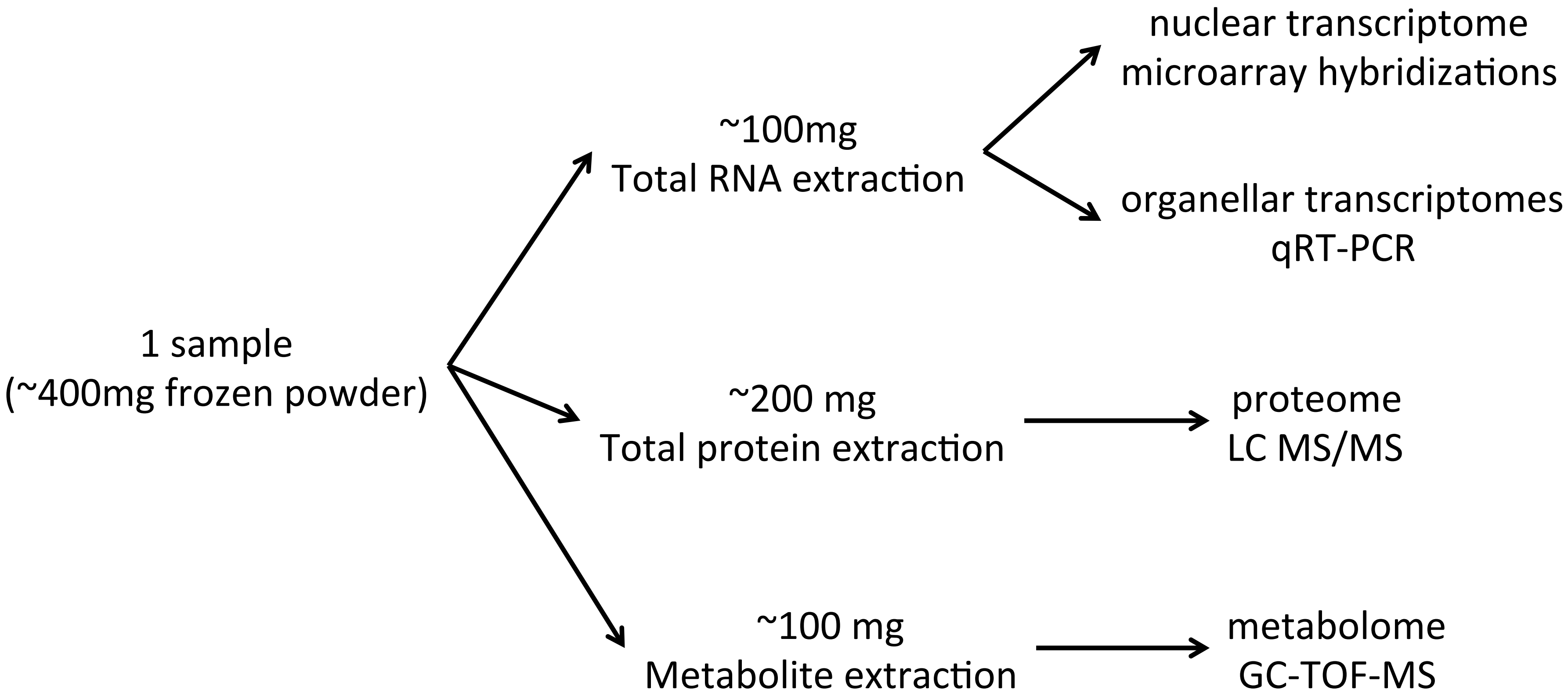




F. Chardon

Towards a molecular understanding of the effects of cytoplasmic exchange

Multilevel molecular phenotyping



1 sample
(~400mg frozen powder)

~100mg
Total RNA extraction

nuclear transcriptome
microarray hybridizations

organellar transcriptomes
qRT-PCR

~200 mg
Total protein extraction

proteome
LC MS/MS

~100 mg
Metabolite extraction

metabolome
GC-TOF-MS

F. Aubé, H. Mireau, E. Delannoy, G. Cueff,
L. Rajjou, A. Lornac, F. Gilard
POPS Transcriptomic Platform (IPS2 Orsay)
BIBS Proteomic Platform (BIA Nantes) & PAPPSO Platform (GV Le Moulon)
Metabolism Metabolom Platform (IPS2 Orsay)

