Genetic mapping of resistance factors to *Melamspora* sp in *Populus* sp

A review

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INRA Experimental unit GBFOR

Véronique Jorge

18/ 04/ 2018

Unit BioForA



Our research objective

Management of forest genetic resources for sustainable timber production in a changing climate context

Individuals

Theme A
Better understand the construction of phenotypes of complex traits

Wood formation | Resistance to diseases | Reproduction (sexual/asexual)

Populations

Theme B
Analyse responses of populations under natural or artificial selection to a changing environment

Management Units (Conservation, Breeding)

Theme C
Optimize sustainable management of genetic diversity in natural and breeding populations

Genetic Resources for research

in- & ex-situ Conservation

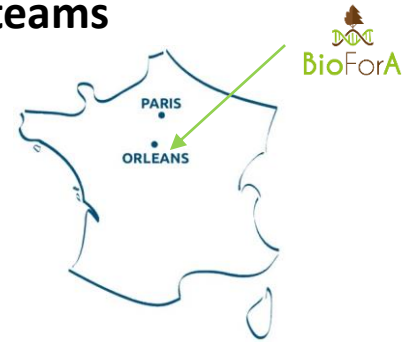
Breeding programmes

Operational Pole « Genetic Resources »

FRM production engineering.

Guidelines for FRM Use

- **Joint unit between INRA and ONF** (French National Forest Service)
- **~50 persons (40 perm.)**
- **4 teams**



Species studied : Poplars, Douglas Fir, Scot Pine, wild cherry, larches, ash

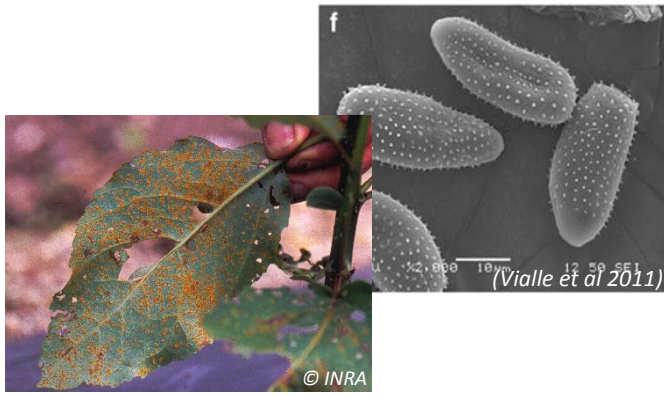
SUMMARY

1. Poplar rust pathosystems
2. Questions and strategies to identify R factors
3. How to measure resistance components ?
4. Variability for resistance components
5. Genetic mapping: from QTL to QTN
6. Comparative genomics
7. Conclusion: potential uses for breeding

1

Poplars / rusts pathosystems

Poplar rusts



- Causes by *Melampsora spp*
- Biotrophic, heterotic cycle.
- Losses in biomass :
  - 32% per year (Dowkiw, 2003)
  - 57% (Steenackers et al. 1996)
- Resistance/tolerance is as major breeding issue
- **Qualitative resistances** deployed in hybrid cultivars overcame

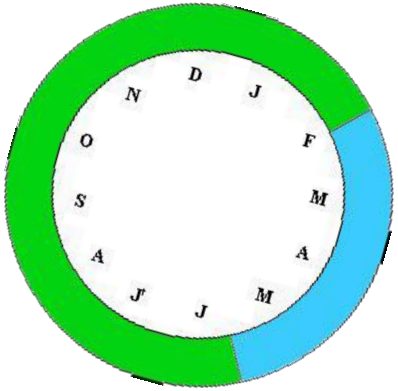




A complex biological cycle …

… favouring rapid pathogen evolution

Poplars

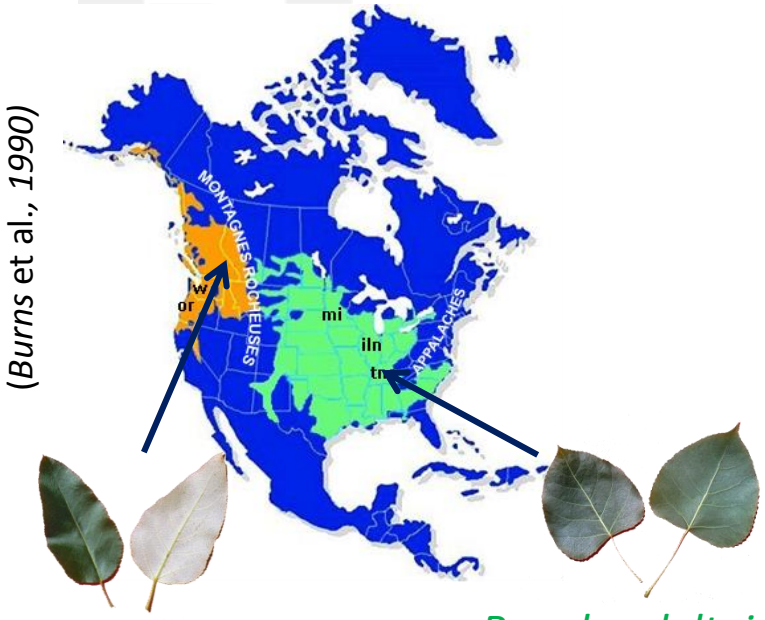


Asexual reproduction
Epidemics

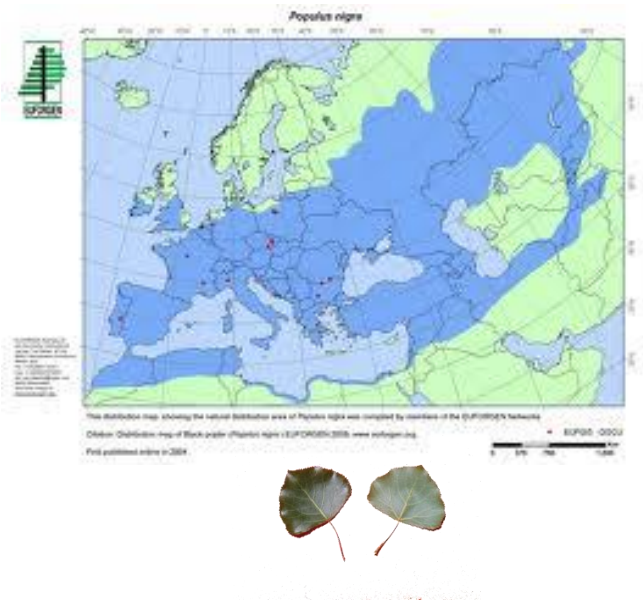
Sexual reproduction

Larches
Pines
Douglas fir

Natural poplars-foliar rusts pathosystems

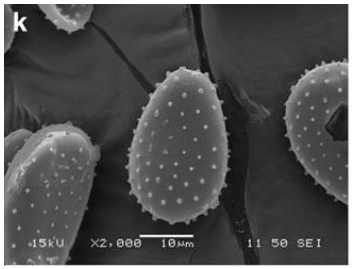


Populus trichocarpa
Melampsora occidentalis

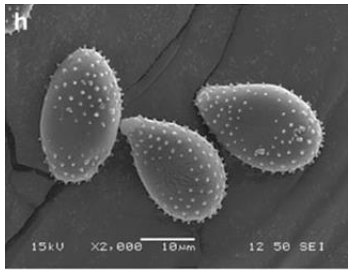


Populus deltoides
Melampsora medusae
f.sp. deltoideae

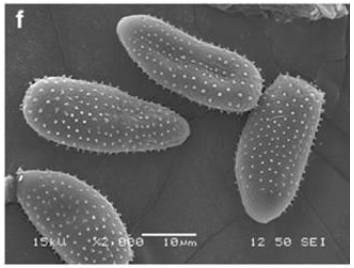
Populus nigra
Melampsora larici-populina



(Vialle et al 2011)



(Vialle et al 2011)



(Vialle et al 2011)

Poplar/rust « exapted » pathosystems



- Use of *Populus* exotic species => showdown of host and pathogen that did not **co-evolved**.
- The interaction is **compatible**

Exapted pathosystem
(Gould et Vrba, 1982; Newcombe 1998)

	Melampsora occidentalis	*Melampsora medusae f.sp. deltoideae*	*Melampsora larici-populina*
Populus trichocarpa	natural	**exapted**	**exapted**
Populus deltoides	?	natural	**exapted**
Populus nigra	?	**exapted**[a]	natural

	Pt	Pd	Pn
Pt	O	O	O
Pd	O	O	O
Pn	O	X	O

Possible interspecific hybridation both in host and pathogen

Mo x *Mmd*

Melampsora x columbiana

[a] Pinon et Frey, 2005, ? : no published data

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Questions and strategies to identify R factors

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SCIENCE & IMPACT

Questions

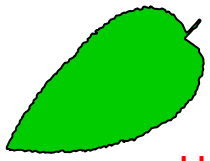
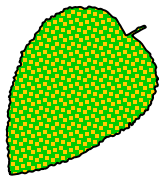
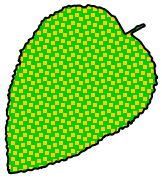
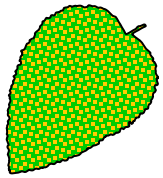
- What is the variability for resistance in the differen type of pathosystems (natural/exapted)?
- What is the genetic determinism (loci and genes)?

Strategies/approaches

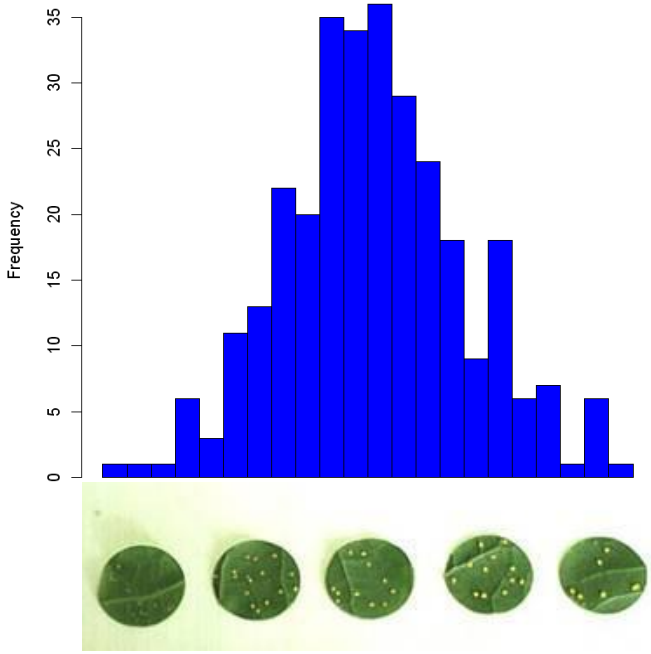
- Identify resistance components
- Evaluate variability for these components
- Map genetic factors : from QTLs to QTNs.
- Comparative genomics

A simple classification of resistance types

Qualitative resistance
Gene for gene hypothesis
(Flor, 1942)

Plant	Pathogen: Avr/Avr Avr/vir	Pathogen: Vir/vir
R/R or R/r	 +HR	
r/r		

Quantitative resistance



3

How to measure resistance components ?

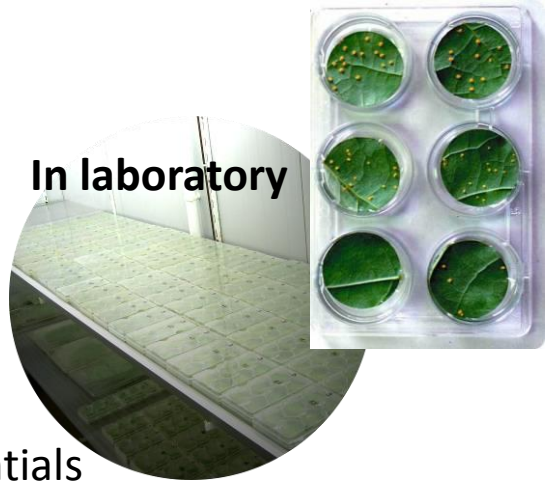
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Qualitative resistance

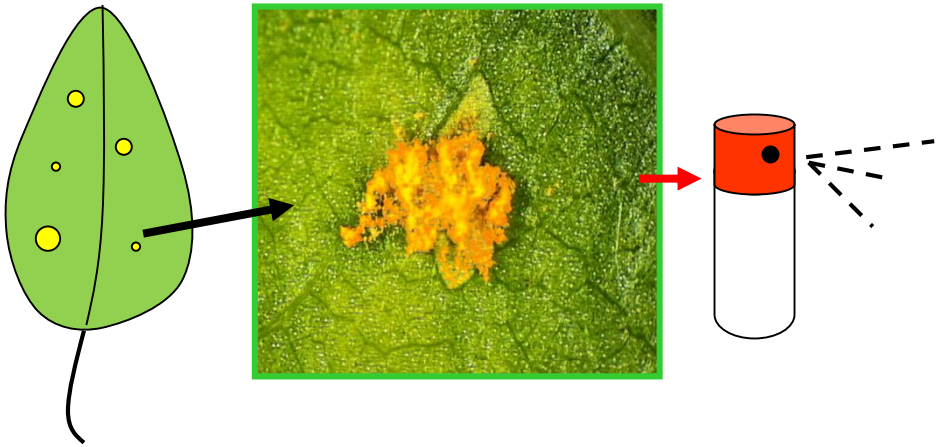
Plant	Pathogen: Avr/Avr Avr/vir	Pathogen: Vir/vir
R/R or R/r	+HR	
r/r		

Specific

Control and characterise
Pathogen variability

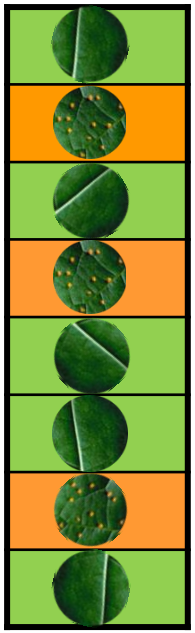


1 uredia = 1 clone of uredospores

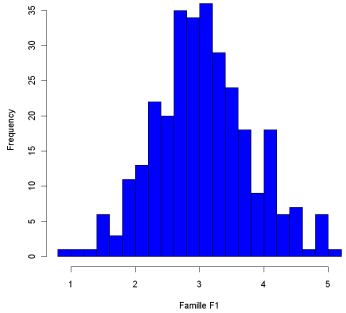


Host differentials

cv. 'Ogy'	R1
cv. 'Candicans'	R2
cv. 'Brabantica'	R3
cv. 'Unal'	R4
cv. 'Rap'	R5
87B12	R6
cv. 'Beaupré'	R7
cv. 'Hoogvorst'	R8



Qualitative resistances / virulences well documented in exapted pathosystem :
Also for TxD hybrids/*Mxc* (Newcombe et al. 2001) *P. deltoides/Mlp*



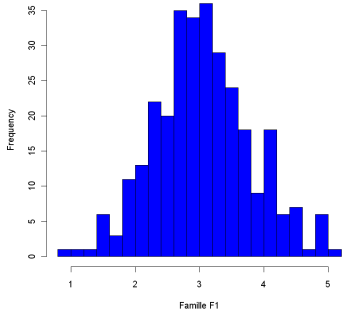
Epidemiological components of quantitative resistance

In laboratory





Field



Epidemiological components of quantitative resistance

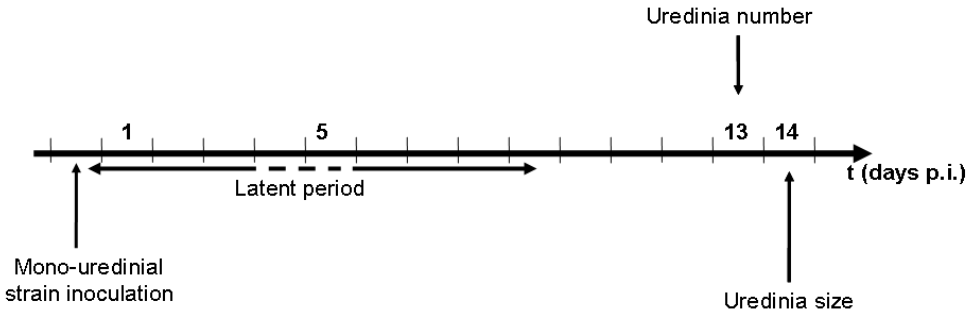
In laboratory

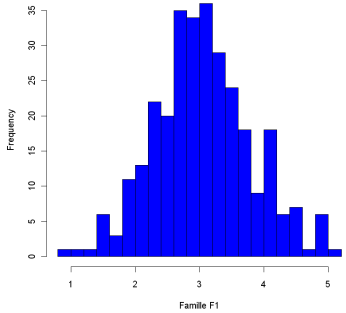




Field







Epidemiological components of quantitative resistance

In laboratory

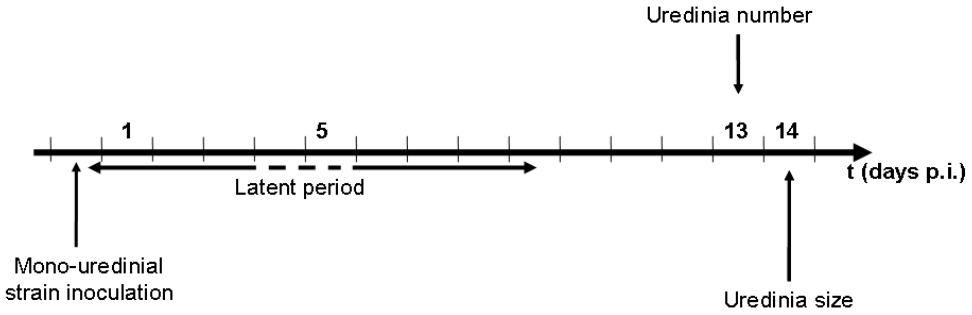




Field







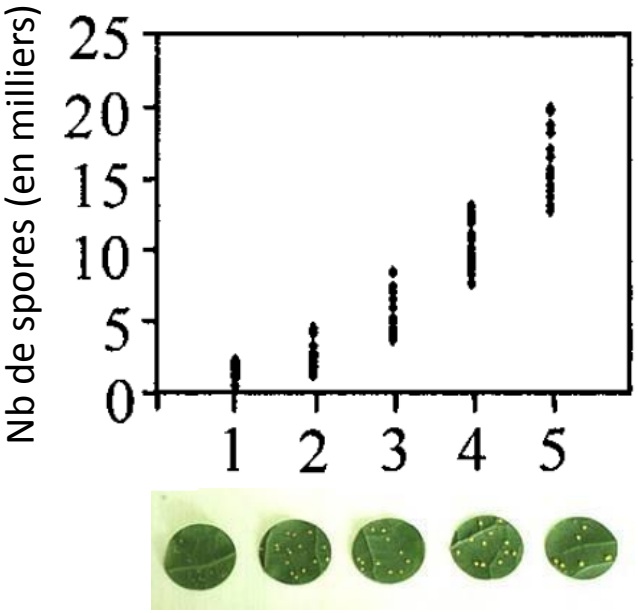


Rust score on the most infected leaf

Epidemiological components of quantitative resistance

In laboratory

1 infection cycle

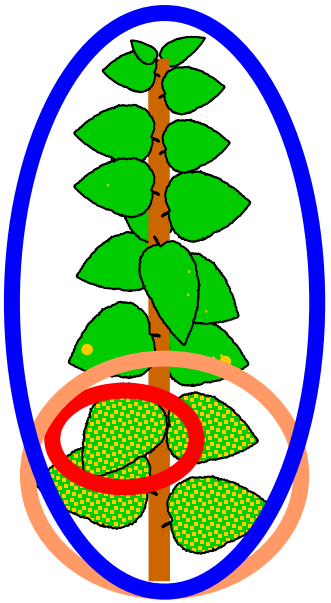


(Dowkiw et al 2003)

Measure of interaction on the pathogen

Field

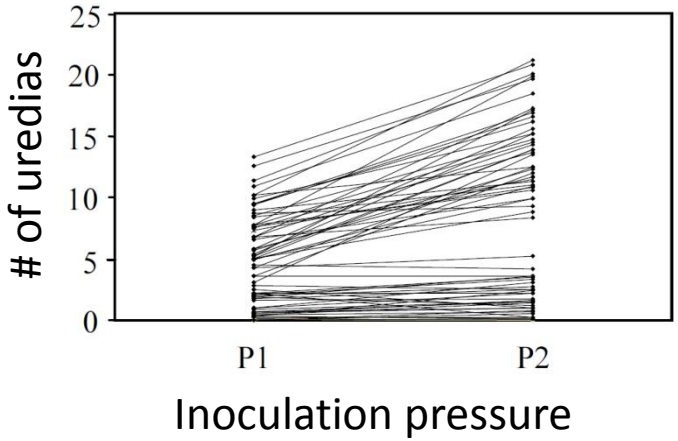
several infection cycles



Measure of interaction on the host plant (symptoms, leaf losses)

Epidemiological components of quantitative resistance

- Conditions of inoculation, scales to be tuned depending on the host and on level of infection:
  - hybrids/pure species
  - Qualitative R / quantitative R
  - period of evaluation (field)
  - age of plants
- We are sure about susceptibility not resistance



(Dowkiw et al 2003)

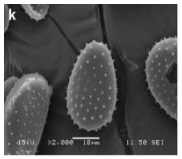
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Variability for resistance components

Natural pathosystems

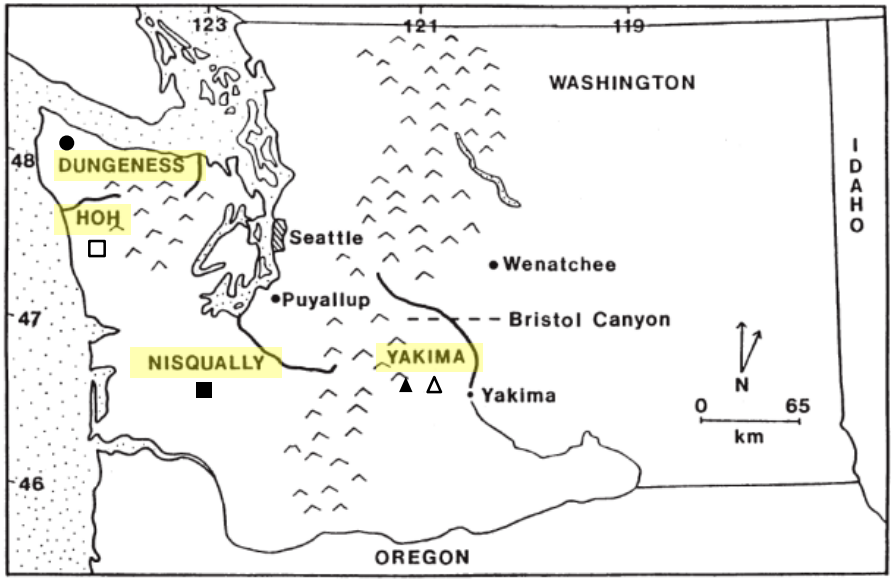
Populus trichocarpa

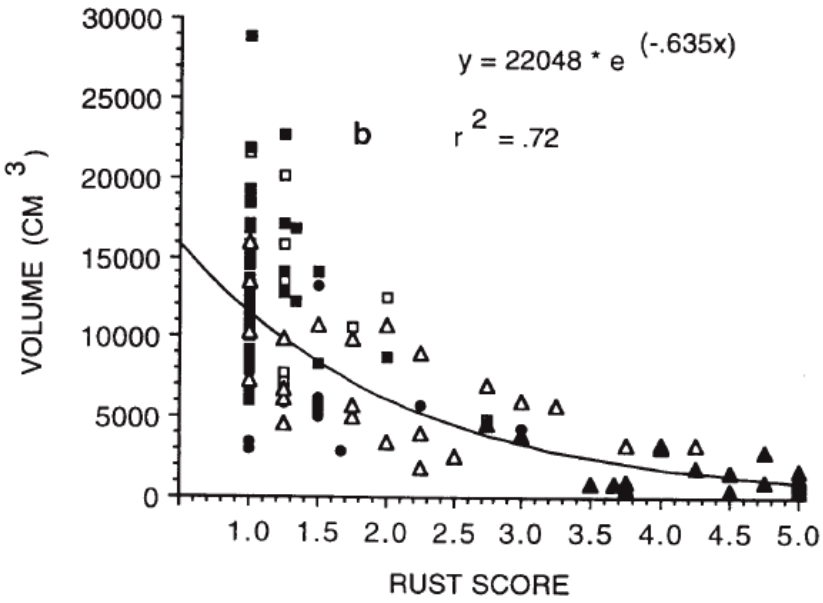




(Vialle et al 2011)

Melampsora occidentalis





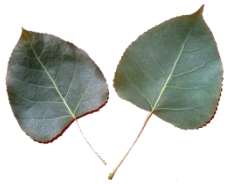
5 provenances, 126 clones
Washington state

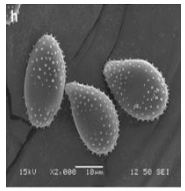
- Field evaluation
- Highly resistant genotypes
- Variability geographically structured

(Dunlap and Stettler, 1998)

Natural pathosystems

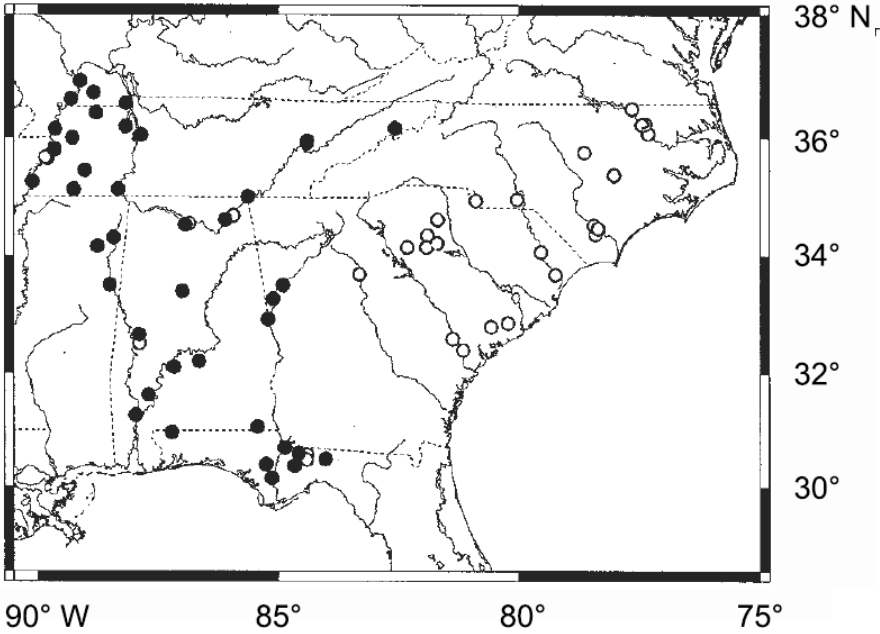
Populus deltoides

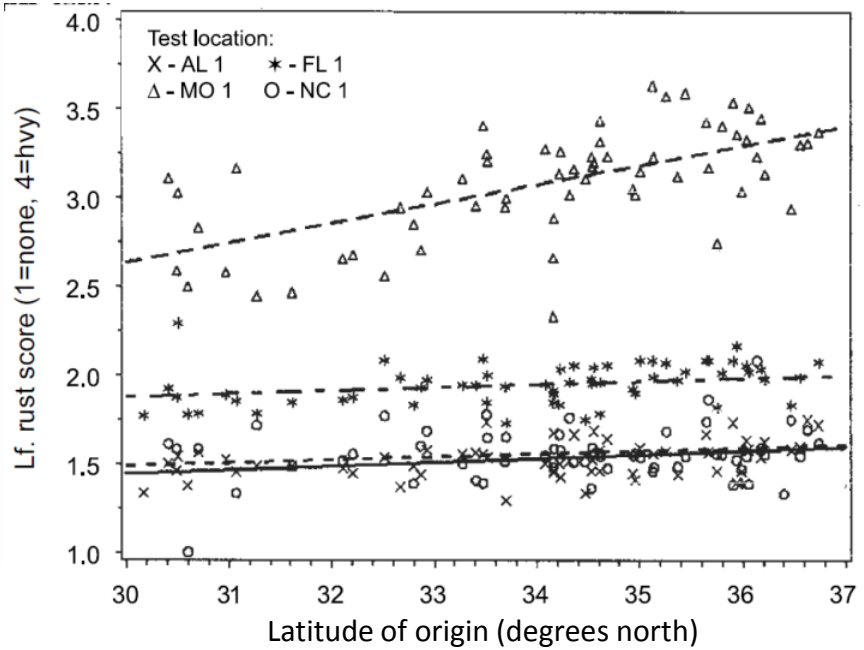




(Vialle et al 2011)

Melampsora medusae f.sp. deltoideae





62 provenances
Eastern USA
4 clonal trials

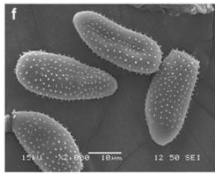
➢ Variability geographically structured

(Land and Jeffreys, 2006)
(Nelson and Tauer, 1986)
(Hamelin et al 1993, 1992)

Natural pathosystems

Populus nigra

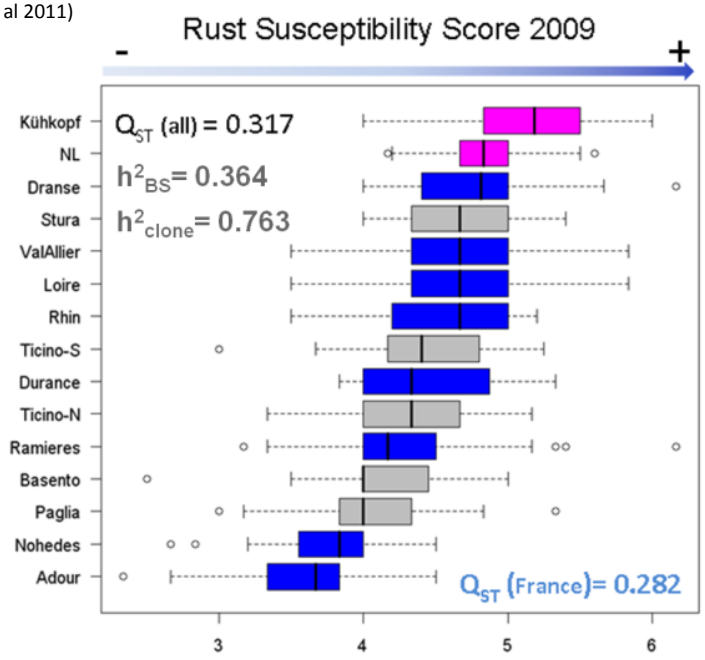




(Vialle et al 2011)