CYTOPHENO

AGENCE NATIONALE DE LA RECHERCHE
ANR

Towards a molecular understanding of the effects of cytoplasmic exchange

metabolome

Metabolite extract

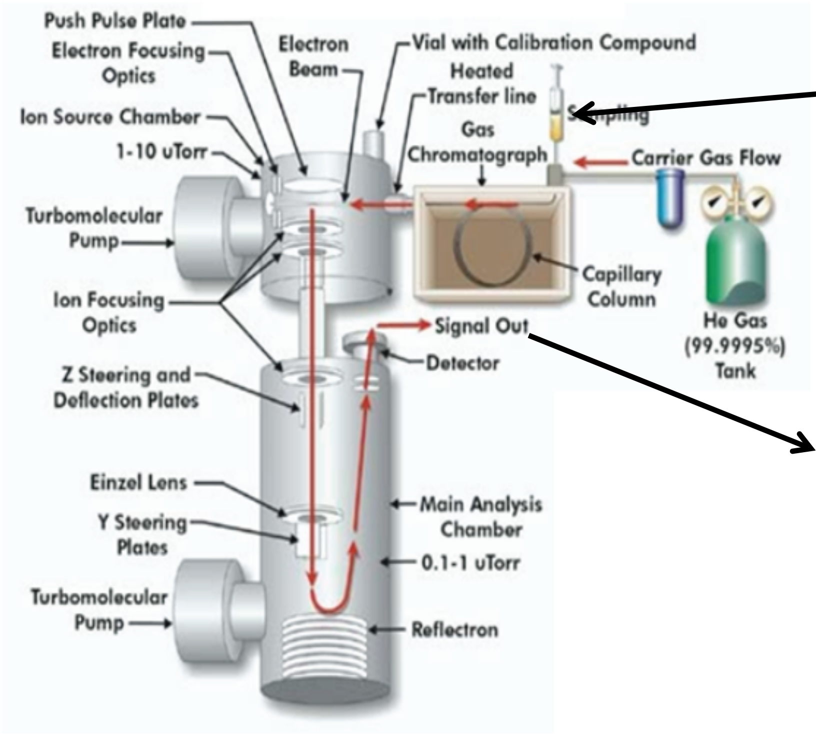
↓

+ internal reference

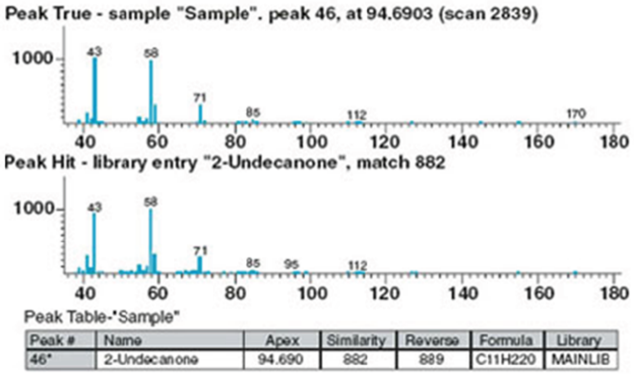
↓

Evaporation and solubilization

randomized injections



Identification of metabolites from spectra and comparison to database (~100 mol.)



Peak Table-"Sample"

Peak #	Name	Apex	Similarity	Reverse	Formula	Library
46*	2-Undecanone	94.690	882	889	C11H220	MAINLIB

↓

81 quantified metabolites

A. Lornac, F. Gilard
Metabolism Metabolom Platform (IPS2 Orsay)

CYTOPHENO

AGENCE NATIONALE DE LA RECHERCHE ANR

Towards a molecular understanding of the effects of cytoplasmic exchange

proteome

Total protein extract

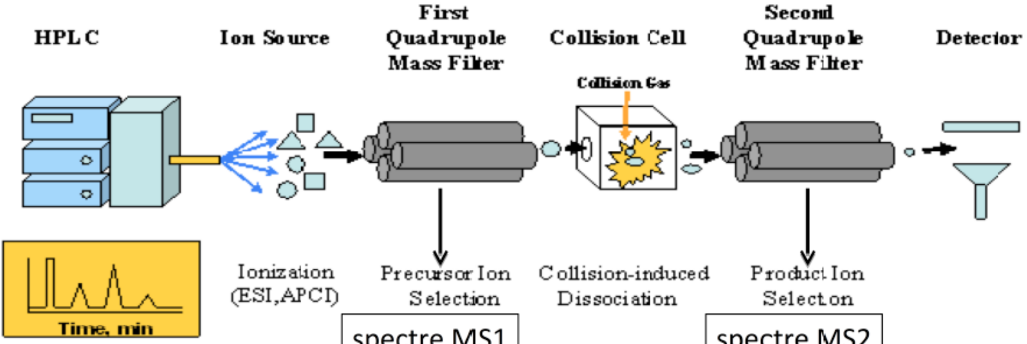
↓

Trypsin digestion

↓

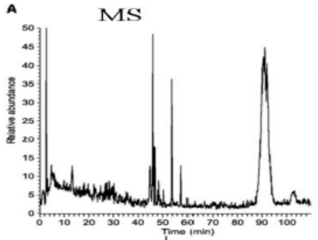
Purification of peptides

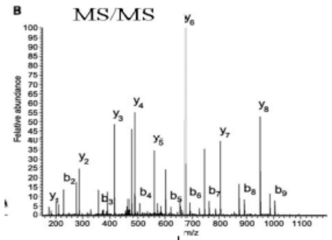
randomized injections



spectre MS1

spectre MS2





quantification

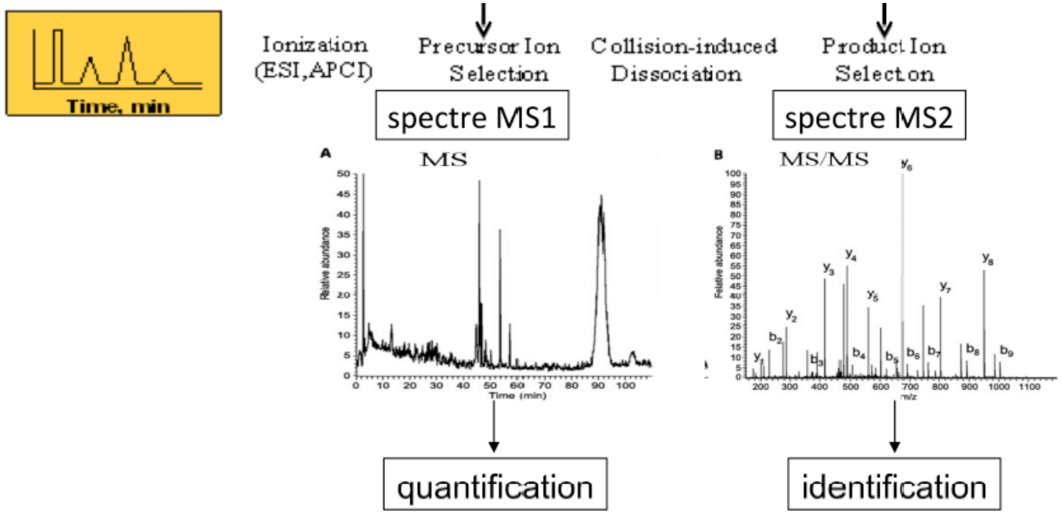
identification

G. Cueff, L. Rajjou
BIBS Proteomic Platform (BIA Nantes)
& PAPPSO Platform (GV Le Moulon)

CYTOPHENO

Towards a molecular understanding of the effects of cytoplasmic exchange

proteome



spectre MS1

spectre MS2

quantification

identification

Database : Tair10_pep_20101214

Protein identification criteria:
- Score peptide <0.001
- Log(e-value) prot <-4
- Min 2 unique peptides