Melampsora larici-populina





14 metapopulations, 1141 clones
Western Europe

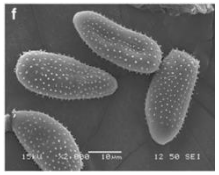
- No immune genotypes but some very resistant.
- Variability geographically structured

(Bastien et al, unpublished)
(Légionnet et al, 1999)

Exapted pathosystems

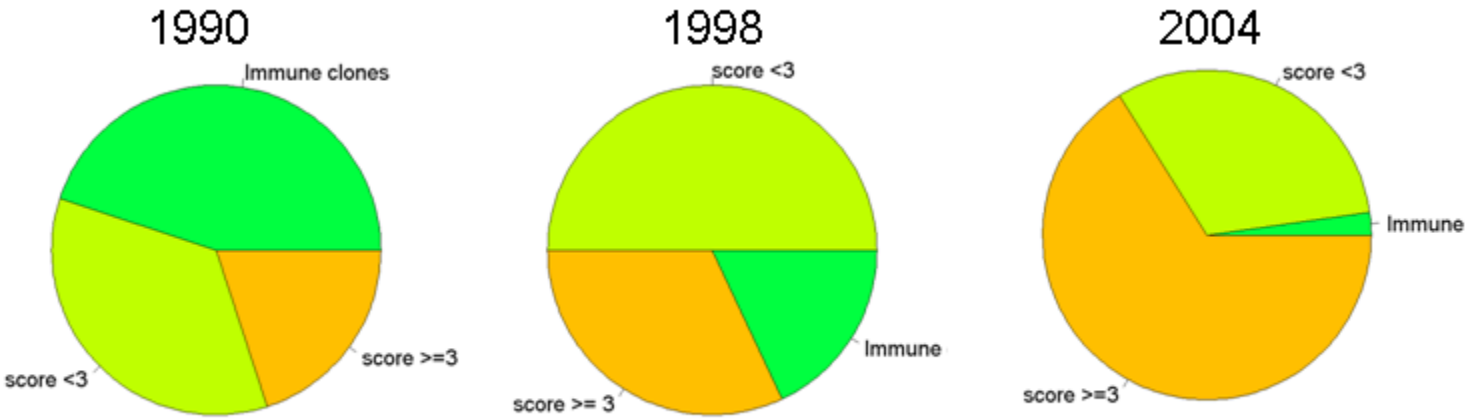
Populus deltoides





(Vialle et al 2011)

Melampsora larici-populina



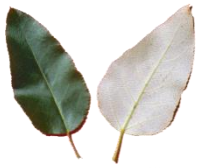
545 *P.deltoides* genotypes – Field rust susceptibility (Orléans)

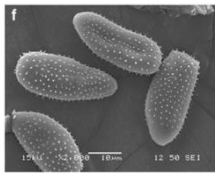
- Qualitative resistances
- Erosion of resistance over time

(Bastien et al, unpublished)

Exapted pathosystems

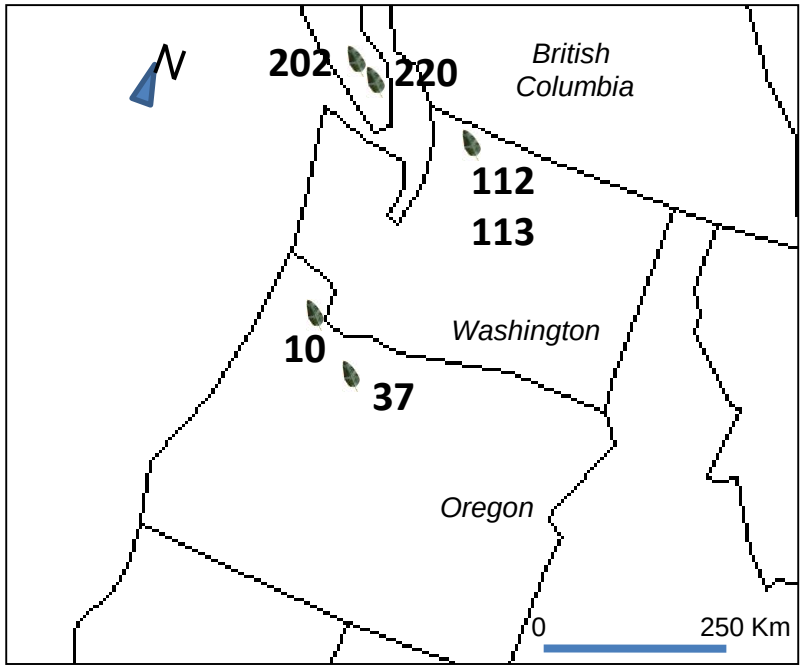
Populus trichocarpa

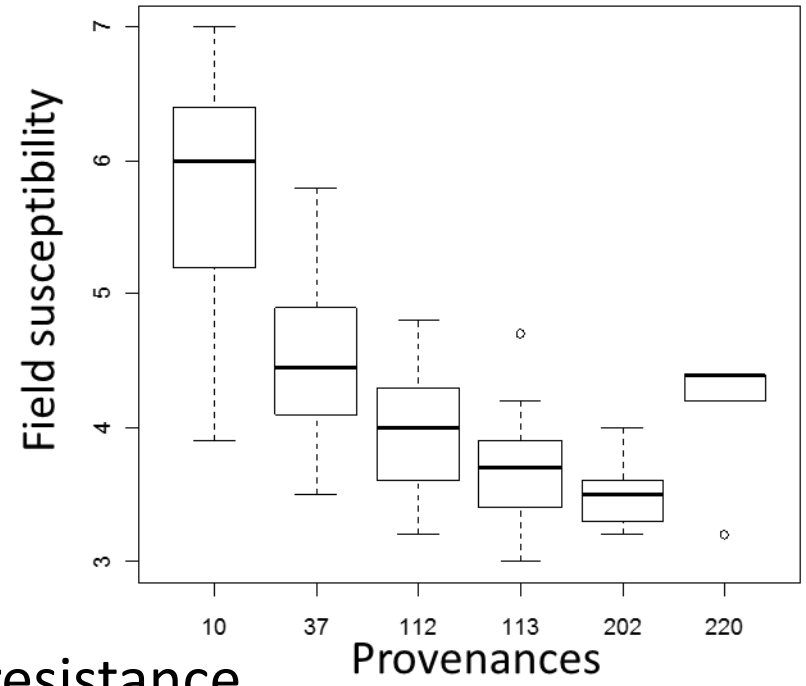




Melampsora larici-populina

(Vialle et al 2011)





6 provenances, 102 clones
Clonal trial, Orléans (F)

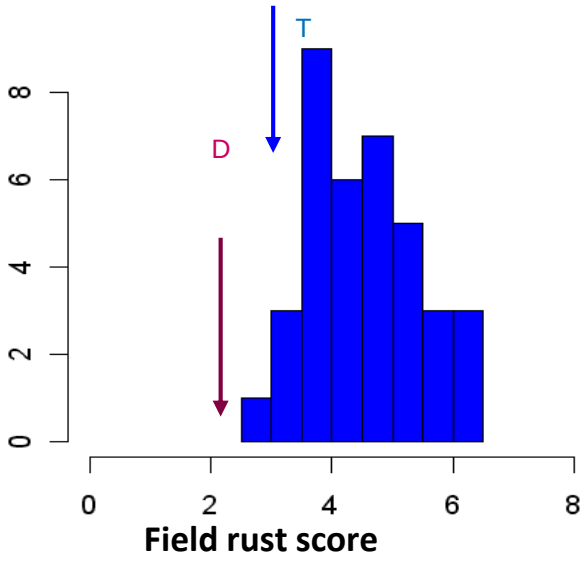
- Quantitative resistance
- Strong geographic structuration
- Idem *P. trichocarpa* / Mxc (La Mantia et al 2013)

(Bastien et al, unpublished)

Exapted pathosystems

Case of interspecific hybridization

Hybrides **DxT** / **DxN** vs ***Mlp***

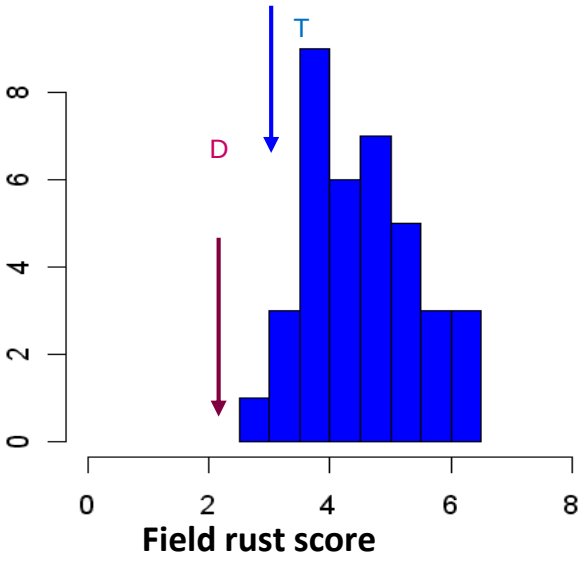


Negative heterosis …..

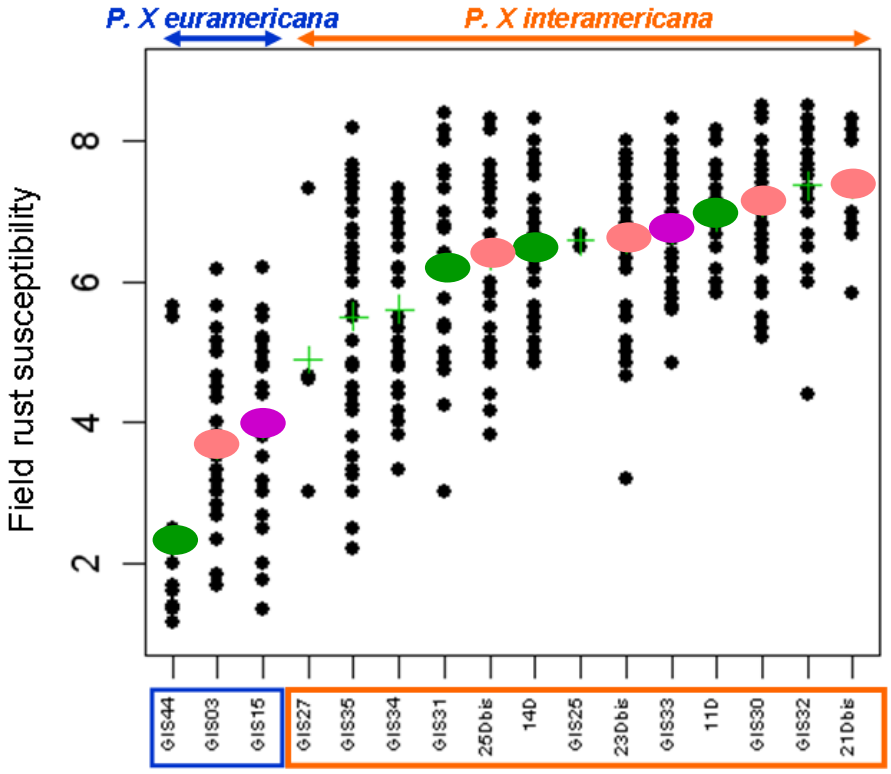
Exapted pathosystems

Case of interspecific hybridization

Hybrides **DxT** / **DxN** vs ***Mlp***



Negative heterosis …..

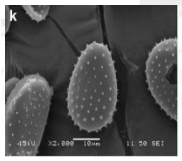


16 F_1 families involving common *P.deltoides* female parents

To sum up about variability !

Natural pathosystems





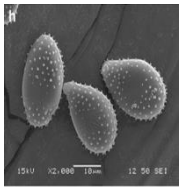
P. trichocarpa

Mo

(Dunlap and Stettler, 1998)

(Vialle et al 2011)





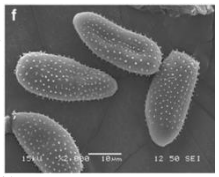
P. deltoides

Mmd

(Land and Jeffreys, 2006)
(Nelson and Tauer, 1986)
(Hamelin et al 1993, 1992)

(Vialle et al 2011)





P. nigra

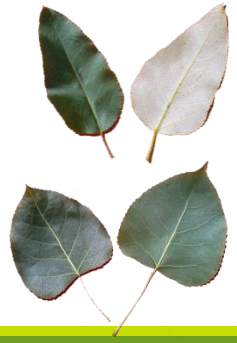
Mlp

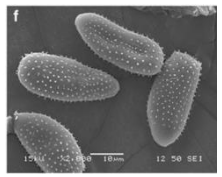
(Bastien et al, in prep.)
(Légionnet et al, 1999)

(Vialle et al 2011)

- Some genotypes strongly resistant, no qualitative resistance
- Quantitative resistance strongly geographically structured

Exapted pathosystems





P. deltoides

P. trichocarpa

Mlp,Mxc

(Bastien et al, unpublished)
(La Mantia et al 2013)

(Vialle et al 2011)

- Qualitative resistances in *P. deltoides* almost all overcomed after 15 years
- Quantitative resistance in both sp. , structured in *P. trichocarpa*