1737 proteins identified from 16302 peptides

Reproductible peptides (<5%NA)
Min 2 quantified unique peptides

665 quantified proteins from 3447 peptides

G. Cueff, L. Rajjou
BIBS Proteomic Platform (BIA Nantes)
& PAPPSO Platform (GV Le Moulon)

CYTOPHENO ANR

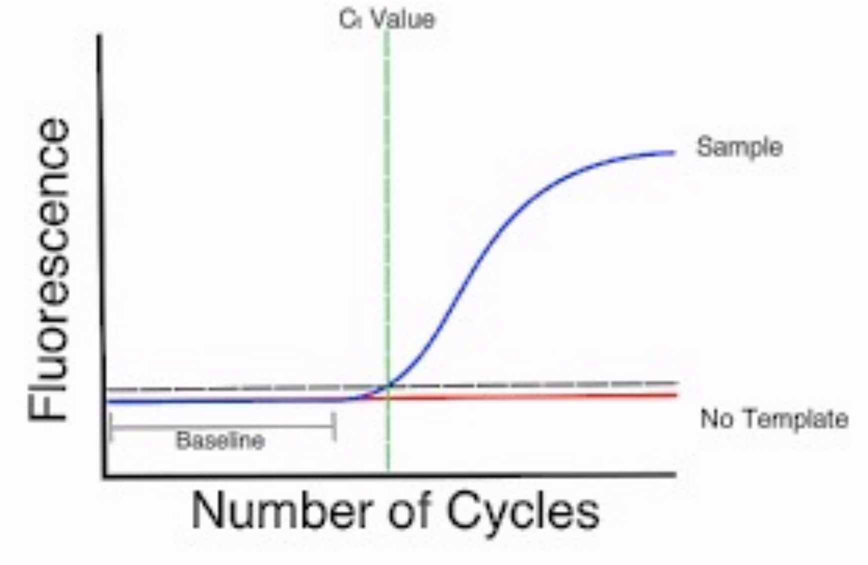
Towards a molecular understanding of the effects of cytoplasmic exchange

organellar transcriptomes

qPCR

80 genes of the chloroplast

32 genes of the mitochondria



F. Aubé, H. Mireau, E. Delannoy

CYTOPHENO

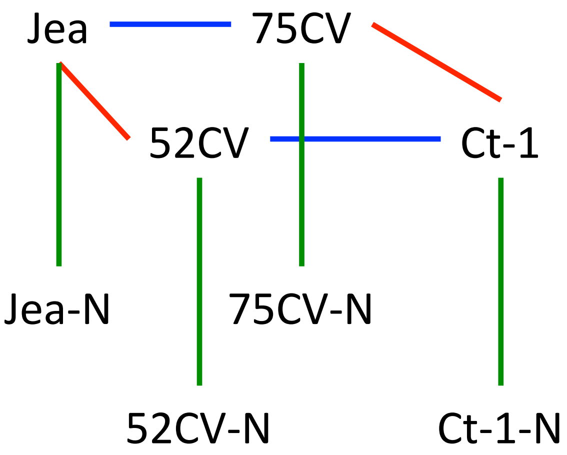
ANR

Towards a molecular understanding of the effects of cytoplasmic exchange

Nuclear transcriptome

CATMA v6 microarray : 60-mers oligonucleotides designed from the Col-0 annotated genome

1 hybridization experiment = 2 labeled samples from the same biological repetition



8 hybridization experiments

🟦 = nucleus ≠ cytoplasm

🟥 ≠ nucleus = cytoplasm

🟩 = genotye ≠ N regime

2 technical repeats <-> dye swap

↓

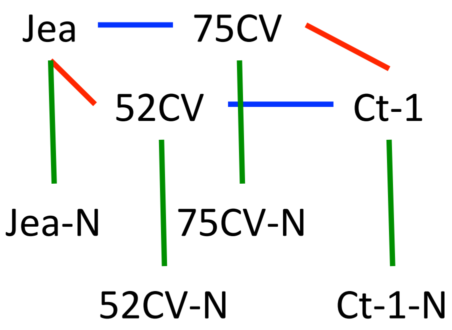
Fluorescence intensity for ~ 32,000 annotated genes
Direct comparisons of genotypes/conditions