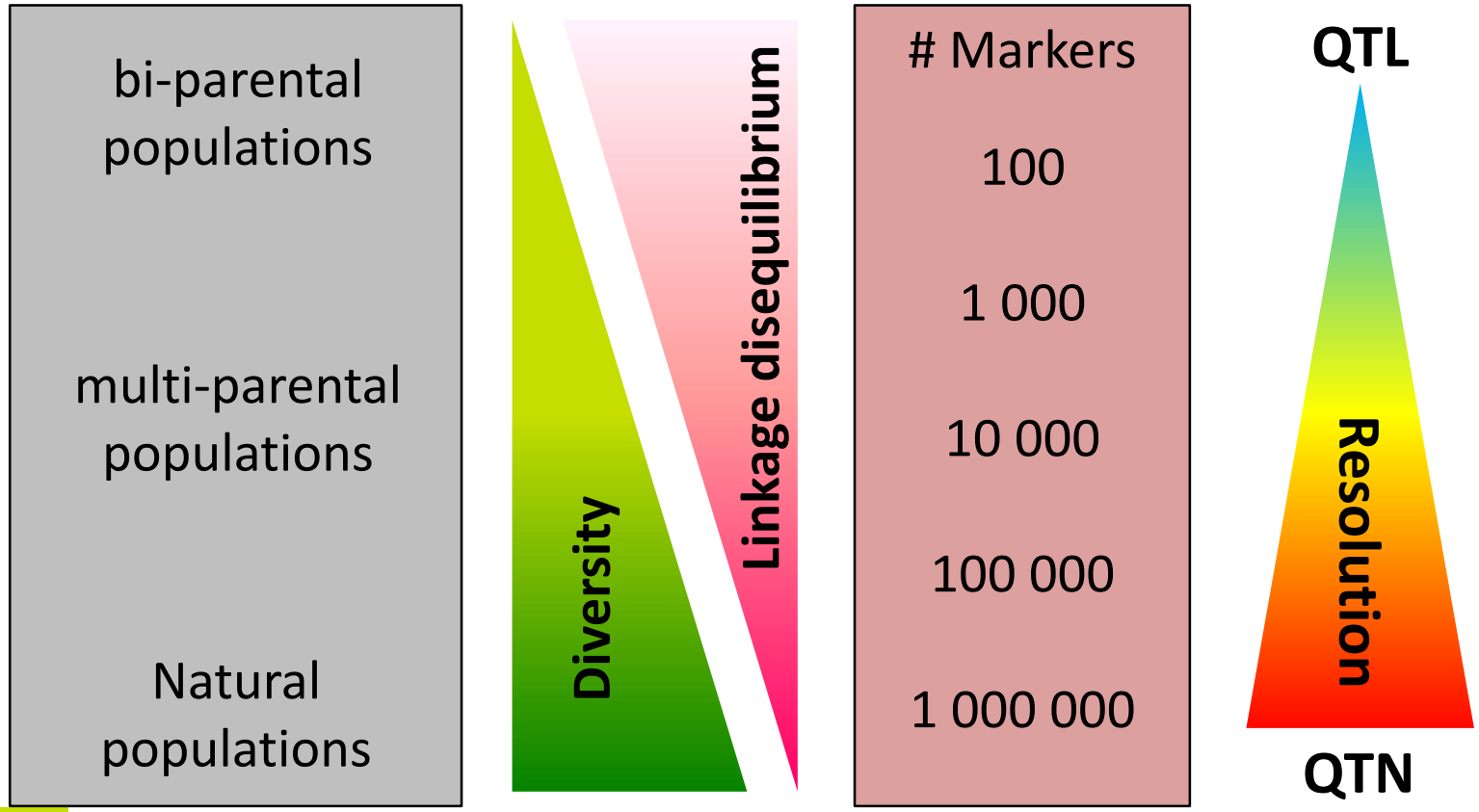
5

Genetic mapping: from QTL to QTN

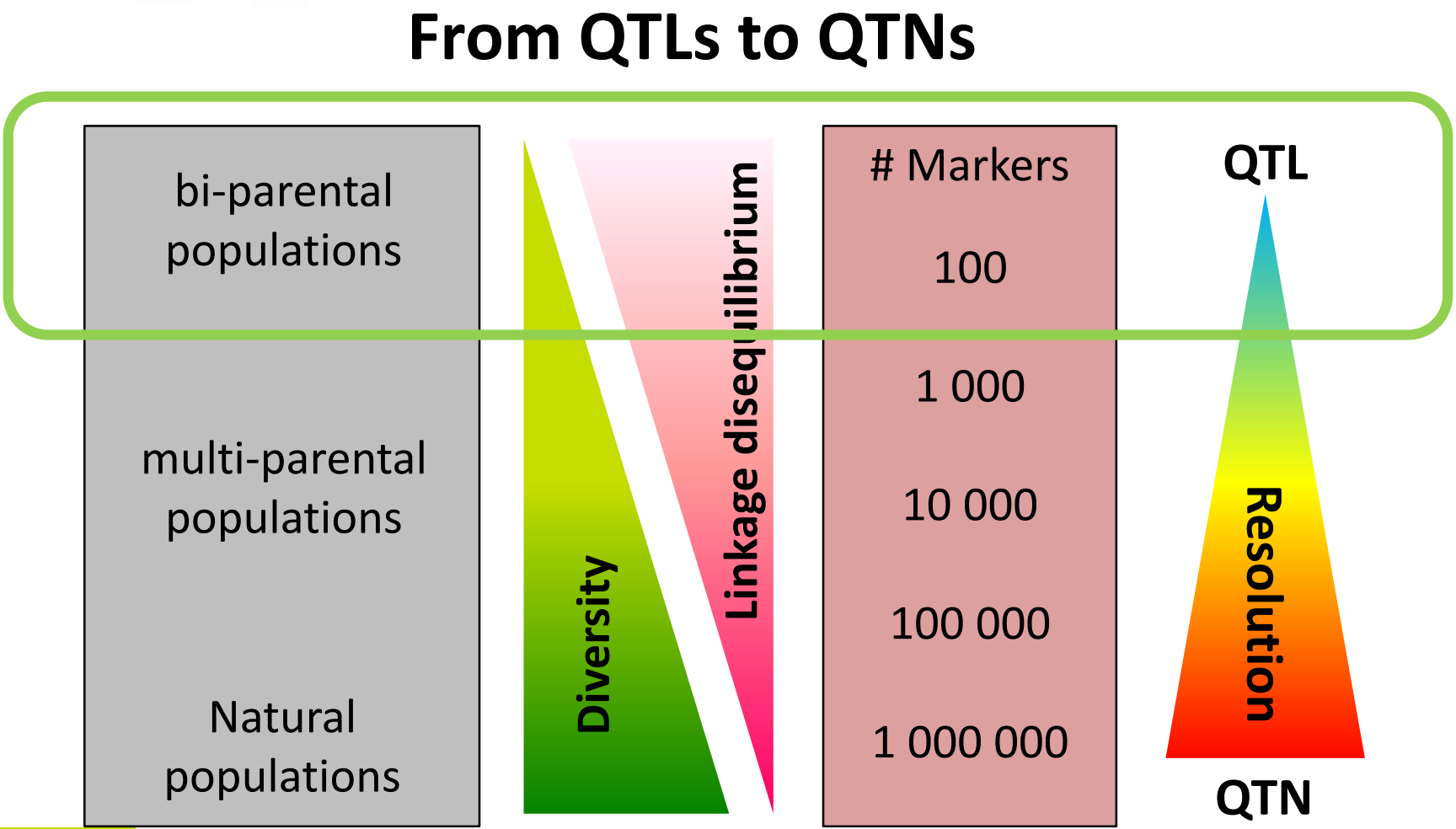
INRA
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Strategies to study genetic architecture of resistance

From QTLs to QTNs



Strategies to study genetic architecture of resistance

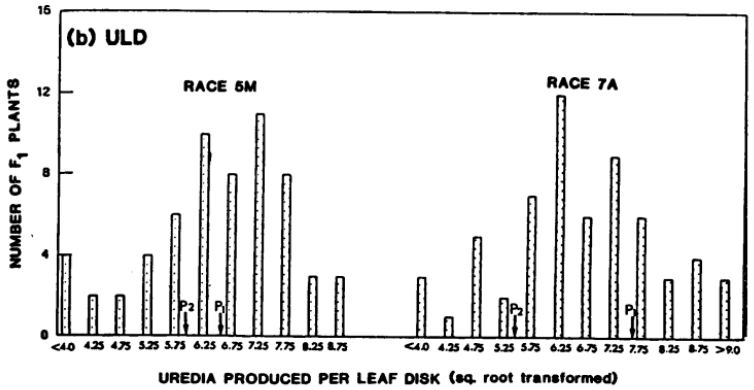


Natural pathosystem

bi-parental populations

P. deltoides x *P. deltoides* F1

2 strains *Mmd*



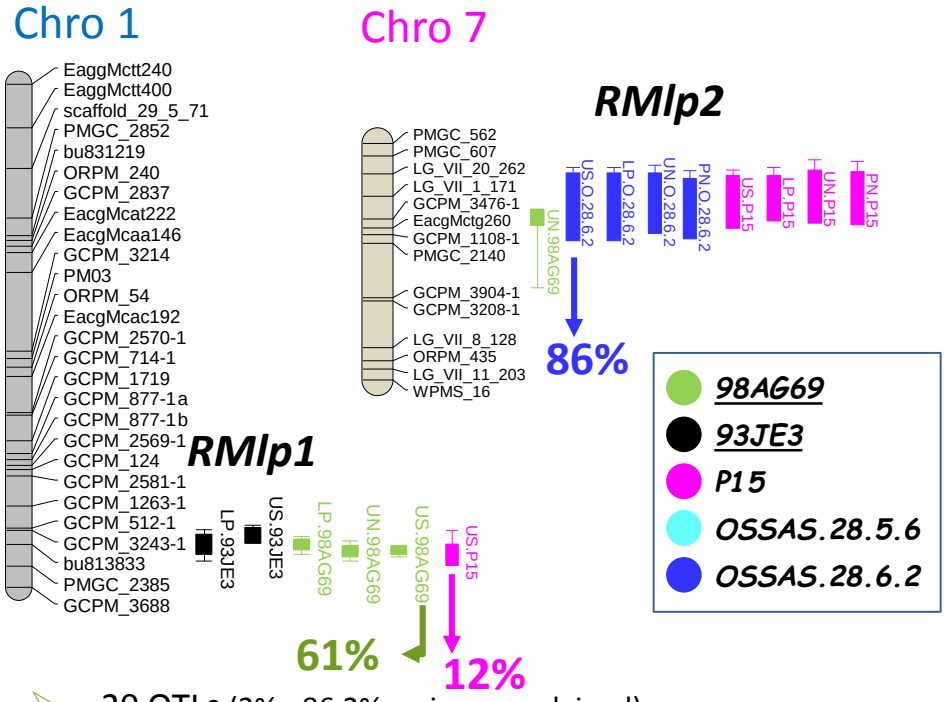
(Prakash & Heather, 1989)

- Qualitative resistance (locus *Lrd1* ; *Tabor et al, 2000*)
- Genetic variability for quantitative resistance (Prakash & Haether 1989a)
- Interaction genotype/strain (Prakash & Heather 1989b, Tabor et al 2000)

No map localisation

P. nigra x *P. nigra* F1

5 strains *Mlp*

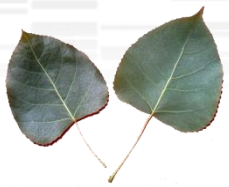


- 20 QTLs (2% - 86.2% variance explained)
- 2 major QTLs (chro 1 & 7, RMlp1, RMlp2)
- QTLs and their effect are strain specific (no QTL for OSSAS.28.5.6).
- Colocalisation with R-genes clusters
- « HR » mainly controlled by 1 locus

(Elmalki, 2012, PhD; Elmalki et al , in prep)

Exapted pathosystem

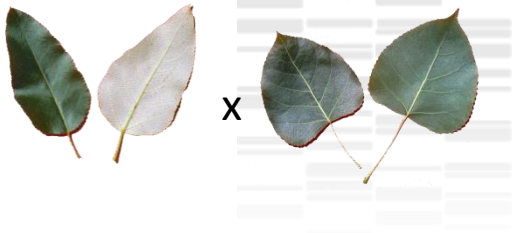
bi-parental populations


x


Mainly hybrid families used


x


Family	Type	Taxon	Challenged against
Family 331	F2	*P. trichocarpa x P. deltoides*	*Mmd* *Mlp*
Family 545	F1	*P. trichocarpa x P. deltoides*	*Mcx*
Family 54	F1	*P. deltoides x P. trichocarpa*	*Mlp*
Family 87001 Family 87002	F1 F1	*P. deltoides x P. trichocarpa* *P. deltoides x P. nigra*	*Mlp*



Exapted pathosystem

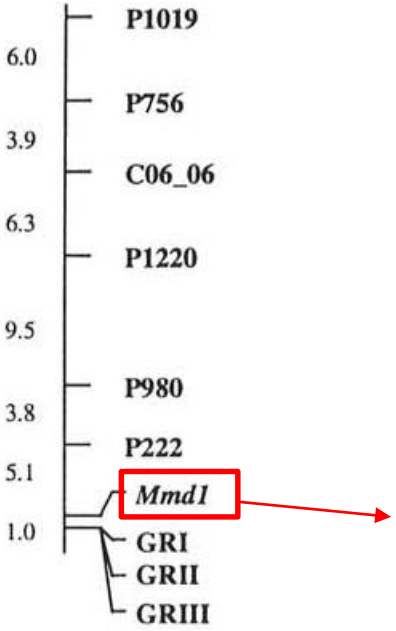
bi-parental populations

Family 331, ***P. trichocarpa*** x ***P. deltoides***

1 ***Mmd*** *strain & field assessment*

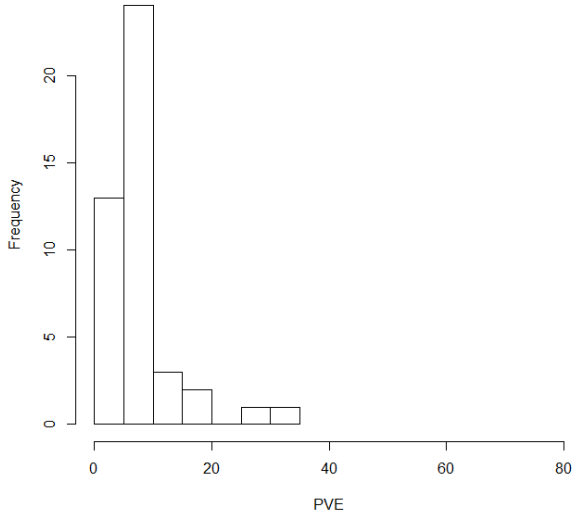
5 Mlp strains

Chromosome 4



Effect on field resistance

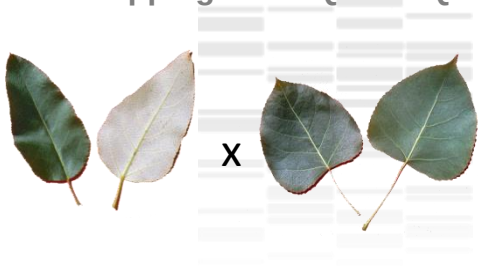
- 17 regions (44 QTLs)
- The largest on chro 15
- Strain specific QTLs



The qualitatives R against ***Mmd*** is not working against ***Mlp*** !

(Newcombe et al 1996; Newcombe, 1998)

(Dowkiw et al., in prep.)