E. Delannoy
POPS Transcriptomic Platform (IPS2 Orsay)

CYTOPHENO

AGENCE NATIONALE DE LA RECHERCHE
ANR

The case of microarray results: dealing with natural polymorphisms



Jea — 75CV

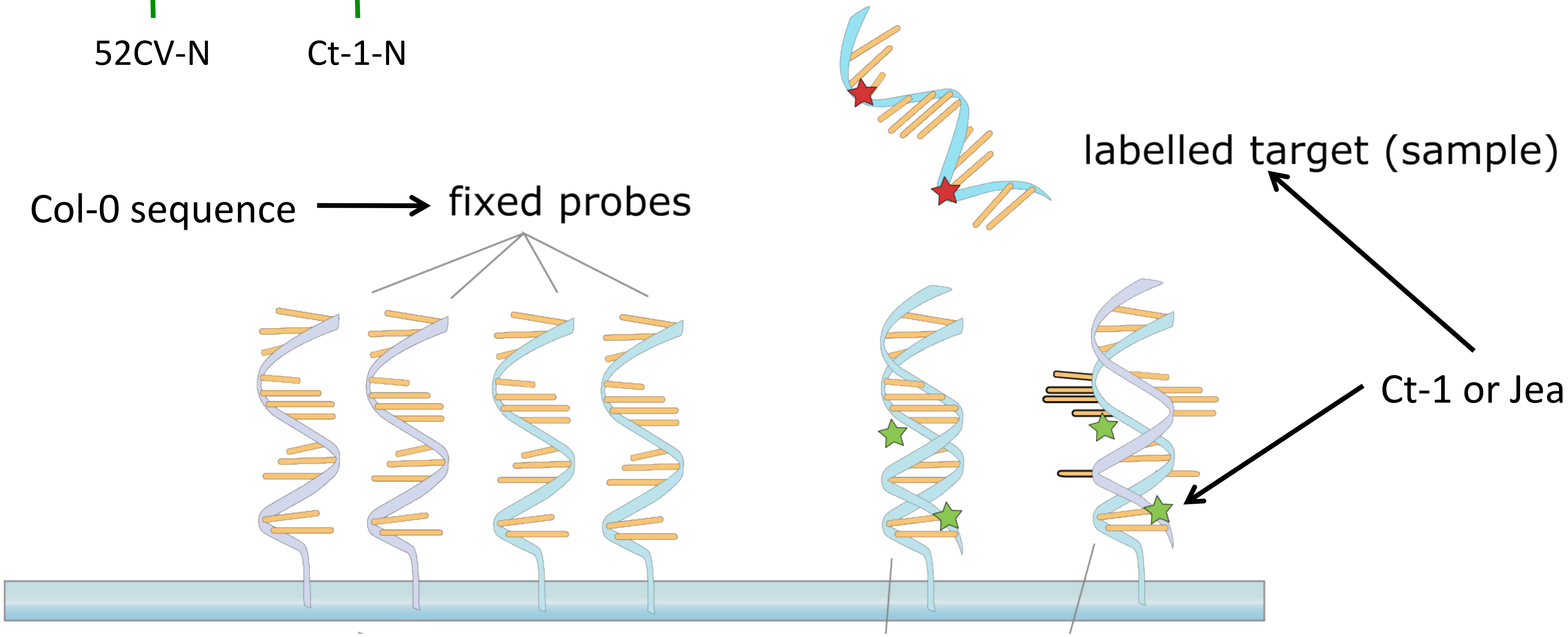
52CV — Ct-1

Jea-N

75CV-N

52CV-N

Ct-1-N



Col-0 sequence → fixed probes

labelled target (sample)

Ct-1 or Jea

Are the results biased for the probes
where Jea and Ct-1 don’t have the same sequence?

The case of microarray results: dealing with natural polymorphisms

1001 Genomes

A Catalog of *Arabidopsis thaliana* Genetic Variation

http://1001genomes.org/

↓

Retrieve positions of polymorphisms

- between Ct-1 and Col-0
- between Jea and Col-0

↓

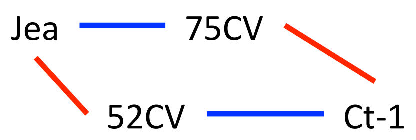
Positions and number of polymorphisms
between Ct-1 and Jea that match on probe sequences