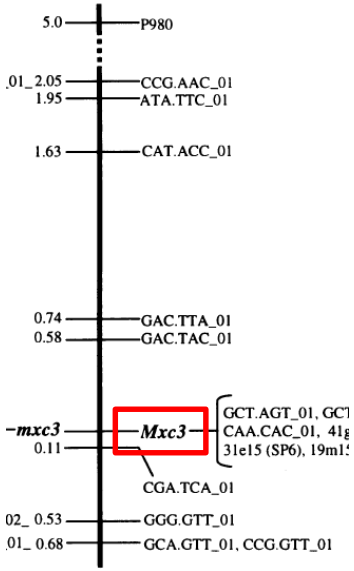


Exapted pathosystem

bi-parental populations

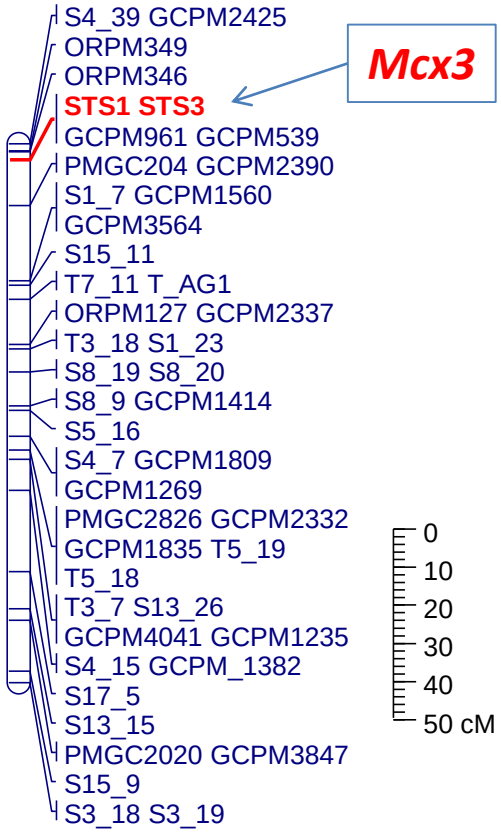
Family 545, *P. trichocarpa* x *P. deltoides*

1 *Mcx* strain



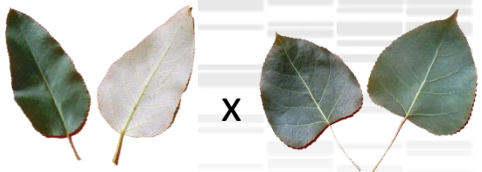
Confirmed location on Chromosome 4

Fam 13



(Stirling et al 2001)

(Yin et al 2004)

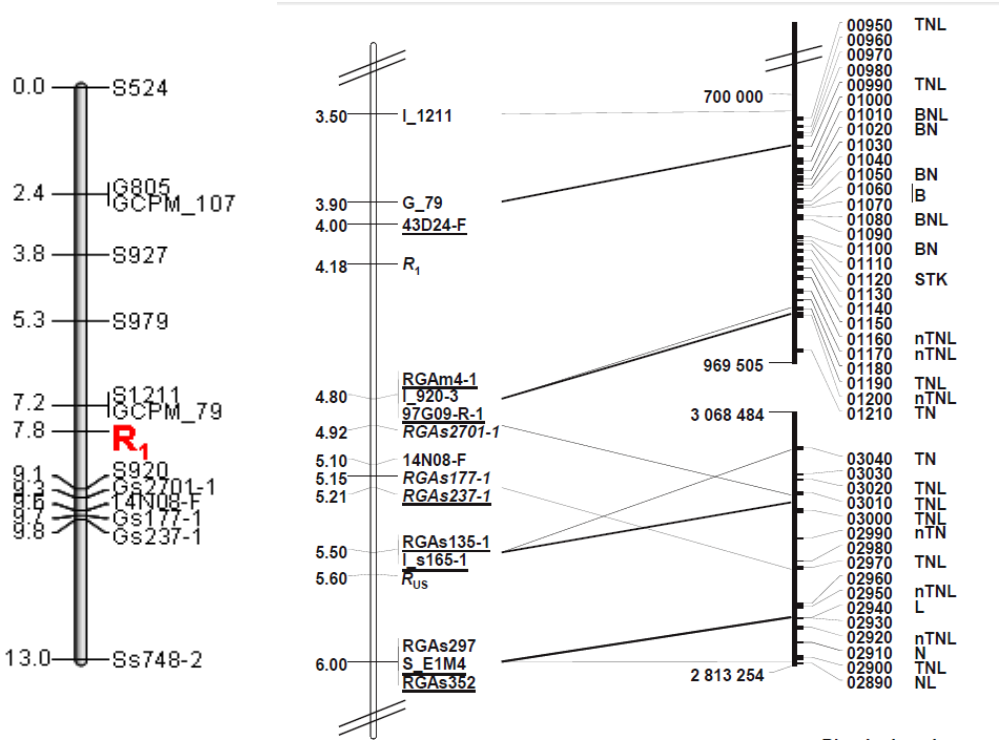


Exapted pathosystem

bi-parental populations

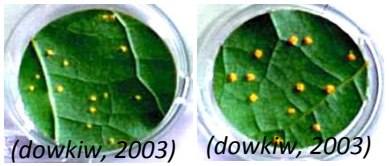
Family 54 ***P. deltoides*** *x* ***P. trichocarpa***

5 ***Mlp*** & field assessment



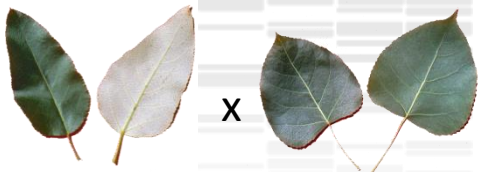
- 2 major factors on Chromosome 19
  - R_1 inherited from *P. deltoides*
  - R_{US} from *P. trichocarpa*
- Several minor QTLs
- Specificity
- Fine and physical mapping, cloning R_{US}
- In major R-gene cluster

R_{us} r_{us}



(dowkiw, 2003) *(dowkiw, 2003)*

(Jorge et al, 2005; Bresson et al, 2011)

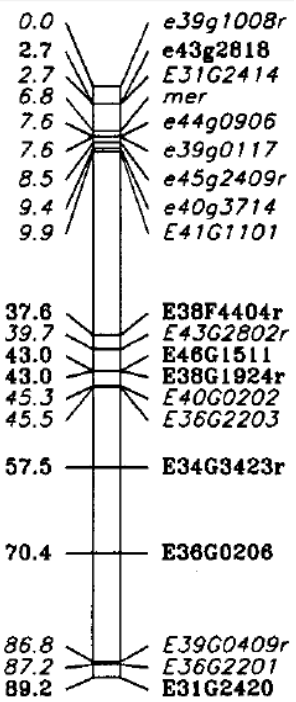


Exapted pathosystem

bi-parental populations

Family 87001 ***P. deltoides*** *x* ***P. trichocarpa***
Family 87002 ***P. deltoides*** *x* ***P. nigra***
3 ***Mlp*** strains

Chromosome 19

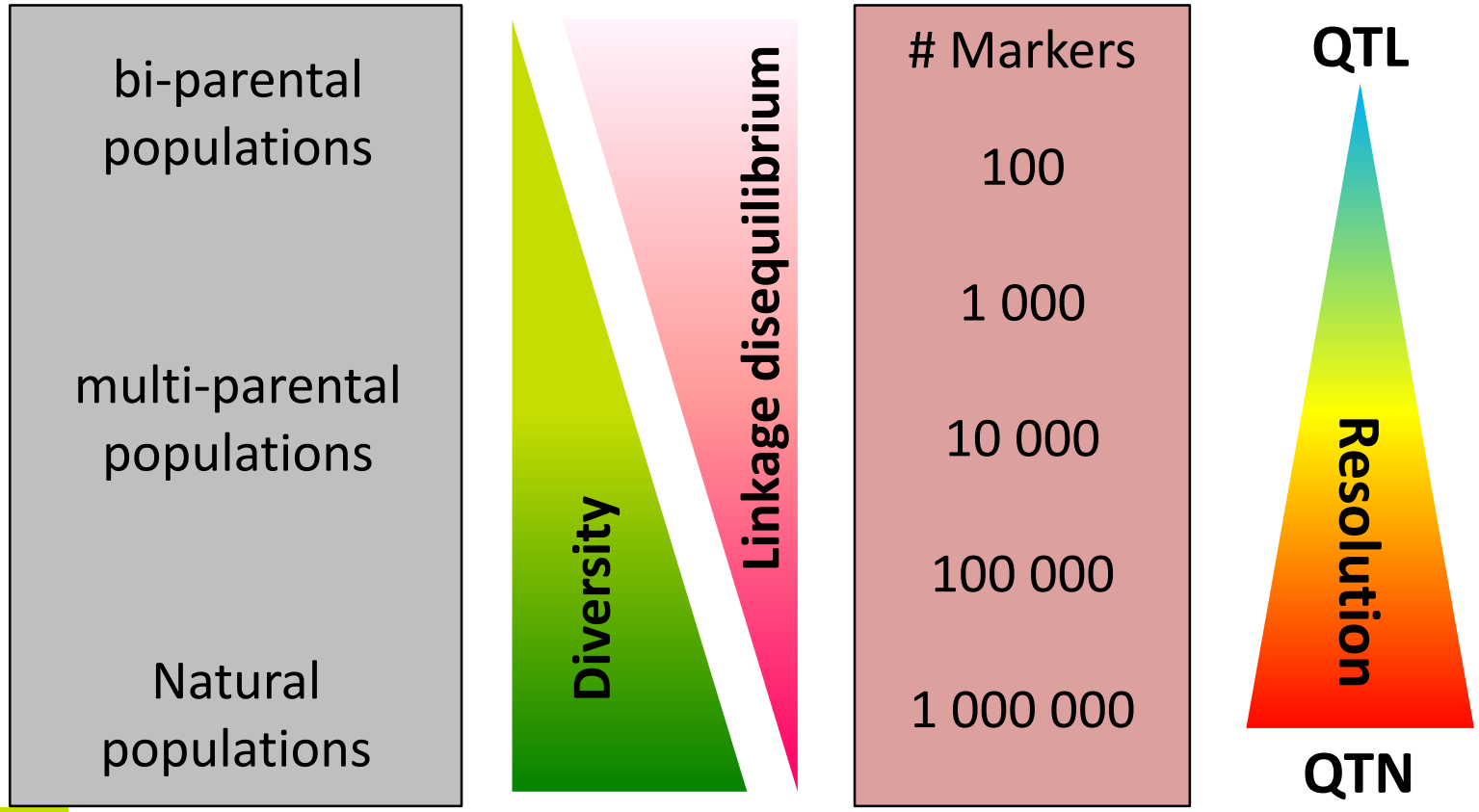


Mer locus inherited from *P. deltoides*
Fine and physical mapping, cloning failed

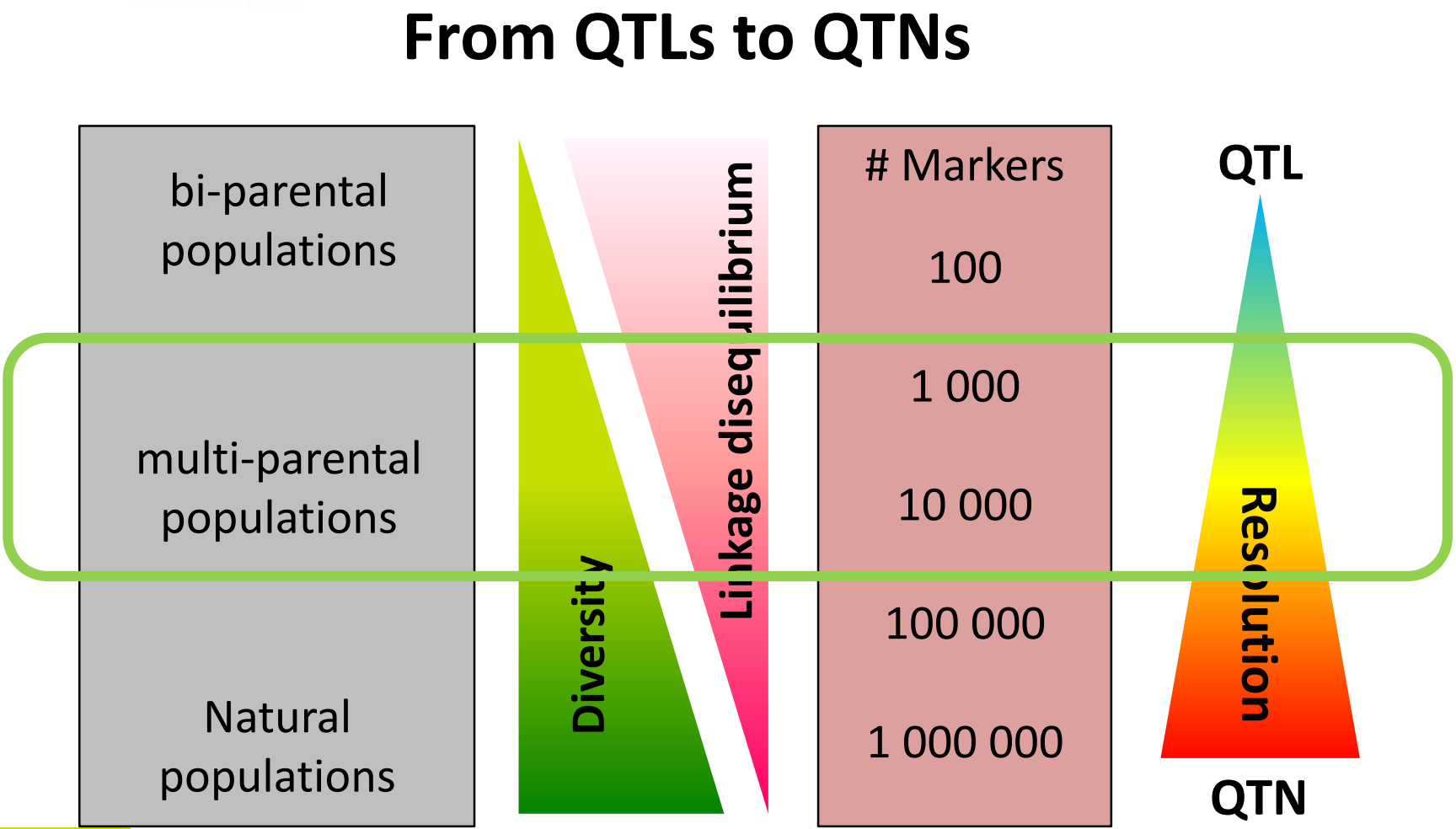
(Cervera et al, 2001; Lescot et al, 2004)

Strategies to study genetic architecture of resistance

From QTLs to QTNs

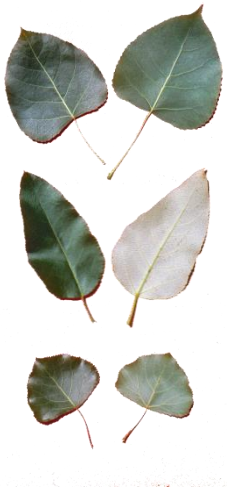


Strategies to study genetic architecture of resistance

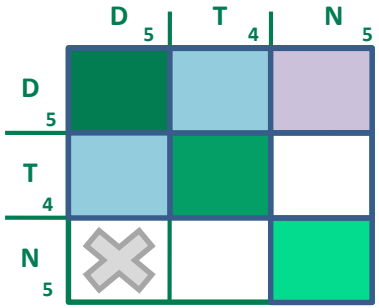


Natural and exapted pathosystem

multiparental populations



14 x 14 **factorial mating design**



118 FS families

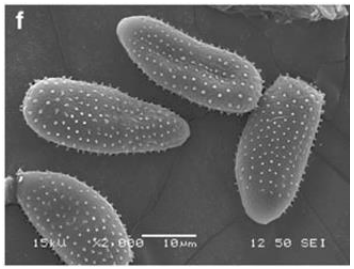
3480 clones

INRA

1988-2000

vs.