↓

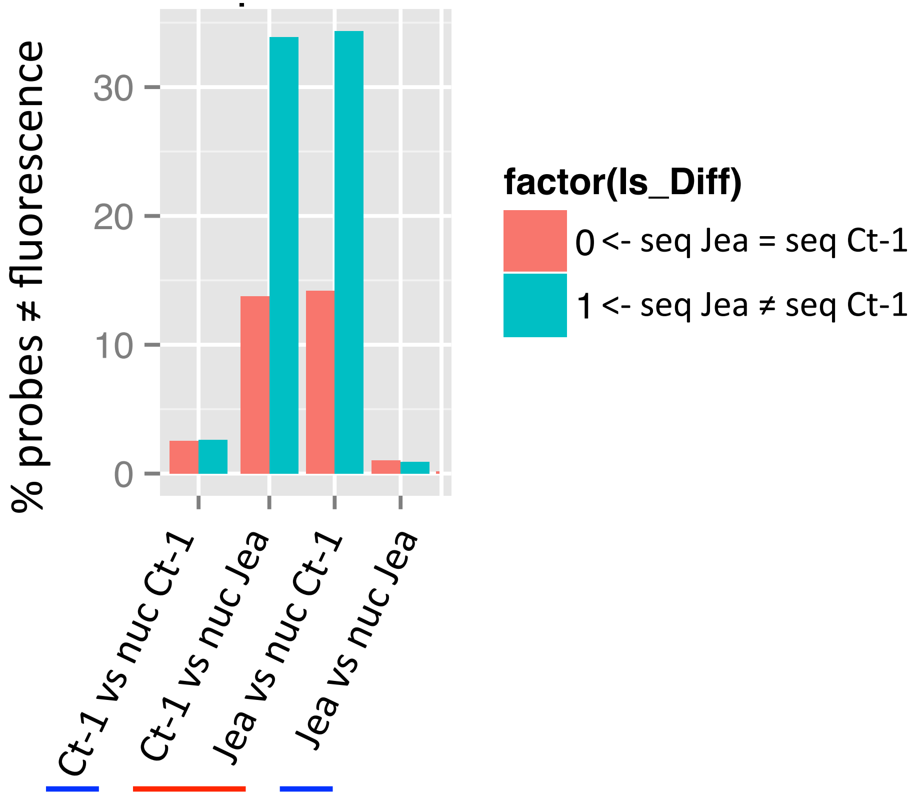
8656 probes representing 5629 genes

V. Kudla

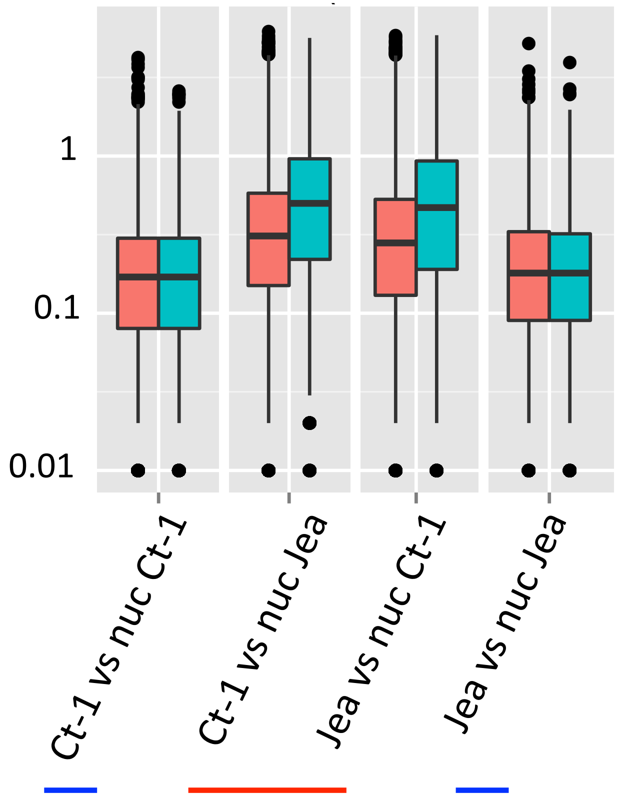
Effect of unequal polymorphisms on



probe fluorescence

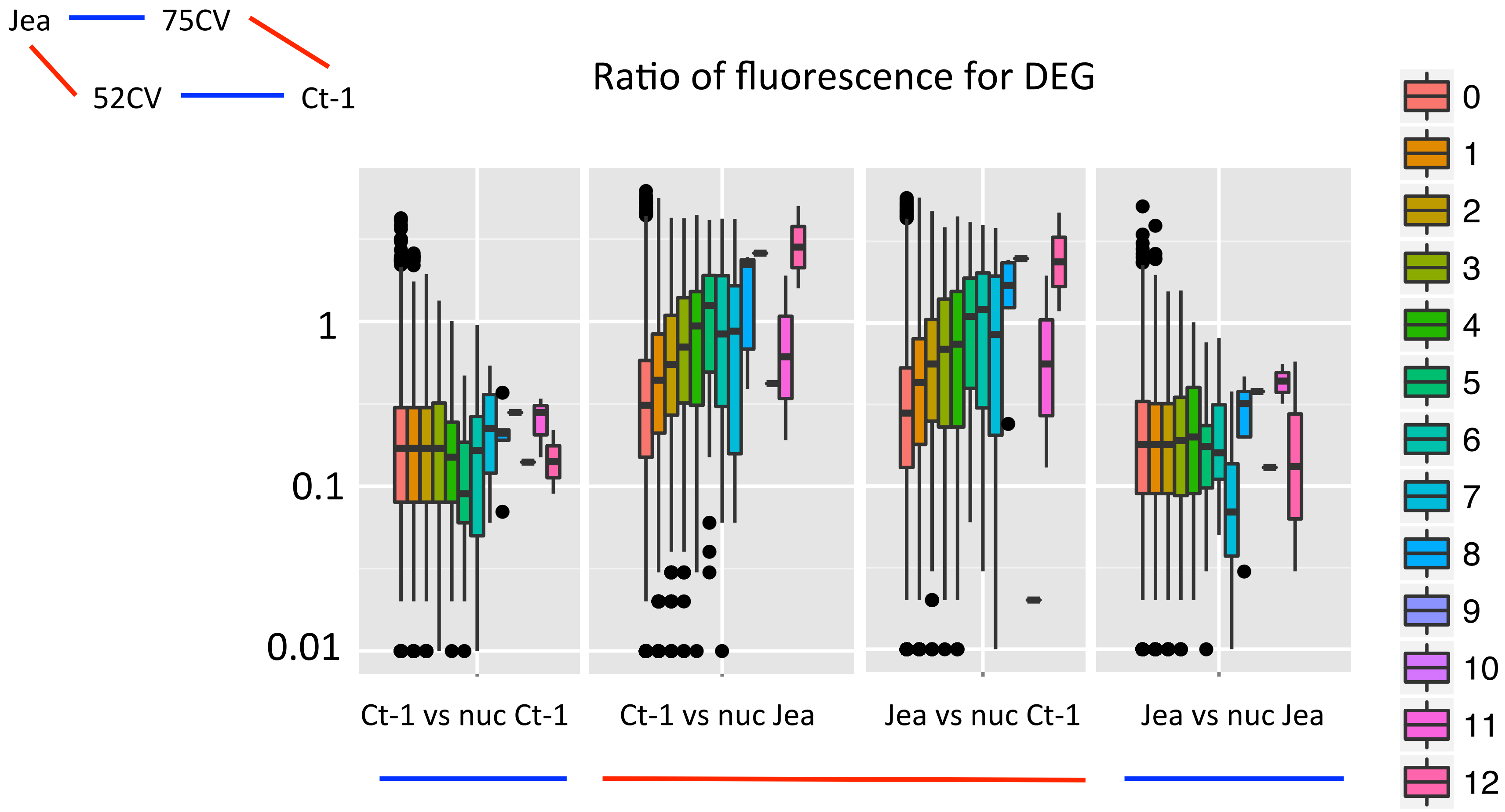


Ratio fluorescence for DEGs



J. Jimenez-Gomez

Effect of the number of unequal polymorphisms



5629 genes removed from the results

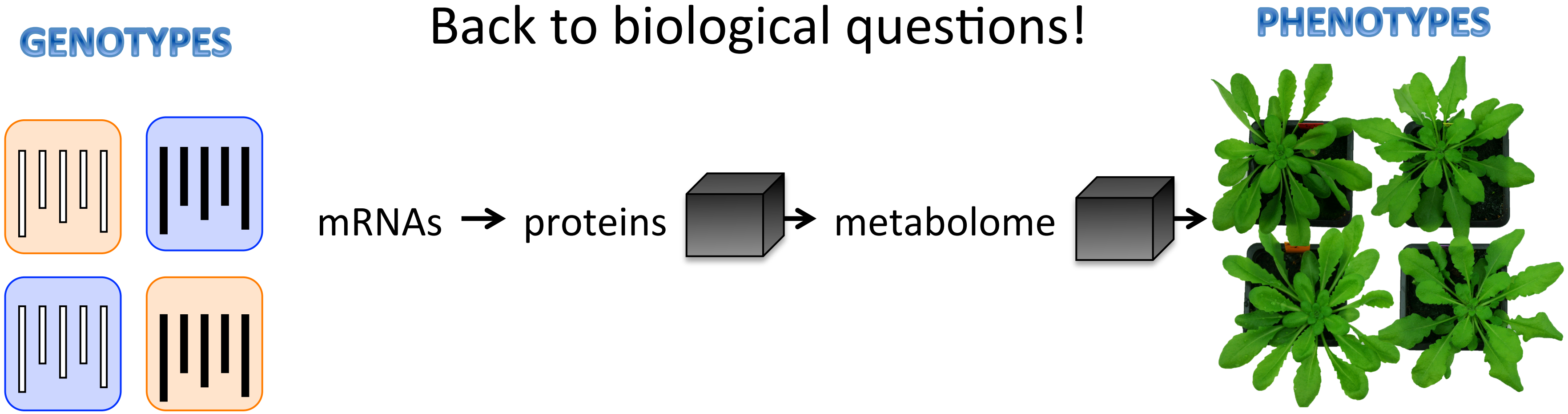
J. Jimenez-Gomez

Towards a molecular understanding of the effects of cytoplasmic exchange

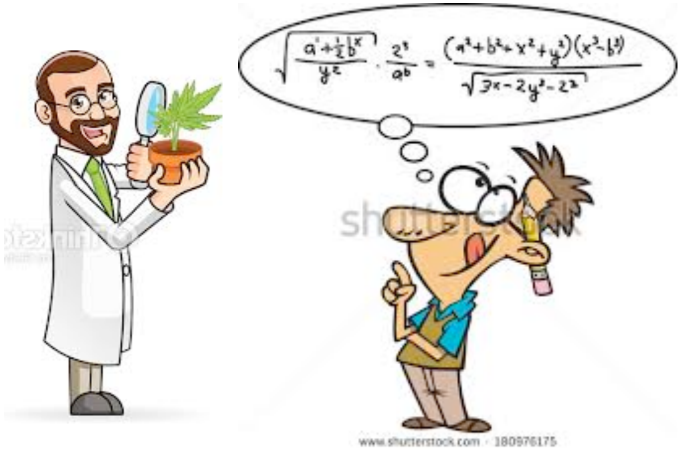
Multilevel molecular phenotyping

Phenotype	Technique	Measure	Output	dataset
Nuclear transcriptome	micro-array (CATMA V6)	Level of dye fluorescence	Relative mRNA abundance for 26,885 genes	Normalized to the median
organelle transcriptomes	qRT-PCR	Nb of cycles (C_t)	Relative mRNA abundance for 80 cp genes and 32 mt genes	Normalized to the median in each organelle
Proteome	LC/MS-MS	Max intensity of MS peaks	Relative abundance of 665 proteins	Normalized to the median, log10 transformed
Metabolome	GC-TOF-MS	Integration of MS peaks	Semi-quantitative abundance of 81 metabolites	Centered reduced, normalized to introduced internal control and to fresh weight

Back to biological questions!



- Is the route from genotype to phenotype changed by the disruption of cytonuclear coadaptation?
- Is there a 'molecular signature' of this change (if any)?
- Does the disruption of genomic co adaptation modify the way plants react to N starvation?



ANOVAs for each measure

Question 1: Do the starved samples show typical molecular response of N starvation?

Validation of the N starvation effect

For each mRNA/protein/metabolite

Y = μ + Nuc + Cyto + Cyto x Nuc

+ **Nitrogen** + Cyto x **Nitrogen** + Nuc x **Nitrogen** + Cyto x Nuc x **Nitrogen**

+E

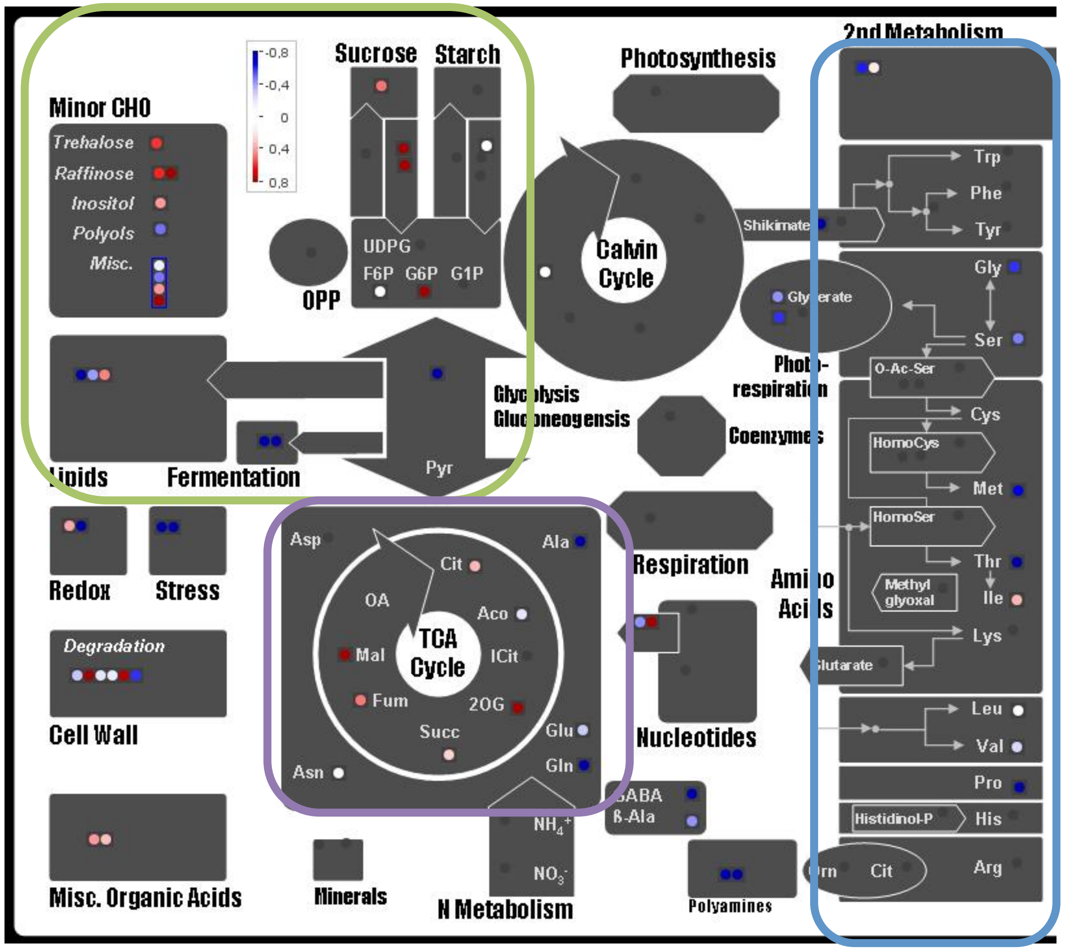
ML Martin-Magniette, F. Chardon

Validation of the N starvation effect

Significant Nitrogen effect on:

51 metabolites :