Melampsora larici-populina

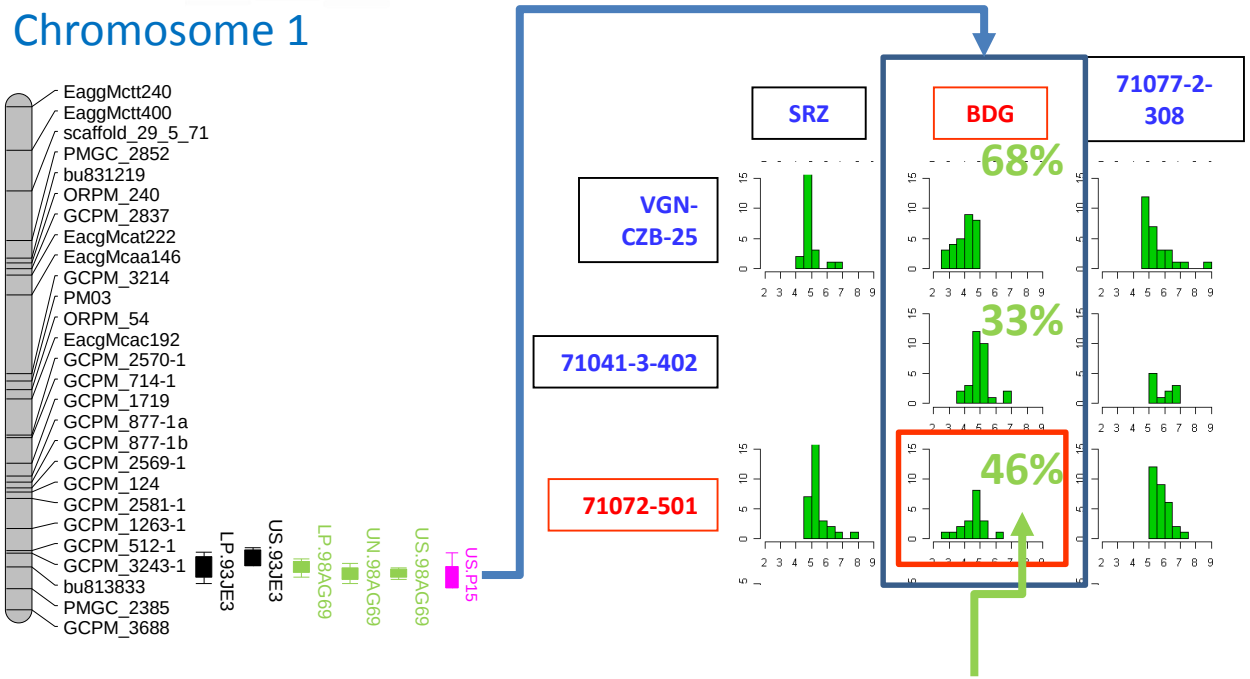


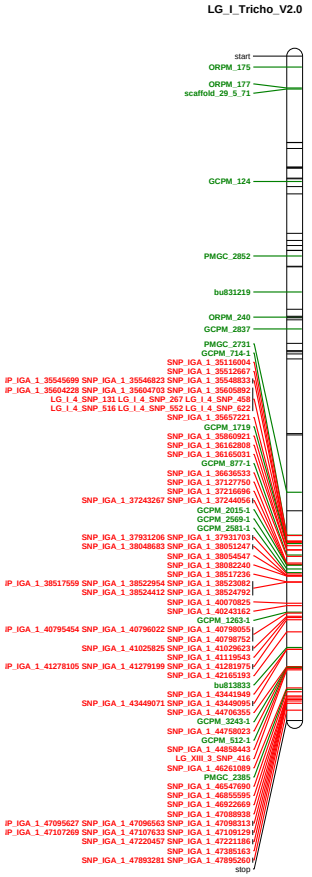
(Vialle et al 2011)

- Several characterized strains
- Field trials

Natural pathosystem

multiparental populations





- In each family, markers explaining the variation for US
- The region of the QTL has been densify in markers (72 SNP on 11 Mb)
- Validation for strain 98AG69

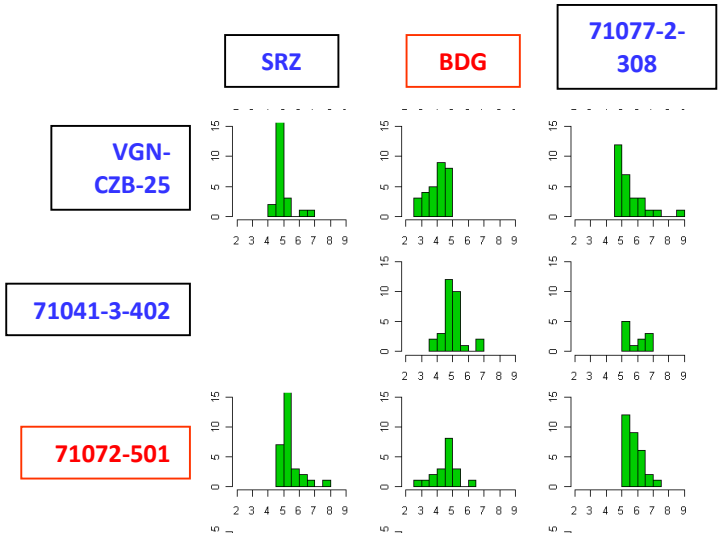
(Elmalki, 2012, PhD; Elmalki et al , in prep.)

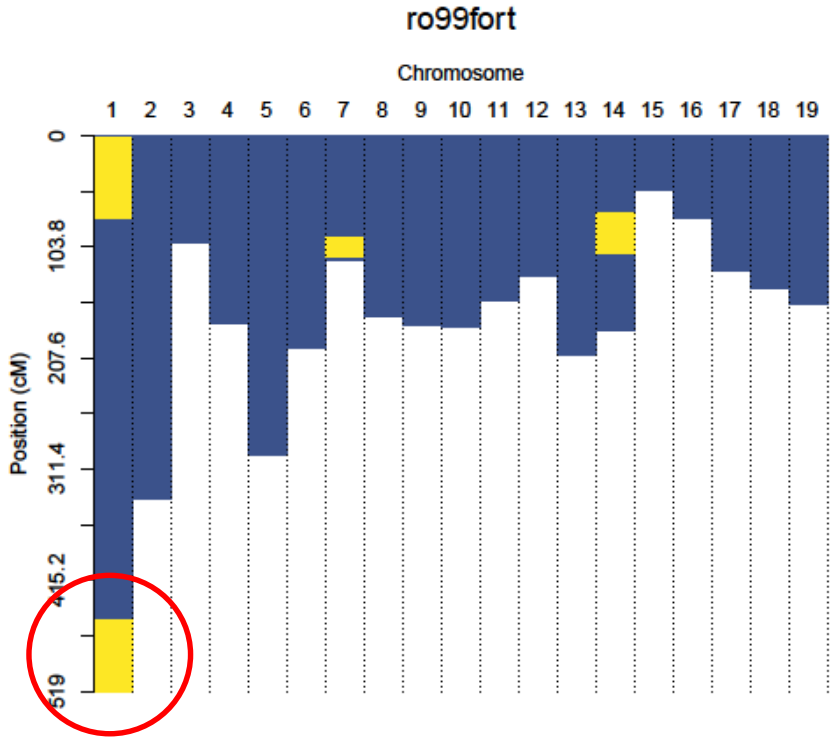
Natural pathosystem

multiparental populations

QTL mapping in complex pedigre using FlexQTL (Bink et al. Univ. Wageningen)

Rust scoring in field trial





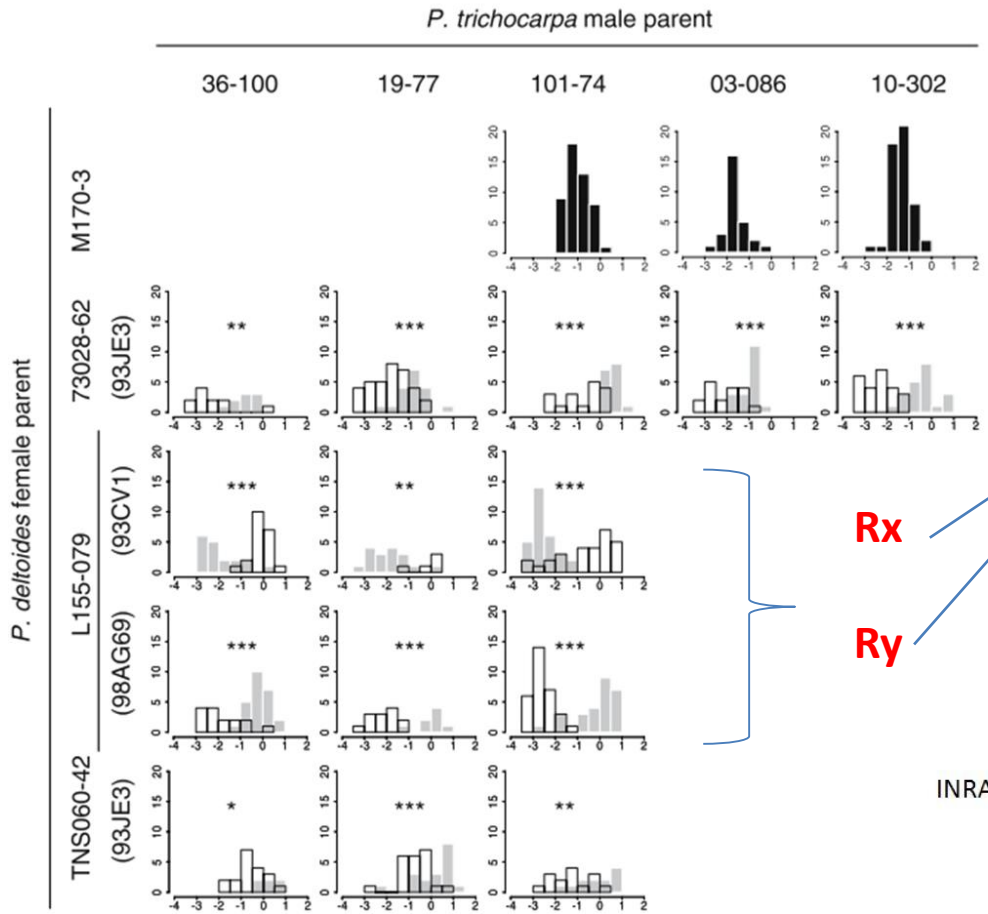
Previously detected

(Mangel et al, unpublished)

Exapted pathosystem

multiparental populations

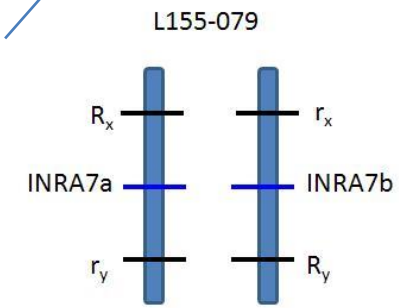
2 *Mlp* strains

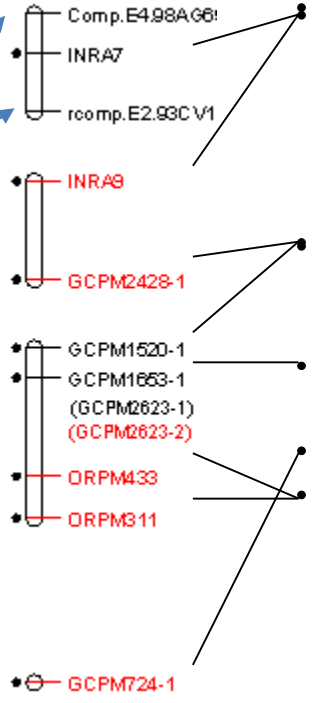


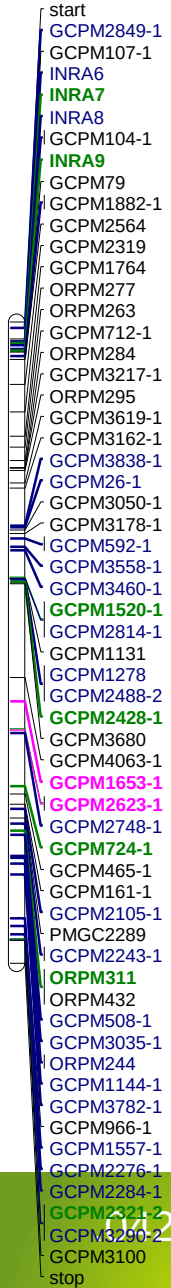
R1
(slide 31)

Rx

Ry







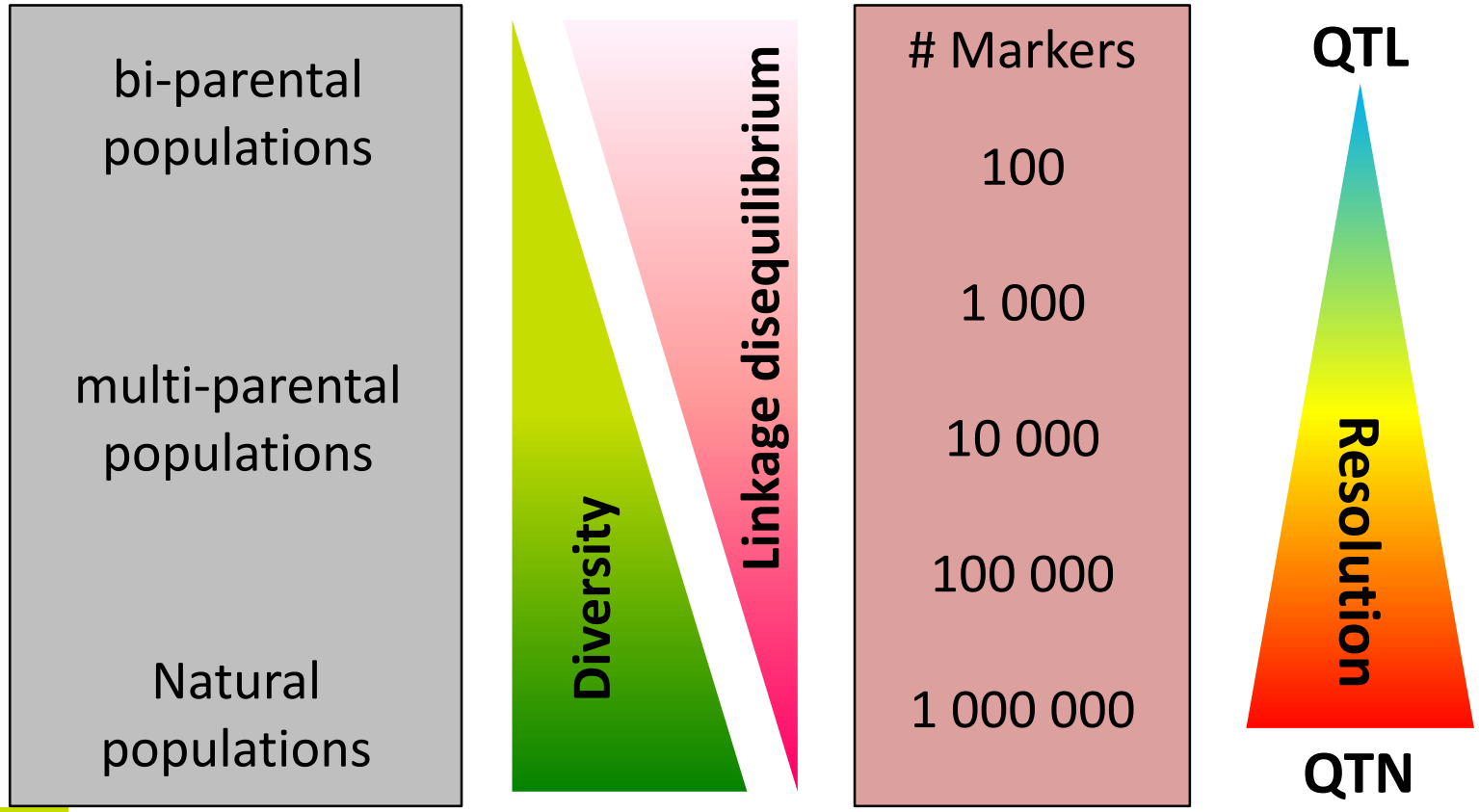
2 additional loci linked in repulsion

(Dowkiw and Bastien, Tree Genetics & Genomes, 2007)