- ↗ of sugars
- ↗ of organic acids
- ↘ of amino acids



Berthomé, Krapp et al. Plant Physiol. 2011

ML Martin-Magniette, F. Chardon

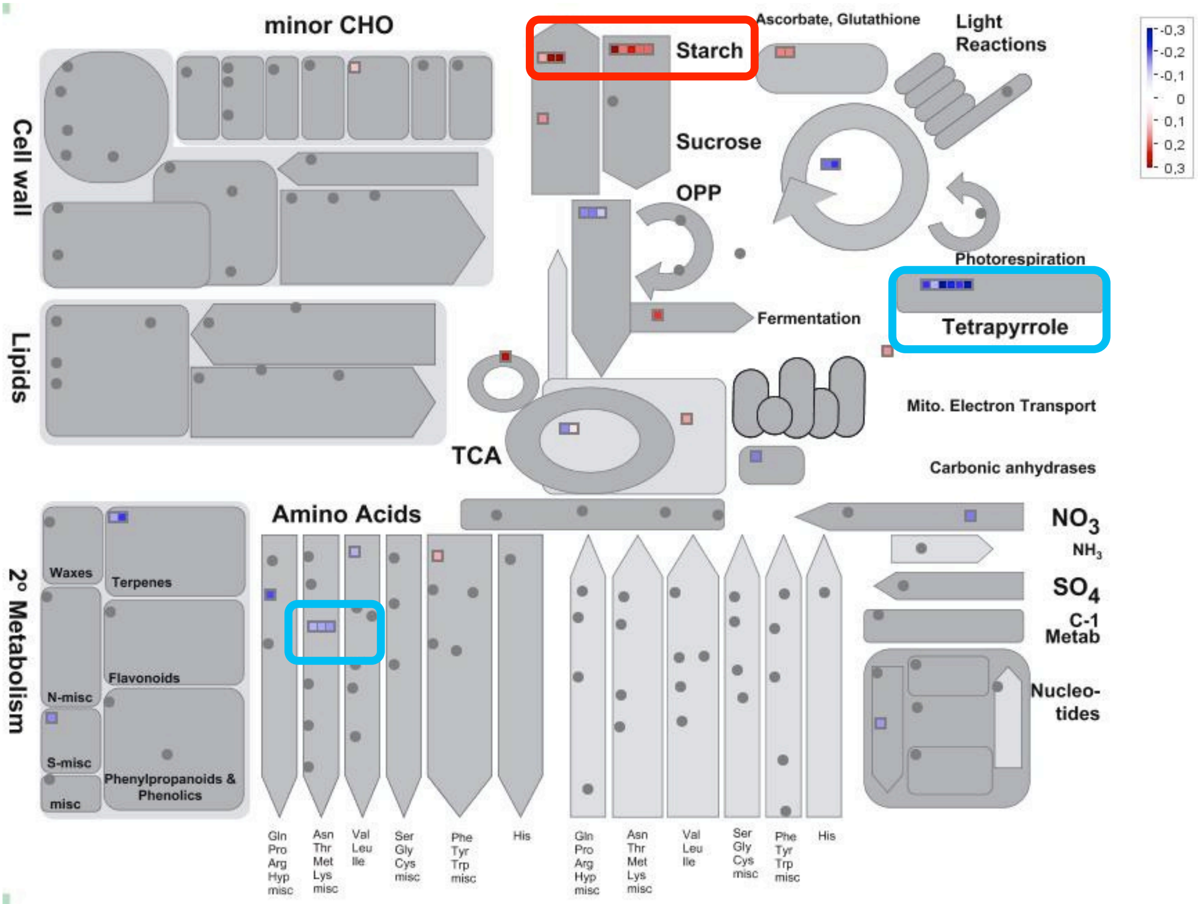
Validation of the N starvation effect

77 proteins :