(Vlainic et al)

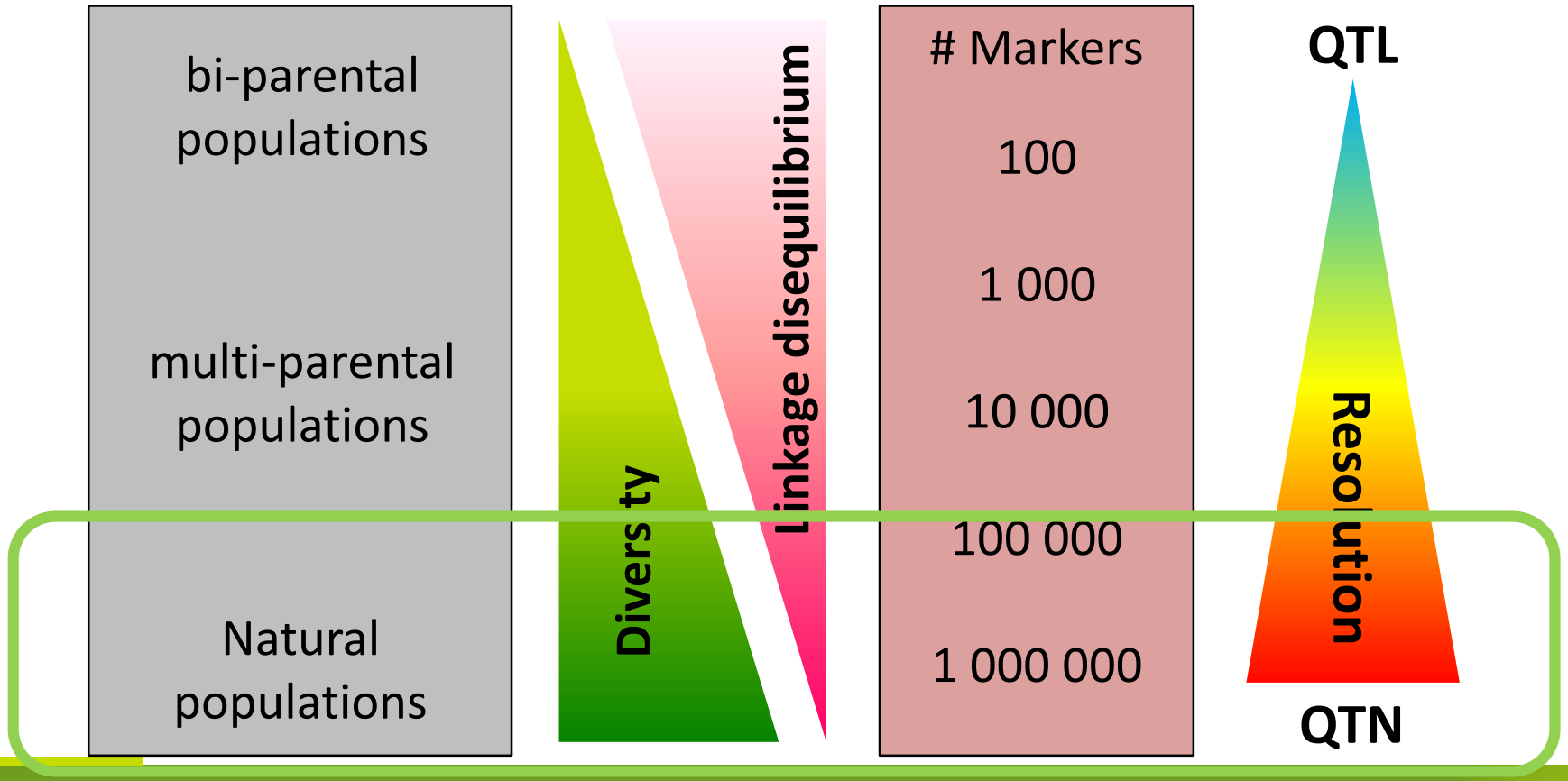
Strategies to study genetic architecture of resistance

From QTLs to QTNs



Strategies to study genetic architecture of resistance

From QTLs to QTNs

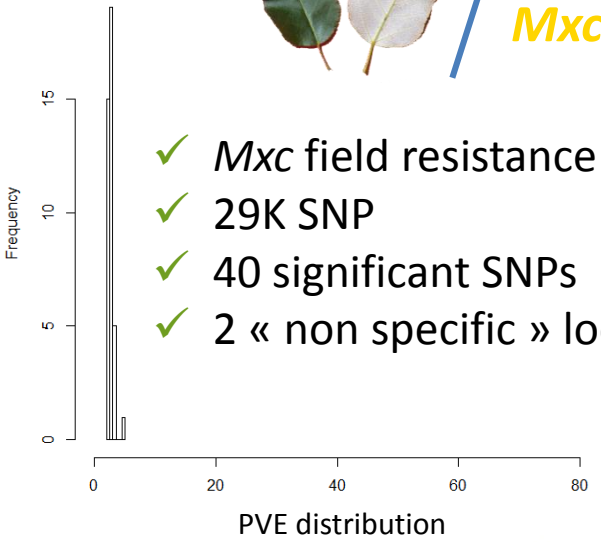


Natural populations
GWAS

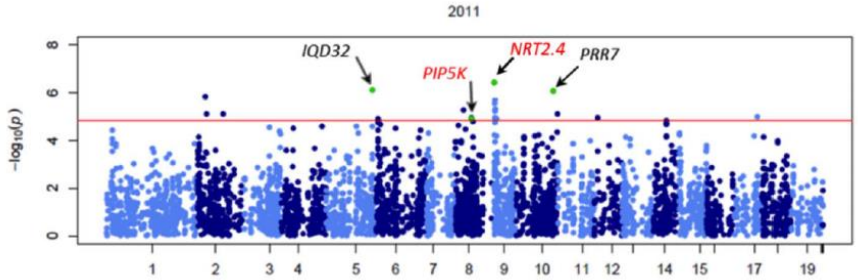
LaMantia et al. PlosONE 2013



Mxc



- ✓ *Mxc* field resistance for 412 *P. trichocarpa*
- ✓ 29K SNP
- ✓ 40 significant SNPs
- ✓ 2 « non specific » loci



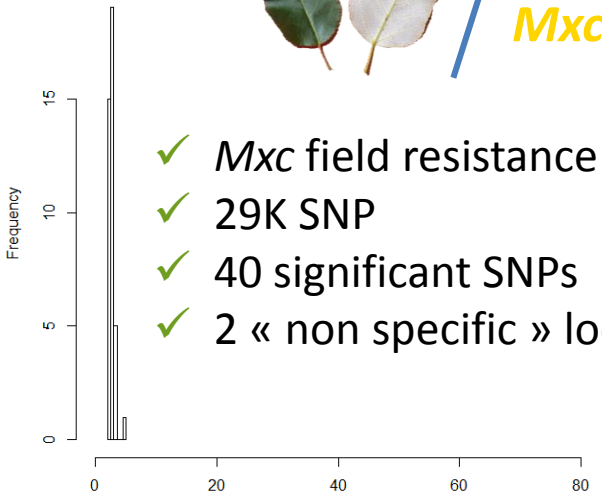
- ✓ 3 sign. SNP match with differentially expressed genes in *P. trichocarpa* x *P. deltoides* cv. Beaupré/*Mlp* interaction

Natural populations GWAS

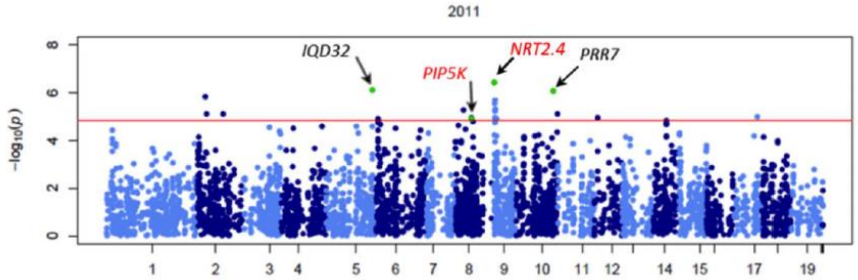
LaMantia et al. PlosONE 2013



Mxc



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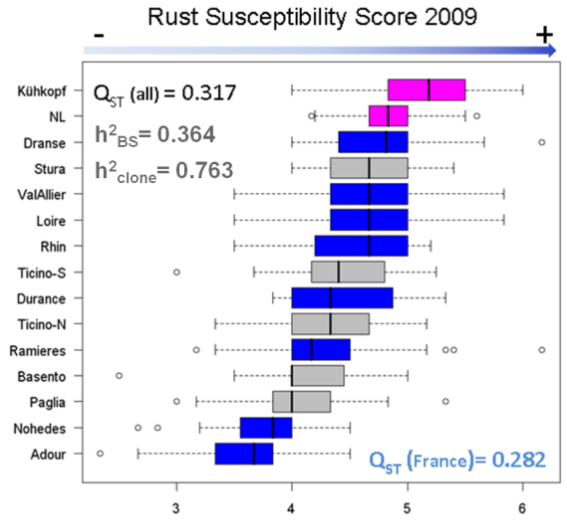
- ✓ 3 sign. SNP match with differentially expressed genes in *P. trichocarpa* x *P. deltoides* cv. Beaupré/*Mlp* interaction

Bastien et al, in prep



Mlp

- ✓ Lab & Field resistance for 1100 *P. nigra*
- ✓ 8K SNP (focused on genes & candidate regions)
- ✓ To be done …



6

Comparative genomics

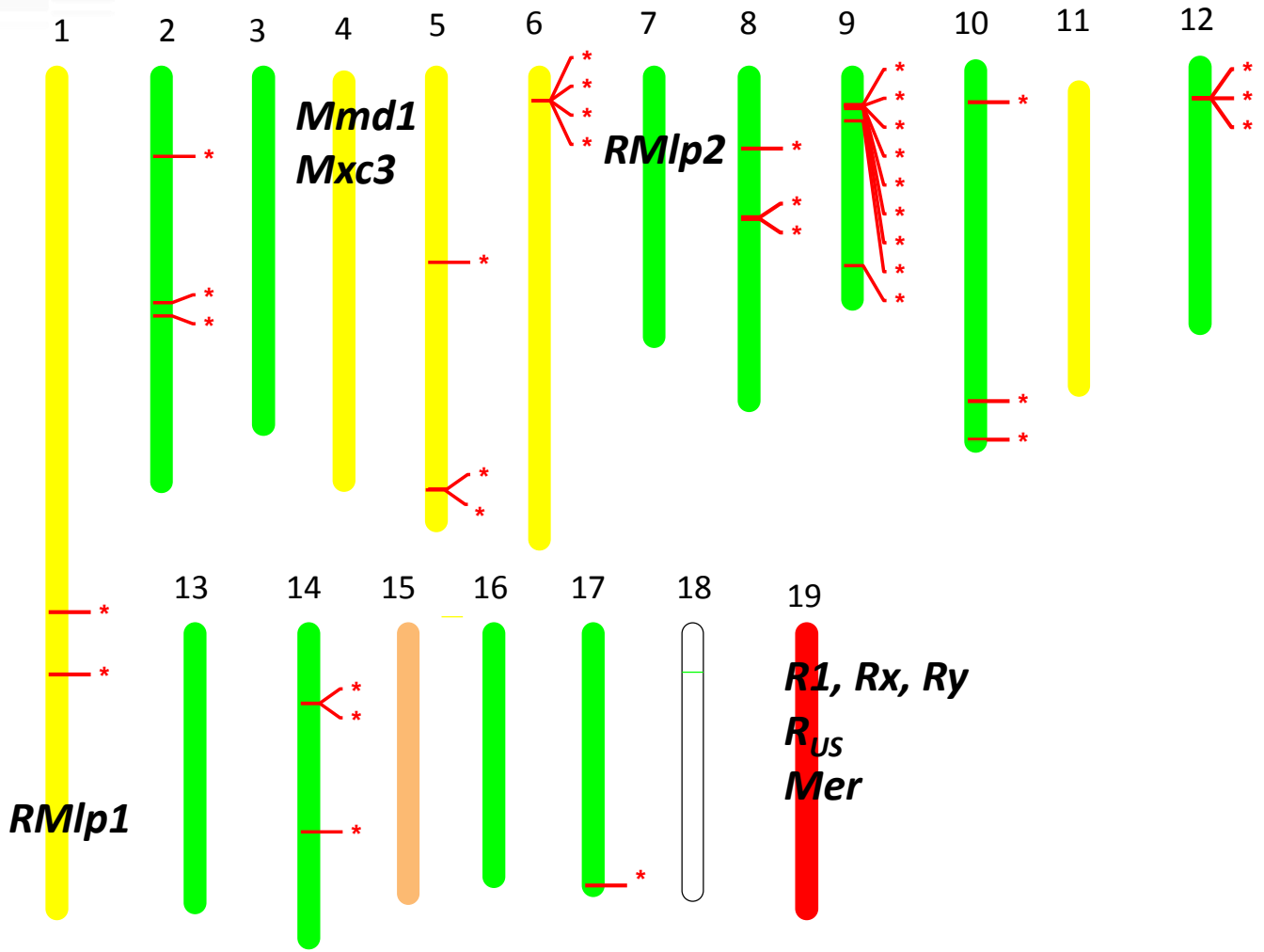
INRA
SCIENCE & IMPACT

Sum up !