↗ starch catabolism

↘ Tetrapyrrole biosynthesis

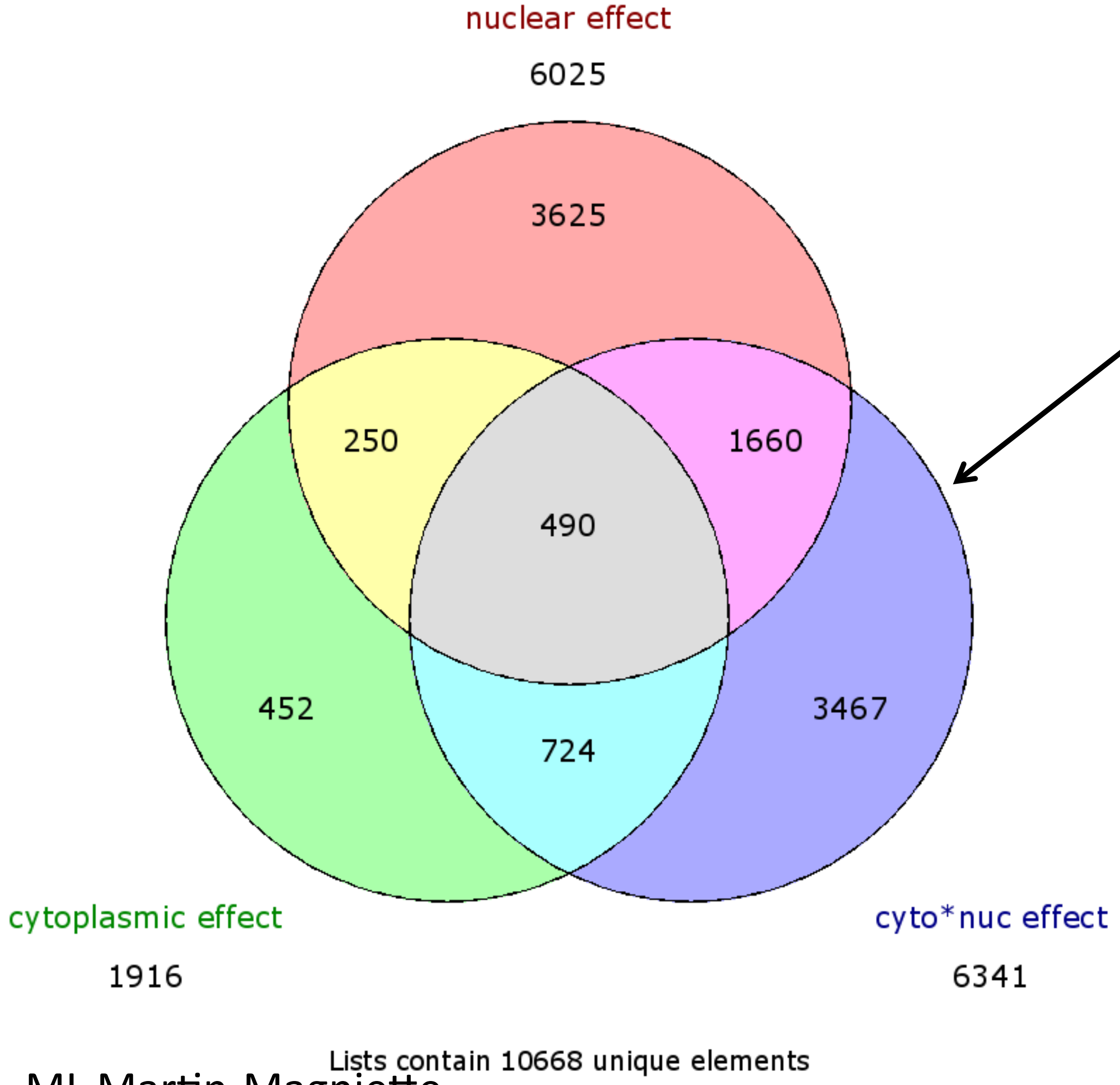
↘ Methionine biosynthesis



ML Martin-Magniette, F. Chardon

Question 2: What are the genes/proteins whose accumulation is different when cytonuclear interaction is disrupted?

Identification of mRNAs and proteins under the influence of cyto*nuclear interaction (only unstarved samples)



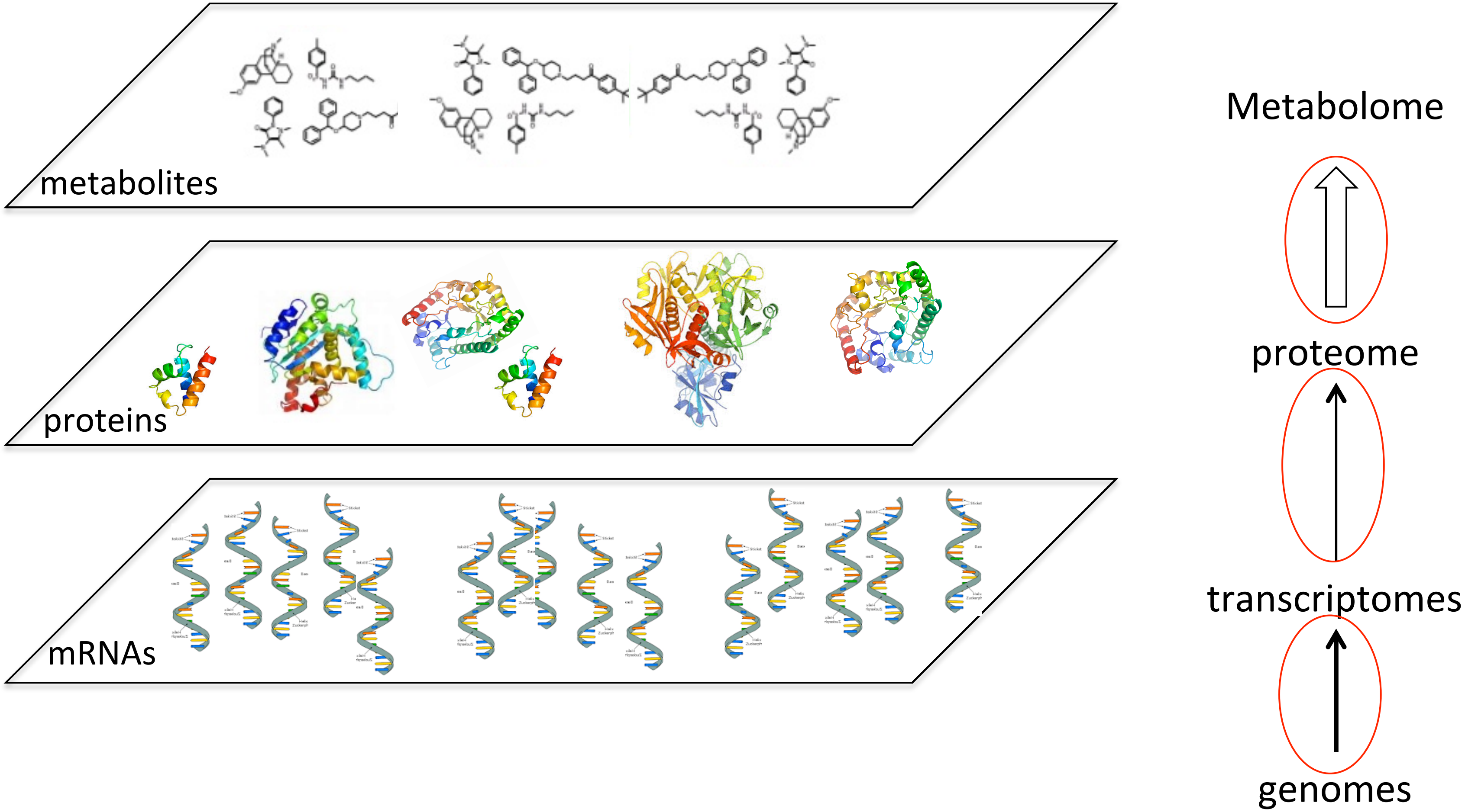
$Y = \mu + Nuc + Cyto + Cyto \times Nuc + E$

10668 nuclear DEG
8 cp DEG
7 proteins

In course : GO enrichments of DEG under the influence of cyto x nuc interaction

ML Martin-Magniette

Can we make links between the different molecular phenotypes?



Metabolome

proteome

transcriptomes

genomes

Can tools integrating omics data help us learning about:

- the route from genotype to phenotype changed by the disruption of cytonuclear coadaptation?
- a 'molecular signature' of this change (if any)?
- modification of the way plants react to N starvation when disruption of co adaptation?
- a 'signature' of the response to N starvation when cytonuclear co adaptation is disrupted?

mixOmics?

…. See you tomorrow !