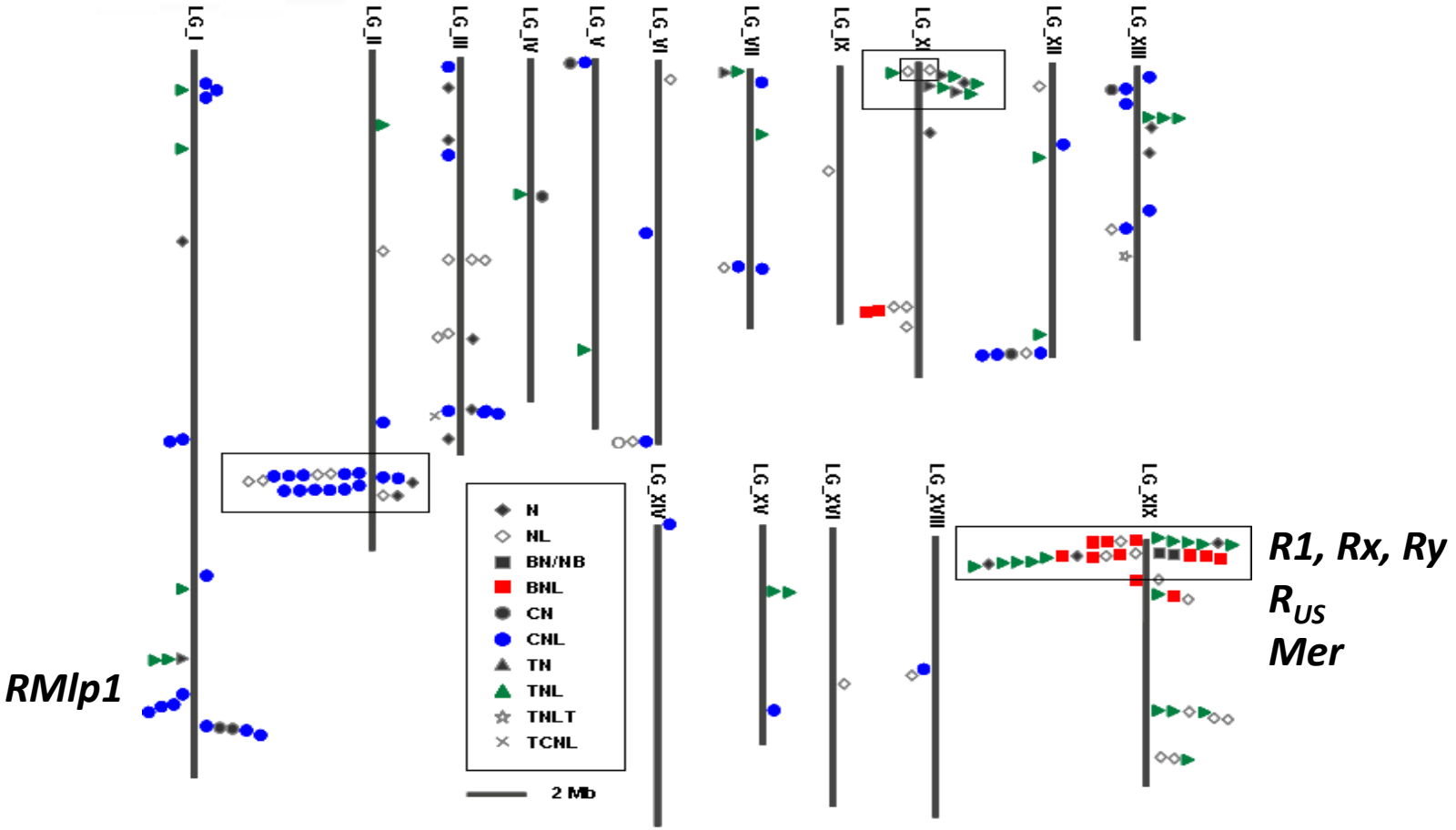
Loci and QTL identification (« Heat map »)

4 mapping pedigrees
Family 331 : TDxTD
Family 54: DxT
87001,2 : DxN, DxT
Family 1311: NxN
Factorial
GWAS (*)

- Several components lab & field
- Several strains

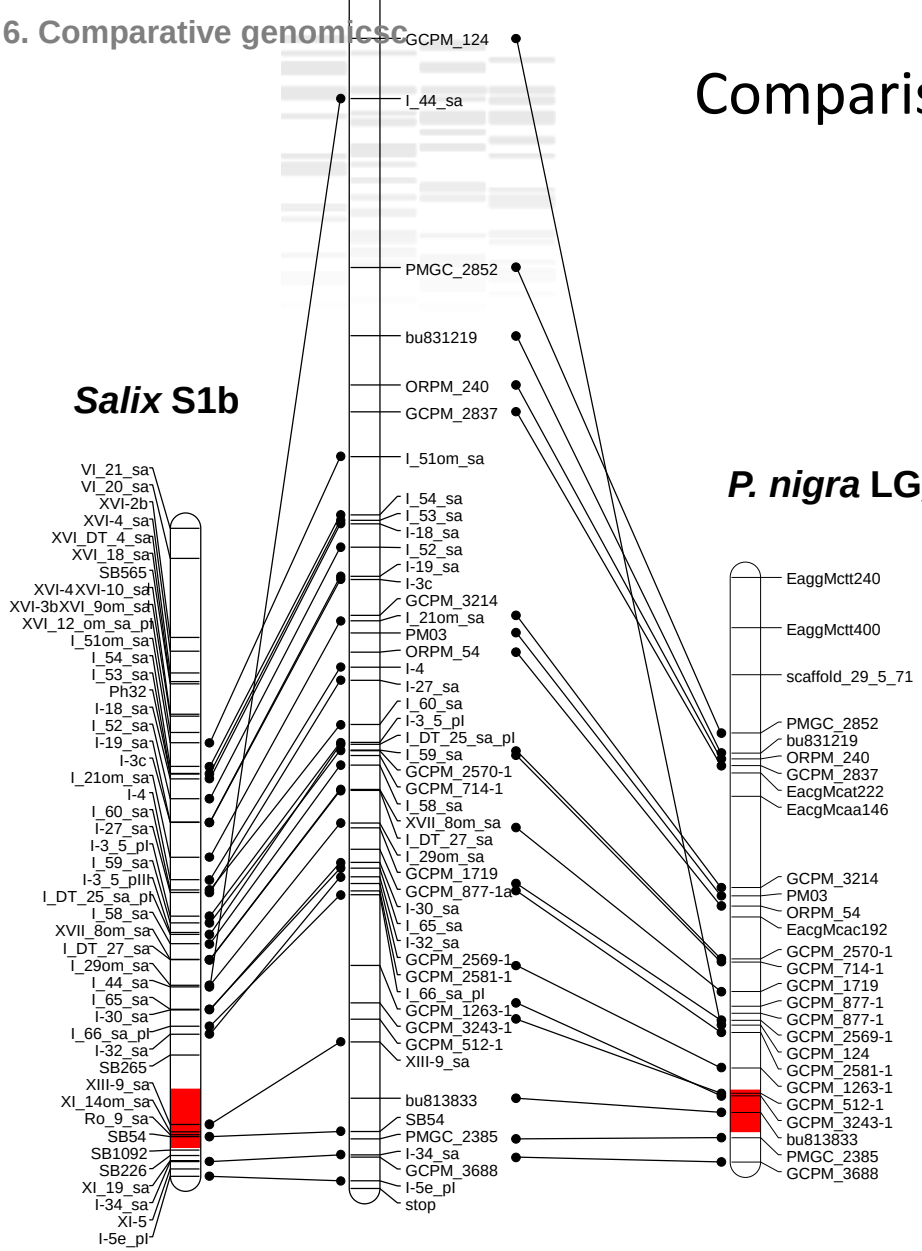


Some RGA clusters co-localise with resistance factors and QTLs described

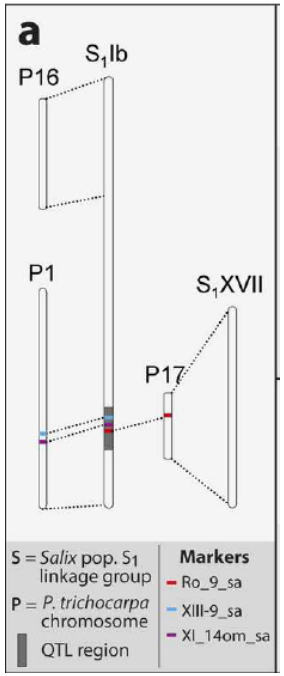


(Kolher et al 2008)

Comparison with *Salix* / *Mle* pathosystem



- Alignement of maps possible thank to co-linearity between *Salix* and *Populus* genomes
- Genetic mapping show co-location
- But physical mapping and cloning not
- Some complex rearrangement between both genus

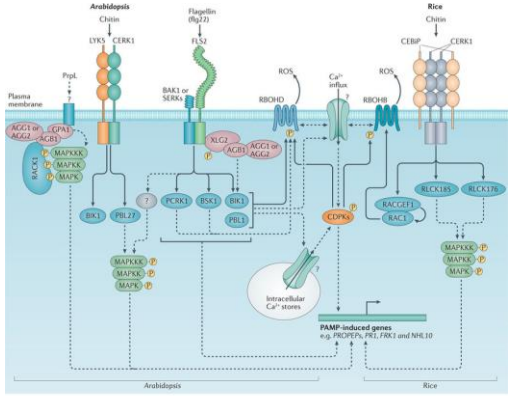


(Samils et al, 2011)
(Bertin et al 2016)

Any pattern of loci identify with natural / exapted classification ?

Major Loci identified	*Melampsora occidentalis*	*Melampsora medusae f.sp. deltoideae*	*Melampsora larici-populina*
Populus trichocarpa	Natural **?**	**Exapted** ***Mmd1***	**Exapted** ***Rus,***
Populus deltoides	?	Natural **?**	**Exapted** ***R1, Rx, Ry***
Populus nigra	?	**Exapted**[a] **?**	Natural *RMlp1,RMlp2*

- Both control quantitative / qualitative resistance
- Both showed specificity
- No overlap between loci identified, expected ? =>

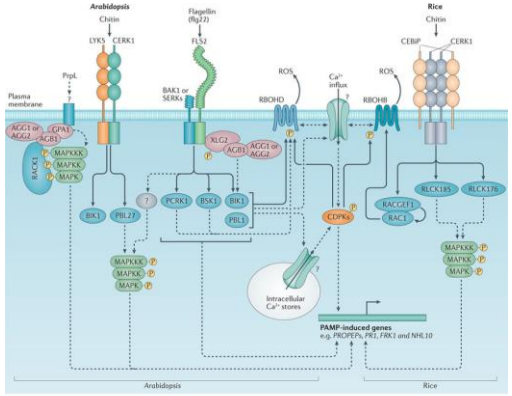


Couto & Zipfel, 2016

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Couto & Zipfel, 2016

What are the function of exapted resistances in the natural area?

7

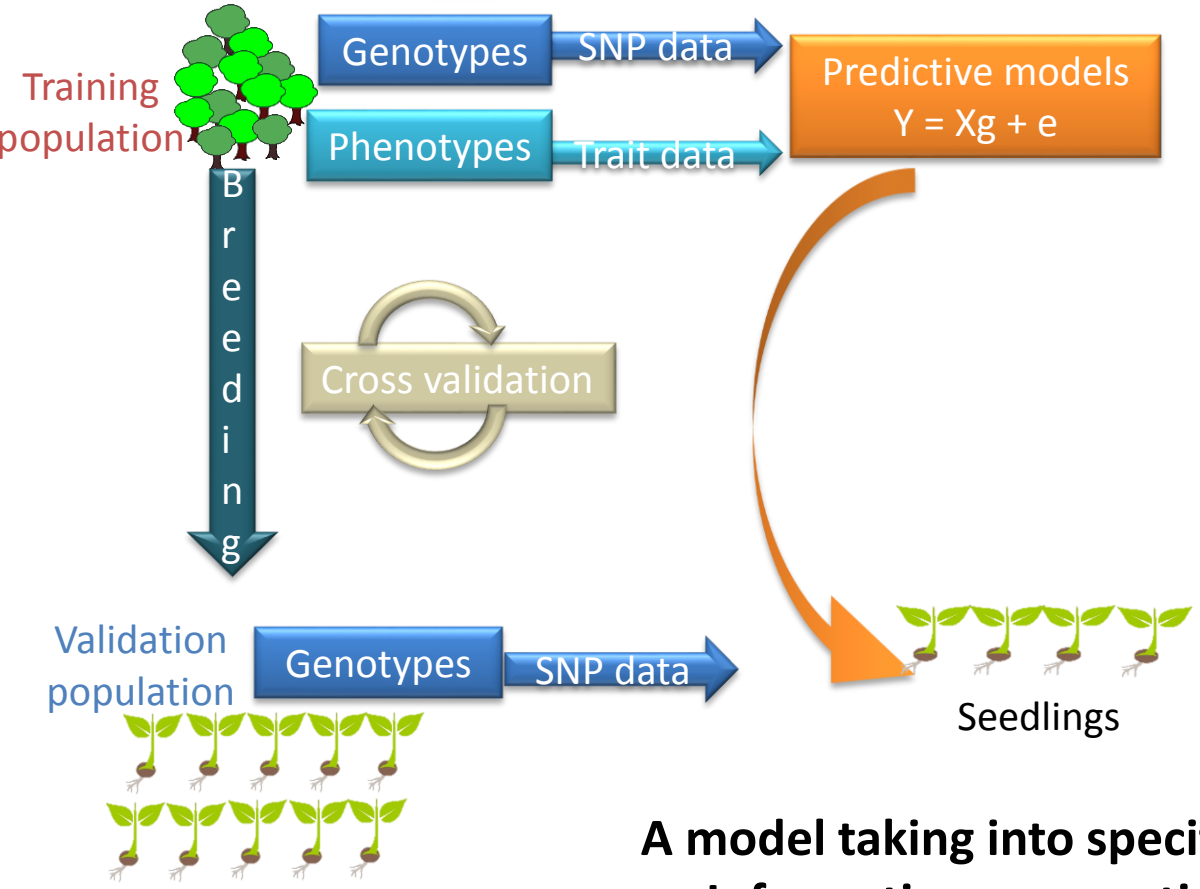
Conclusion: potential uses for breeding

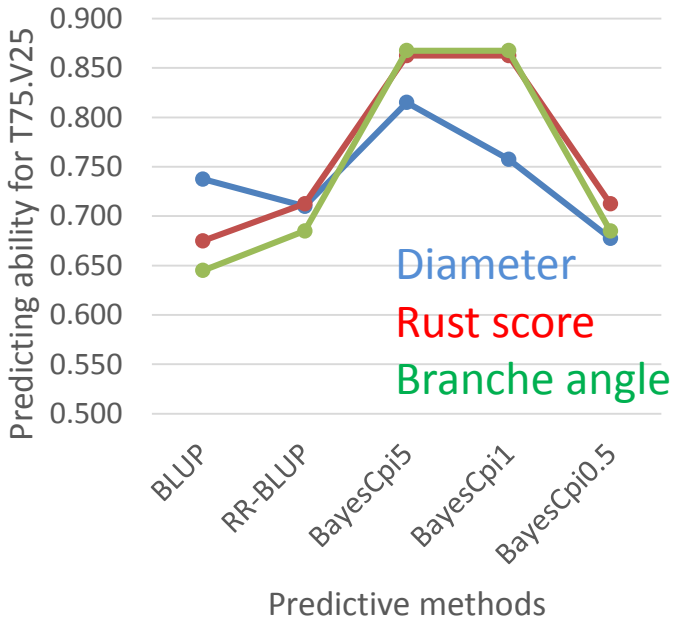
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A case study of genomic selection in *Populus nigra*

Which is the best predictive model for rust resistance ?





A model taking into specific marker effects on the trait.
=> Information on genetic determinism is useful!

(Pegard et al , in prep.)

Research in Poplar genetics and genomics at INRA

(BioForA-Orleans & URGV-Evry)

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Cv 'italica'



M. Villar

P. nigra

Natural populations

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With focus on :

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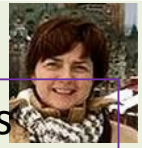
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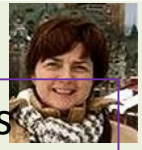
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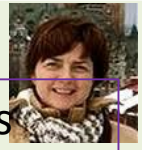
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Long term management of genetic diversity


L. Sanchez

genetic/genomic prediction

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Melampsora- poplar interaction
(R durability, selection pressures)

Wild/Cultivated Interactions


A. Dowkiw

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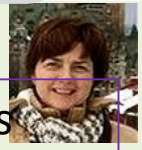

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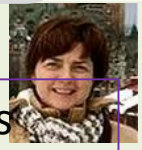
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A. Dowkiw


C. Bastien

French national Breeding Program
(D, DxN, DxT,)

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